

Letter to the Editor

T_H17-associated cytokines (IL-17A and IL-17F) in severe asthma*To the Editor:*

Asthma affects approximately 8% of the adult population and as many as 20% of children worldwide.¹ Most of the studies have been conducted on patients with mild to moderate asthma and have shown airway inflammation to be associated with T-cell activation, eosinophil accumulation, and T_H2-type cytokine production. Severe asthma represents 10% of the asthmatic population but has greater morbidity and mortality and uses a disproportionate amount of health care expenditures. Furthermore, the subpopulation of patients with severe asthma appears to manifest a different pattern of airway inflammation that is not associated with either classic T_H1 or T_H2 cells. For example, the neutrophil chemoattractant IL-8 is prominently expressed in the airways of such patients.² These observations suggest the possibility of a role for other T-cell subsets. Regulatory T cells have been shown to be reduced in severe asthma, but a recently described subset of T cells, the T_H17 cell, has not been investigated to date.

T_H17 cells produce a number of cytokines, but in particular IL-17A and IL-17F.³ These cells have been implicated in the pathogenesis of a number of autoimmune diseases, including psoriasis and rheumatoid arthritis.³ We have reported previously that IL-17A is upregulated in asthma and in nasal polyposis, and in the latter condition, its expression is resistant to topical steroids,⁴ consistent with a recent report showing an association between a T_H17-driven murine model of asthma and resistance to corticosteroids. A similar steroid hyporesponsiveness appears to be a feature of severe asthma. T_H17 cells also produce TNF- α , which has been reported to be an important mediator of severe asthma and has itself been implicated in the induction of a number of proinflammatory cytokines. To induce T_H17 cells, there is a requirement for IL-6 and TGF- β , and all of these cytokines have also been reported in association with severe asthma.⁵ IL-23 is an important cytokine involved in the regulation of T_H17 cells, but it is principally associated with the maintenance rather than

induction of this phenotype.⁶ The T_H17-derived cytokines are important in the induction and activation of neutrophils and could also be involved in promoting the proinflammatory properties of structural cells, exemplified by the secretion of IL-6 and IL-8 from airway epithelial cells. The objectives of our study were to investigate the expression of IL-17A and IL-17F in the airways of patients with asthma of varying severity.

Airway tissues were obtained by bronchoscopy performed under light sedation following American Thoracic Society guidelines for biopsies of patients with severe asthma (n = 10), moderate asthma (n = 10), and mild asthma (n = 10), and controls without asthma. The diagnosis and severity of asthma were based on American Thoracic Society criteria. Subjects were recruited from the Montreal Chest Institute and Sacré Coeur Hospital. The protocols for the study were reviewed and approved by the Ethics Committees of both hospitals, and informed consent was obtained from all participating subjects.

To detect the expression of IL-17A and IL-17F, immunocytochemistry was performed as previously described⁷ using polyclonal goat antihuman IL-17F antibody (catalog no. AF1335, concentration 0.33 μ g/mL [1:300]; R&D Systems, Minneapolis, MN) with antigen retrieval. Polyclonal IL-17A goat antihuman antibody (catalog no. AF-317-NA, concentration 0.83 μ g/mL [1:120]; R&D Systems) was used to detect IL-17A immunoreactivity. The number of immunoreactive cells infiltrating the mucosa was assessed and expressed per square millimeter of tissue. Epithelial expression of IL-17F was assessed by using a scoring technique based on the percentage of positive epithelium. To confirm the results, real-time PCR was performed by using a LightCycler PCR machine with SyberGreen reagents (Roche, Mannheim, Germany) on RNA samples extracted from tissues from 5 cases per group with specific primers for IL-17A and IL-17F. IL-17A was expressed in all groups, and it was almost exclusively expressed in mononuclear cells present in the subepithelial tissue within clusters of inflammatory cells (Fig 1). Some immunoreactive cells were present in the epithelium and within smooth muscle bundles. The number of IL-17A⁺ cells was

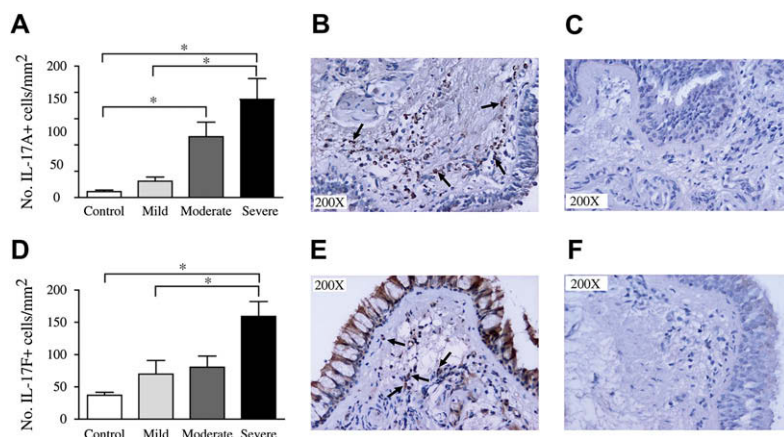


FIG 1. Increased IL-17A and IL-17F immunoreactivity in lung tissue sections from patients with severe asthma. The numbers of IL-17A-immunoreactive (A) and IL-17F-immunoreactive (D) cells are expressed as the number of cells per square millimeter of subepithelial surface. Representative immunocytochemistry stainings for IL-17A (B) and IL-17F (E) from patients with severe asthma. Negative control Goat IgG isotype staining representing IL-17A (C) and IL-17F (F) in bronchial biopsies. **P* < .05 (ANOVA, Dunn multiple comparison tests).

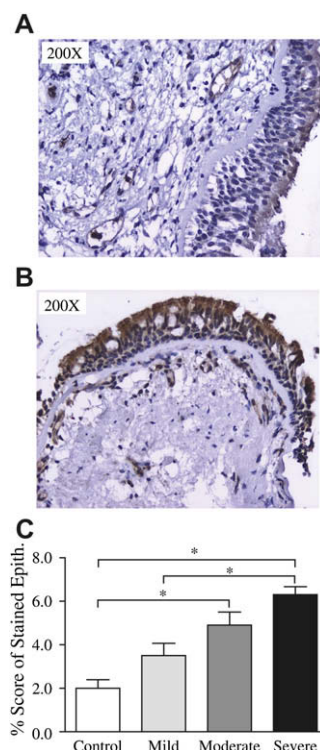


FIG 2. Evidence for IL-17F immunoreactivity in airway epithelium from subjects with asthma. Representative immunocytochemistry staining for IL-17F in bronchial epithelium from a normal control (A) and a patient with severe asthma (B). C, The intensity of IL-17F immunoreactivity was graded by blind scorers and expressed as scores of stained epithelium (0 = no staining; 8 = 100% staining). * $P < .05$.

significantly higher in the patients with severe asthma compared with all other groups. There was also an increase in these cells in subject with moderate but not in mild asthma compared with controls. In contrast, IL-17F was expressed in both epithelial cells and inflammatory infiltrates (Fig 2). IL-17F⁺ cells in the subepithelial compartment were significantly elevated only in the subjects with severe asthma, whereas the epithelial expression was also elevated in moderate subjects compared with controls. To confirm the ability of the epithelium to produce IL-17F, we performed laser capture on 3 different biopsies and extracted RNA for PCR analysis, in which all samples showed a positive signal. Results from real-time quantitative PCR performed on airway biopsies confirmed an increase in mRNA for both IL-17A and IL-17F in the severe asthma groups (Fig 3). We found an increase in expression in TGF- β , TNF- α , and IL-6 (unpublished data) in a similar group of subjects with severe asthma.

This is the first report of the expression of T_H17-related cytokines in the airway tissues in severe asthma. Although we did not perform colocalization studies, the pattern of the immunoreactive cells in the submucosa suggests that this new subset of T cells may be involved in the inflammatory process in severe asthma. IL-17 has been associated with the activation of epithelial cells *in vitro* and the induction of IL-6 and IL-8 with downstream effects on neutrophil recruitment and activation.⁷ We and others have reported an upregulation of IL-8 in severe asthma.⁸ Neutrophils were also shown to be increased in severe asthma by many groups,⁹ and this phenomenon may be IL-17-driven. We have also previously reported that IL-17 is increased in chronic

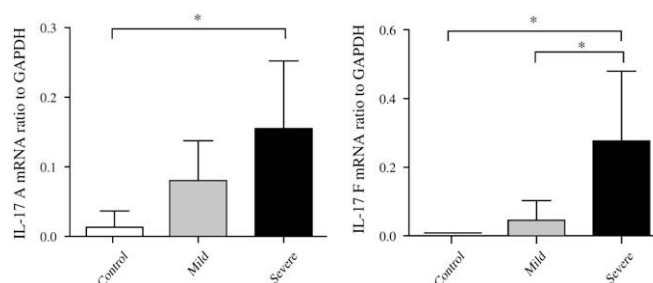


FIG 3. IL-17A and IL-17F cytokine mRNA expression in bronchial biopsies from normal controls and patients with mild, moderate, and severe asthma, relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as determined by quantitative real-time PCR (n = 5 per group). * $P < .05$.

sinusitis and that its expression is resistant to steroids.⁴ Steroid unresponsiveness in severe asthma has been attributed to the presence of neutrophilic inflammation and an upregulation of the glucocorticoid receptor β isoform. T_H17-related cytokines have been implicated in the pathogenesis of a number of diseases that do not respond well to corticosteroids. Recently McKinley et al¹⁰ have shown in a murine model that T_H17 cells not only are proinflammatory cells but also may induce steroid resistance. It is possible that steroid hyporesponsiveness in subjects with severe asthma may also relate to the presence of IL-17A and IL-17F. IL-17 has also been reported to affect structural cells and to stimulate the production of profibrotic cytokines and extracellular matrix proteins. This feature of airway remodeling in severe asthma may be attributable to an excess of these cytokines. If so, targeting IL-17 cytokines may be of value in the therapy of severe asthma, in which steroid resistance, neutrophilic inflammation, and airway remodeling are substantial.

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