

# Images in Allergy and Immunology

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## Role of eosinophils in airway remodeling

**Editor's note:** This feature, Images in allergy and immunology, is designed to highlight current concepts of the immunopathology of allergic diseases and other common immunologically mediated diseases. The presentation will appear as sets of images that involve cross-pathology, histopathology, and molecular pathology and will cover a range of topics of interest to allergists and immunologists.

Eosinophilic inflammation is the major hallmark of allergic disease (Fig 1). Eosinophils have the potential to cause damage to the airway mucosa and associated nerves in asthma through the release of granule-associated basic proteins, lipid mediators, and reactive oxygen species.

There is increasing evidence that the eosinophil may also be important in the pathophysiology of airways remodeling. Thickening of the subepithelial basement membrane has been shown to be associated with increases in bronchial mucosal eosinophils in patients with severe asthma.<sup>1</sup> Eosinophil degranulation and tissue eosinophilia have also been associated with several fibrotic syndromes. Moreover, eosinophils are a source of a number of profibrotic cytokines and fibrogenic mediators that are implicated in airway remodeling such as IL-11, IL-17, IL-17E (IL-25), TGF- $\alpha$ , TGF- $\beta$ 1, and matrix metalloproteinase (MMP)–9.

TGF- $\beta$  is a potent profibrotic cytokine that stimulates fibroblasts *in vitro* to promote the synthesis and secretion of many proteins of the extracellular matrix (ECM) such as collagen I and III, fibronectin, proteoglycans, and tenascin. The major source of TGF- $\beta$ 1 mRNA and immunoreactivity in asthma is

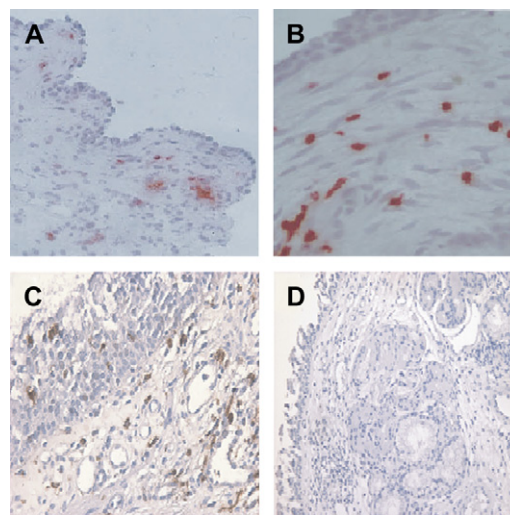


**FIG 1.** Immunostaining for EG2-positive cells in bronchial biopsies from subjects with mild (A) and severe (B) asthma. Reaction was developed with Fast red. Positive staining for EG2 in the nasal epithelium and submucosa of a subject with allergic rhinitis (C) compared with the absence of immunoreactive cells in a control subject (D). EG2 immunoreactivity was developed with diaminobenzidine. Magnification  $\times 200$ .

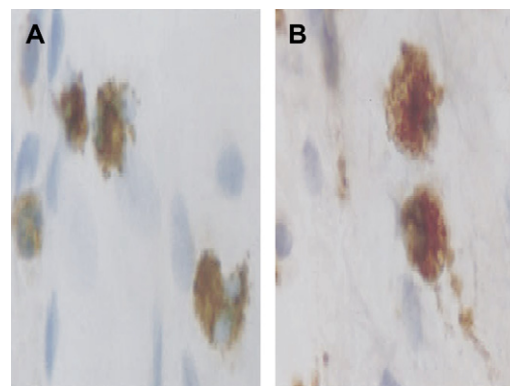


**FIG 2.** TGF- $\beta$ 1 immunoreactive protein in a bronchial biopsy of a patient with moderate asthma (A) and double immunostaining showing the presence of TGF- $\beta$ 1 immunoreactivity in EG2-positive eosinophils (B).

activated eosinophils (Fig 2). Basal levels of the cytokine are increased in bronchoalveolar lavage fluids of subjects with asthma, and these levels are further increased in response to allergen exposure. Expression of TGF- $\beta$ 1 in the airways of patients with asthma correlates with disease severity and degree of subepithelial fibrosis,<sup>2</sup> which suggests a link between chronic allergic inflammation with structural remodeling and the decline in FEV<sub>1</sub> observed in asthma.

IL-11 is a member of the IL-6 family of cytokines that has fibrogenic potential, and its expression within the airways has been localized predominantly to epithelial cells and major basic protein (MBP)-positive eosinophils. Like TGF- $\beta$ 1, IL-11 expression is significantly higher in subjects with moderate and severe

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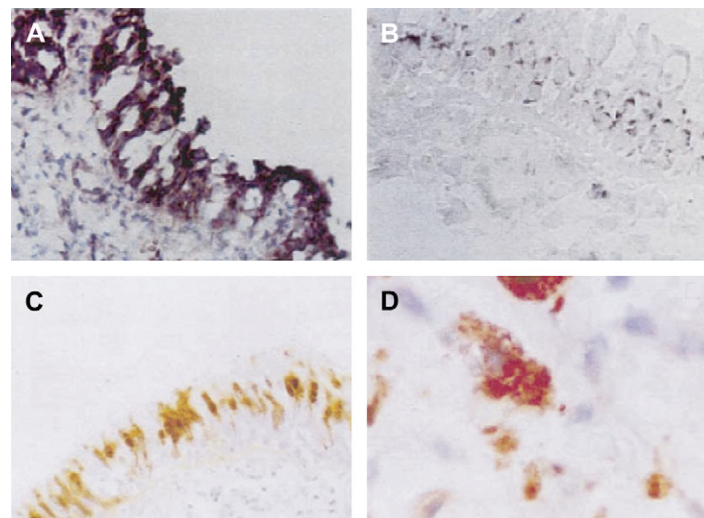


**FIG 3.** *In situ* hybridization with digoxigenin for IL-11 mRNA (dark brown/purple staining) in epithelium and subepithelium of bronchial biopsy specimens from a patient with severe asthma (**A**) and a normal control subject (**B**). **C**, Immunostaining for IL-11 in individual with severe asthma. **D**, Colocalization of IL-11 immunoreactivity (brown) and eosinophil-specific marker (MBP, red staining) in a subject with severe asthma.

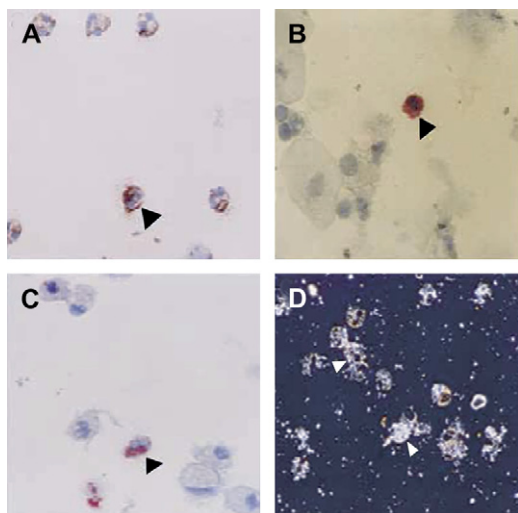


**FIG 4.** Photomicrographs show IL-17 protein and mRNA expression. IL-17 immunostaining in purified blood eosinophils (**A**), sputum (**B**), and bronchoalveolar lavage fluid (**C**) from a subject with asthma; arrows indicate immunostained eosinophils. Positive hybridization for IL-17 mRNA of purified blood eosinophils from a subject with asthma (**D**).

asthma compared with mild disease and control subjects (Fig 3).<sup>3</sup> The inverse correlation with FEV<sub>1</sub> measurements in asthma suggests that IL-11 expression is associated with abnormal lung physiologic function. TGF- $\beta$ 1 is a potent stimulator of IL-11 protein production, mRNA accumulation, and gene transcription in human fibroblasts, alveolar and bronchial epithelial-like cells, and smooth muscle cells. Therefore, it is possible that TGF- $\beta$ 1 also exerts an autocrine-paracrine action and might be a stimulant for eosinophil IL-11 production within asthmatic airways. Regardless of whether such a pathway exists, eosinophil-derived

MBP has previously been shown to participate in the production of IL-11 by lung fibroblasts. The finding of IL-11 expression by eosinophils provide further evidence to support the involvement of these cells in the development of structural remodeling of the airways in asthma.

Chronic skin lesions in atopic dermatitis are also associated predominantly with TGF- $\beta$ 1 and IL-11 expression produced mainly by eosinophils. This further supports the concept that these cells might be involved in the development of structural remodeling in the injured skin of patients with atopic dermatitis. Recently, Aceves et al<sup>4</sup> reported that pediatric patients with eosinophilic esophagitis demonstrate many of the structural remodeling features observed in the airways in asthma such as subepithelial fibrosis and increased vascularity. The degree of eosinophilia correlated positively with the severity of the disease, degree of expression of TGF- $\beta$ 1, phosphorylated SMAD2/3 (its downstream signaling molecule), and vascular endothelial adhesion molecule-1.

Peripheral blood, sputum, and bronchoalveolar lavage eosinophils are a source of IL-17, another profibrotic cytokine that is upregulated in asthma compared with control subjects (Fig 4). Through upregulation of IL-6 and IL-11 by bronchial fibroblasts derived from asthmatic biopsies, IL-17 may play an indirect role in airway remodeling because both these cytokines have been shown to enhance the accumulation of collagen and tissue inhibitor of metalloproteinase 1, thereby preventing ECM breakdown and favoring fibrosis.

Human eosinophils have the capacity to generate IL-6 (Fig 5), a cytokine that has been strongly implicated in tissue remodeling and fibrogenesis because its expression is highly correlated with fibrosis in tissues

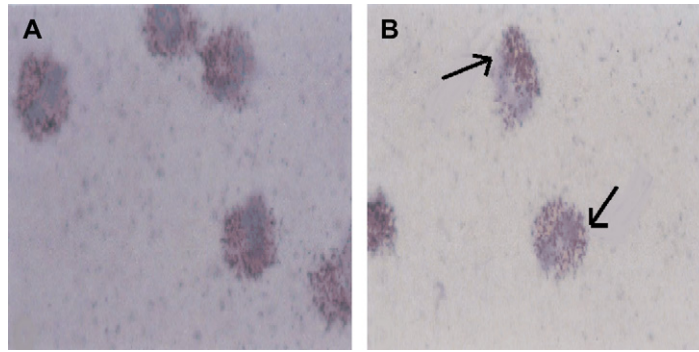


**FIG 5.** Detection of IL-6 mRNA in human eosinophils. Subcellular localization of IL-6 mRNA in human eosinophils stimulated with IFN- $\gamma$  (500 U/mL), stained with carbo1 chromotrope 2R (**A**), or immunostained simultaneously using EG2 and alkaline phosphatase-antialkaline phosphatase (**B**, arrow). Adapted from Hamid Q, Barkans J, Meng Q, Ying S, Abrams JS, Kay AB, et al. Human eosinophils synthesize and secrete interleukin-6, in vitro. *Blood* 1992;80:1496-501.

such as the liver, skin, and lung. In keeping with the close association of IL-11 with IL-6, it is interesting to note that the colocalization of IL-11 to eosinophils is consistent with the production of IL-6 by these cells.<sup>3</sup>

IL-25 (IL-17E) is a newly described cytokine that induces eosinophil recruitment and structural changes associated with airway remodeling when expressed in the lungs of mice. In humans, peripheral blood eosinophils constitutively express IL-25, and expression levels are higher in subjects with asthma compared with control subjects (Fig 6). IL-25 was also detected at the mRNA and protein levels in nasal and bronchial biopsies of subjects with allergic rhinitis and asthma, respectively, but not in controls. The expression of IL-25 in these tissues was predominantly associated with inflammatory cells, in particular eosinophils. These data suggest that eosinophils may contribute to airway remodeling via IL-25 expression and release.

The receptor for IL-25 (IL17BR) is abundantly expressed by airway smooth muscle cells (ASMCs; Fig 7), and on stimulation with IL-17E produced by eosinophils, it increases ASMC expression of ECM components, namely procollagen I and lumican mRNA. This suggests a potential proremodeling effect of IL-25 on ASMCs through the induction of ECM and suggests that its receptor is upregulated by proinflammatory conditions. Human primary lung fibroblasts constitutively express IL-17BR. Further studies are required to evaluate whether IL-25 is capable of directly inducing cell proliferation and production of ECM proteins in these cells.

IL-5 is a key cytokine in eosinophil differentiation, maturation, recruitment, and activation at sites of inflammation. Its specificity for eosinophils in humans is probably conferred by the restriction of expression of the IL-5 receptor  $\alpha$ -chain to eosinophils and basophils. Although produced primarily by activated T cells, it is also secreted by eosinophils as well as other cells. Flood-Page et al<sup>5</sup> showed that treatment with anti-IL-5 antibody specifically decreased the number of airway

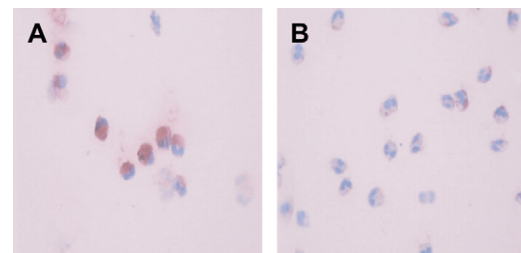


**FIG 6.** Immunocytochemistry showing basal expression of IL-25 in freshly purified peripheral blood eosinophils from a donor with asthma (**A**) and a control subject (**B**). Eosinophils from the subject with asthma show increased immunoreactivity for IL-25 compared with control.

eosinophils in asthma and significantly reduced the deposition of ECM proteins in the bronchial subepithelial basement membrane of patients with mild atopic asthma (Fig 8). The expression of tenascin, lumican, and procollagen III was significantly reduced compared with placebo. Furthermore, these changes were associated with significant reductions in TGF- $\beta$ 1 expression by airway eosinophils. These data suggest a role for eosinophils in airway remodeling in asthma through regulation of ECM deposition in the airways. Similarly, anti-IL-5 mAb significantly reduced eosinophil infiltration and tenascin deposition in allergen-challenged human atopic skin at 48 hours, providing further evidence for the involvement of eosinophils in the remodeling process associated with allergic inflammation.<sup>6</sup>

An imbalance between the expression of proteases and antiproteases and particularly between MMPs and their inhibitors (tissue inhibitors of metalloproteinases) is believed to generate tissue destruction or abnormal tissue repair. Eosinophils constitutively produce and store MMP-9, a protease implicated in tissue remodeling observed in asthma.<sup>7</sup> MMP-9 is required for the migration of eosinophils across basement membrane components, and increased levels

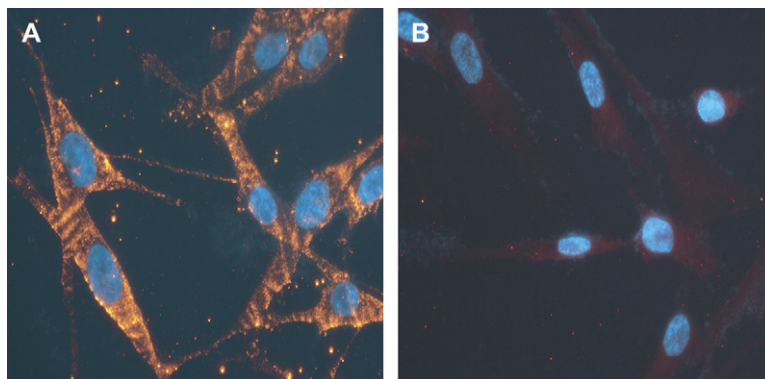


**FIG 7.** Immunofluorescence demonstrating expression of the IL-25 receptor (IL-17BR) in cultured ASMCs. Detection of IL-17BR in cultured ASMCs by immunofluorescence using an Alexa Fluor 555-labeled anti-IL-17BR antibody (Molecular Probes, Eugene, Ore) (**A**) versus isotype control (**B**). Magnification  $\times 200$ .

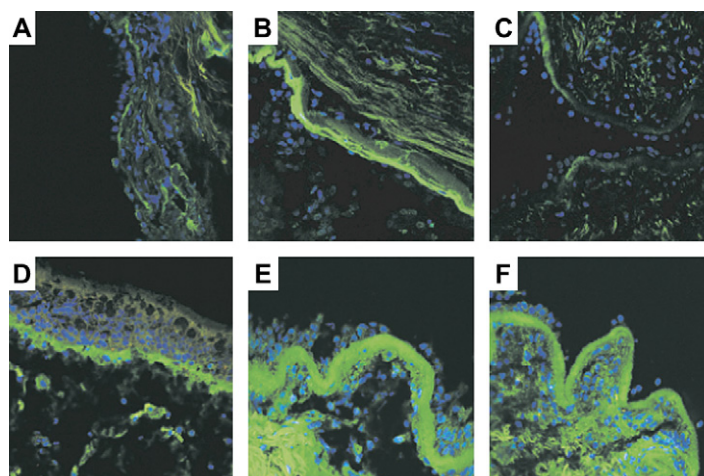


**FIG 8.** Representative photomicrographs of tenascin immunoreactivity in the reticular basement membrane from a normal subject (**A**) and from a subject with asthma before (**B**) and after (**C**) treatment with anti-IL-5. Lumican immunoreactivity from a normal subject (**D**) and from pretreatment (**E**) and posttreatment (**F**) with anti-IL-5 is also shown. Immunoreactivity of ECM proteins is shown in green. Adapted with permission from COPIBEC ([www.copibec.qc.ca](http://www.copibec.qc.ca)), Montreal, Quebec, Canada.

have been found in bronchial biopsy specimens and in bronchoalveolar lavage fluid of untreated patients with asthma compared with control populations. Inhibition of MMP-9 and MMP-2 activity is associated with decreased airway infiltration of eosinophils in a murine model of asthma. Wiehler et al<sup>8</sup> reported rapid release of MMP-9 from peripheral blood eosinophils following stimulation with TNF- $\alpha$ . Tissue remodeling in asthma is characterized by a loss of the MMP-9 substrate type IV and an elevation in levels of MMP-9 in the lung.

With regard to the usefulness of inhaled corticosteroid medications in preventing or reversing the features of airway remodeling in asthma, a recent animal study by Leung et al<sup>9</sup> showed both inhaled ciclesonide and fluticasone propionate to be inhibitors of eosinophilic airway inflammation and airway wall

remodeling in chronic allergic asthma. The novel inhaled corticosteroid, ciclesonide, produced these effects without having an effect on body weight and the hypothalamic-pituitary-adrenal axis.

#### REFERENCES

1. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;160:1001-8.
2. Minshall EM, Leung DYM, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF- $\beta$ 1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326-33.
3. Minshall E, Chakir J, Laviolette M, Molet S, Zhu Z, Olivenstein R, et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. *J Allergy Clin Immunol* 2000;105:232-8.

4. Aceves SS, Newbury RO, Dohill R, Bastian JF, Broids DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2007;119:206-12.
5. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029-36.
6. Phipps S, Flood-Page P, Menzies-Gow A, Ong YE, Kay AB. Intravenous anti-IL-5 monoclonal antibody reduces eosinophils and tenascin deposition in allergen-challenged human atopic skin. *J Invest Dermatol* 2004;122:1406-12.

7. Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998;102:783-8.
8. Wiehler S, Cuvelier SL, Chakrabarti S, Patela KD. p38 MAP kinase regulates rapid matrix metalloproteinase-9 release from eosinophils. *Biochem Biophys Res Commun* 2004;315:463-70.
9. Leung SY, Eynott P, Nath P, Chung KF. Effects of ciclesonide and fluticasone propionate on allergen-induced inflammation and remodeling features. *J Allergy Clin Immunol* 2005;115:989-96.