

Images in Allergy and Immunology

David Préfontaine, MSc, and Qutayba Hamid, MD, PhD, Editors

Airway epithelial cells in asthma

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.

Structural cells of the airways—in particular, epithelial cells—are increasingly appreciated as key players in the pathogenesis of asthma. Changes in the epithelial cells are a major feature of airway remodeling. Epithelial cells have also been shown to contribute to the initiation and maintenance of inflammatory responses.¹

The airway epithelium is composed of ciliated and secretory columnar cells and basal cells. One of the prominent features of the asthmatic airways is the loss of epithelial integrity and the detachment of columnar cells. These observations were recently attributed to the fragility of epithelial cells in asthma. Epithelial cell aggregates are found in increased numbers in sputum from asthmatic patients compared with sputum from control subjects. It is also not uncommon to observe squamous cell metaplasia, especially in severe asthma, even in the absence of a smoking history (Fig 1). Bronchial epithelial cells express increased levels of surface markers and receptors associated with tissue injury and repair, such as the adhesion molecule CD44² (associated with the loss of cell-cell contact) and of the epidermal growth factor receptor (Fig 2),³

From Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada.

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

Received for publication September 4, 2007; revised September 25, 2007; accepted for publication September 25, 2007.

Reprint requests: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, McGill University, 3626 St Urbain St, Montreal H2X2P2, Quebec, Canada. E-mail: qutayba.hamid@mcgill.ca.

J Allergy Clin Immunol ■■■■■.

0091-6749/\$32.00

© 2007 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2007.09.041

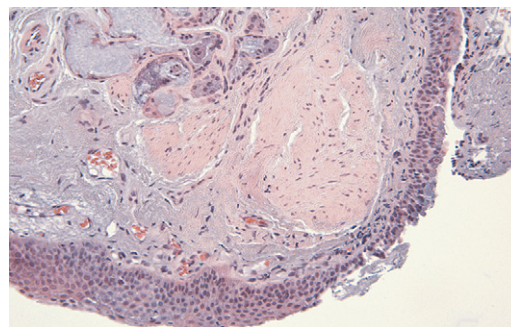


FIG 1. Squamous cell metaplasia in an endobronchial biopsy section from a patient with severe asthma.

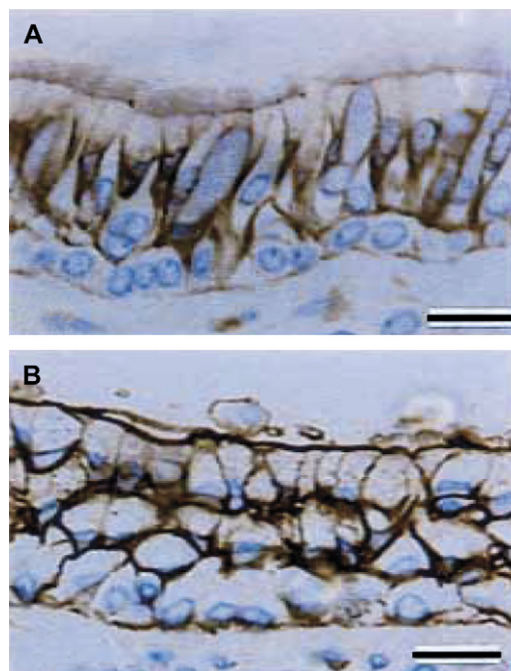


FIG 2. Epidermal growth factor receptor immunoreactivity in bronchial epithelium from a healthy individual (A) compared with a morphologically intact epithelium from an asthmatic patient (B). Note that epidermal growth factor receptor is localized throughout the epithelium in the asthmatic biopsy specimen, whereas it appears confined to the subluminal layers in normal tissue. Scale bars = 20 μ m. Adapted with permission from Polosa et al.³

respectively. The epithelium lining the asthmatic airways also undergoes significant apoptosis, as assessed by staining of double-stranded DNA breaks using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (Fig 3). By using an *in vitro* coculture model of primary epithelial cells, T cell-derived IFN- γ and activated eosinophil-derived TNF- α were shown to separately induce epithelial cell apoptosis, as seen in asthmatic airways.⁴

IL-4 and IL-13 produced by infiltrating T lymphocytes of the asthmatic airways were shown to activate epithelial cells. These T_H2 cytokines activate

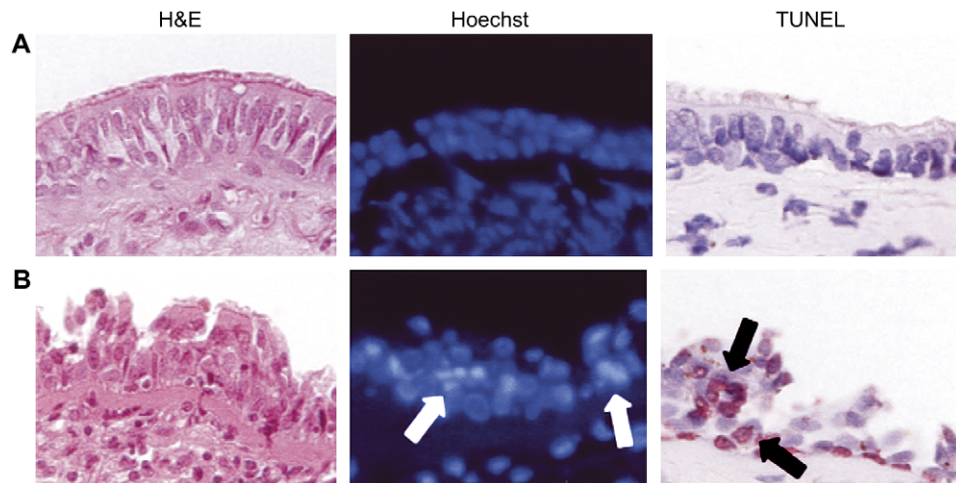


FIG 3. Epithelial cell shedding and apoptosis in the bronchial mucosa of a patient with asthma compared with that of a healthy control subject. As opposed to the healthy patient (**A**), the bronchial epithelium from the patient with asthma (**B**) displays epithelial cell exfoliation (hematoxylin and eosin [*H&E*] stain) and apoptosis: bright condensed nuclei observed with Hoechst staining and red nuclei seen with the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay indicate cells undergoing apoptosis. Adapted with permission from Trautmann et al.⁴

2

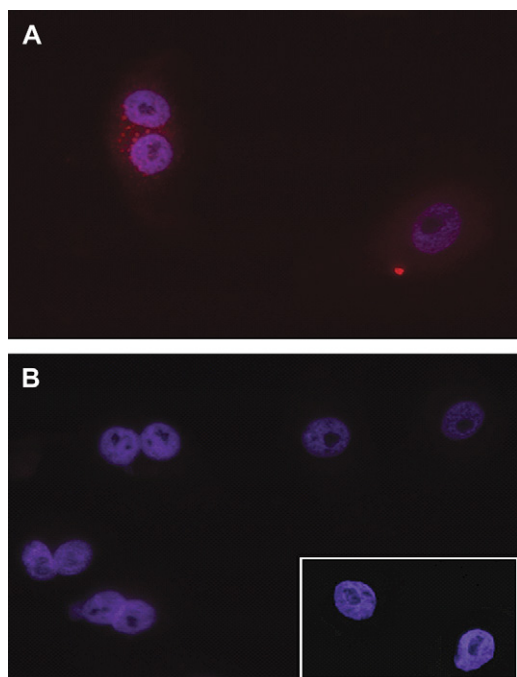


FIG 4. Immunofluorescence staining showing GATA-3 expression by primary normal bronchial epithelial cells incubated in the presence (**A**) of or absence (**B**) of recombinant IL-4. The insert represents typical staining with an isotype control antibody. The authors acknowledge A. K. Mogas for technical assistance.⁵

specific transcription factors, such as signal transducer and activator of transcription (STAT) 6 and GATA-3 (Fig 4).⁵ Increased expression of STAT-6 protein was detected in the airway epithelium from patients with severe asthma compared with that from control subjects (Fig 5).⁶ STAT-6 activation in primary airway epithelial cells could also drive

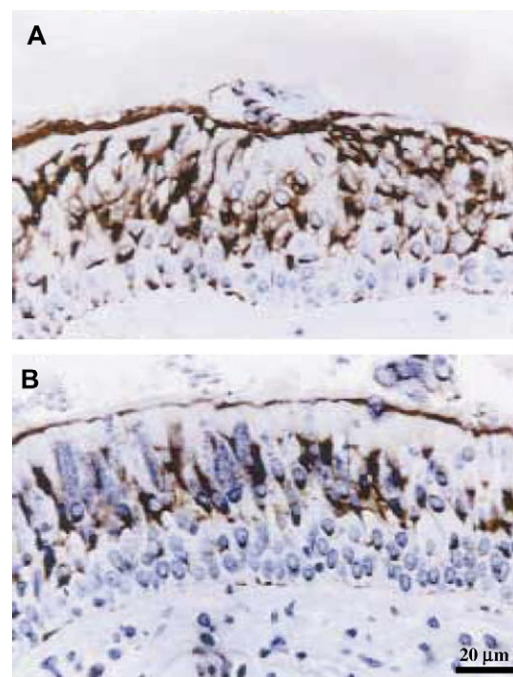


FIG 5. Evidence of increased STAT-6 expression in the bronchial epithelium from a patient with severe asthma (**A**) compared with that from a healthy control subject (**B**). Immunoreactivity was particularly seen in the ciliated columnar cell layer of the epithelium, which appeared to be a major site of STAT-6 expression. Adapted with permission from Mullings et al.⁶

the production of chemokines, such as IL-8 (CXCL8).⁶ Primary cultures of bronchial epithelial cells from asthmatic patients exhibited increased expression of other chemokines, including RANTES (CCL5) and eotaxin (CXCL11), compared with cells from healthy subjects.

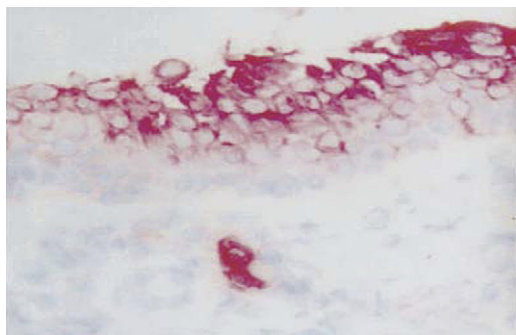


FIG 6. Immunocytochemical analysis of MCP-4 (CCL13) expression in allergen-challenged upper airway mucosa. Patients with allergic rhinitis were intranasally challenged with ragweed extract before biopsy collection. Positive immunostaining was obtained by using the alkaline phosphatase-antialkaline phosphatase method. Adapted with permission from Christodoulopoulos et al.⁷

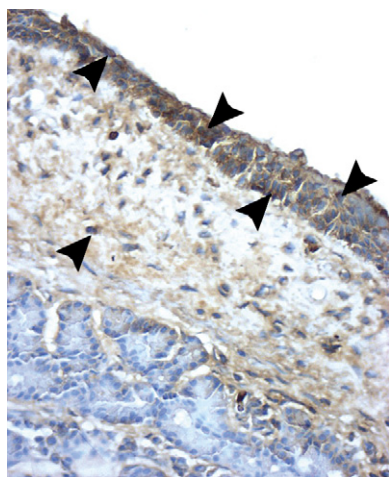


FIG 7. Immunocytochemical analysis of TLR-4 expression in upper airway epithelial cells. Positive immunoreactivity (arrows) was obtained by using biotinylated LPS molecules, and staining was developed with diaminobenzidine (original magnification $\times 200$).

Epithelial cells in allergic inflammation are reportedly an important source of a wide variety of proinflammatory mediators, including cytokines like IL-6, chemokines like eotaxins (CCL11, CCL24, and CCL26), and monocyte chemotactic proteins (MCPs; MCP-3, MCP-4, CCL7, and CCL13, respectively; Fig 6),⁷ growth factors, reactive oxygen or nitrogen species, oxidative arachidonic acid metabolites, vasoactive peptides, and nitric oxide synthase.⁸ Recent advances regarding epithelial cell activation relate to the recognition of microbe-associated molecular patterns. Airway epithelial cells were shown to express several Toll-like receptors (TLRs).⁹ Recognition of microbe-associated patterns, such as bacterial LPS, through TLRs (in this case, TLR-4; Fig 7) induces production of proinflammatory mediators, including cytokines (ie, IL-6, IL-10, TNF- α , and type I interferons),

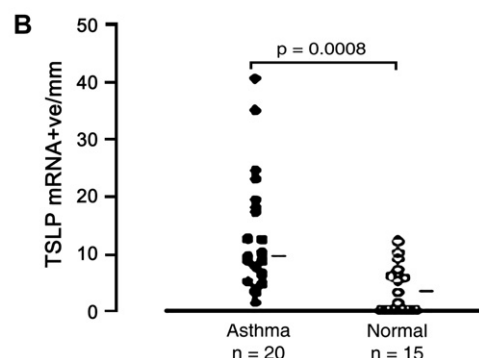
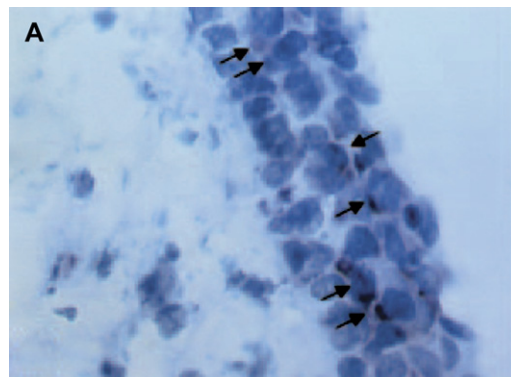


FIG 8. **A**, Detection of TSLP mRNA-positive cells in a bronchial biopsy specimen from an asthmatic patient, as assessed by means of single *in situ* hybridization with digoxigenin-labeled TSLP antisense riboprobes. **B**, The increased number of TSLP mRNA-positive cells in the epithelium of asthmatic patients was expressed as the number of positive cells per millimeter of basement membrane. Adapted with permission from Ying et al.¹²

chemokines (macrophage inflammatory protein 3 α [CCL20] and IL-8 [CXCL8]), adhesion molecules (intercellular adhesion molecule 1), and β -defensins.

An important cause of nonallergic asthma exacerbations are viral respiratory tract infections (rhinoviruses, influenza strains, or respiratory syncytial virus). Virus-derived double-stranded RNA activates primary airway epithelial cells through TLR-3.¹⁰ Furthermore, double-stranded RNA increases the epithelial cell production of the IL-7-like cytokine thymic stromal lymphopoietin (TSLP), whereas IL-4 and IL-13 upregulate its gene transcription.¹¹ Epithelial cells are the main source of TSLP, and the asthmatic airway epithelium is enriched in TSLP-expressing cells compared with healthy control cells (Fig 8).¹² The growing field of TLR-mediated microbial pattern recognition and the recent appreciation of epithelium-derived TSLP linking innate and adaptive immunity¹³ together provide attractive therapeutic targets for regulating epithelial cell activation in asthma.

REFERENCES

- Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol* 2000;105:193-204.

2. Leir SH, Baker JE, Holgate ST, Lackie PM. Increased CD44 expression in bronchial repair following mechanical damage or plating at low cell densities. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L1129-L1137.
3. Polosa R, Puddicombe SM, Krishna T, Tuck AB, Howarth PH, Holgate ST, et al. Expression of c-erbB receptors and ligands in the bronchial epithelium of asthmatic subjects. *J Allergy Clin Immunol* 2002;109:75-81.
4. Trautmann A, Schmid-Grendelmeier P, Kruger K, Cramer R, Akdis M, Akkaya A, et al. T cells and eosinophils cooperate in the induction of bronchial epithelial cell apoptosis in asthma. *J Allergy Clin Immunol* 2002;109:329-37.
5. Mogas AK, Lajoie-Kadoch S, Joubert P, Chakir J, Hamid Q. Functional importance of GATA-3 in human airway epithelial cells [abstract]. *Am J Respir Crit Care Med* 2006;173:A695.
6. Mullings RE, Wilson SJ, Puddicombe SM, Lordan JL, Bucchieri F, Djukanovic R, et al. Signal transducer and activator of transcription 6 (STAT-6) expression and function in asthmatic bronchial epithelium. *J Allergy Clin Immunol* 2001;108:832-8.
7. Christodoulouopoulos P, Wright E, Frenkiel S, Luster A, Hamid Q. Monocyte chemotactic proteins in allergen-induced inflammation in the nasal mucosa: effect of topical corticosteroids. *J Allergy Clin Immunol* 1999;103:1036-44.
8. Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, et al. Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510-3.
9. Greene CM, McElvaney NG. Toll-like receptor expression and function in airway epithelial cells. *Arch Immunol Ther Exp* 2005; 53:418-27.
10. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by toll-like receptor agonists. *Am J Respir Cell Mol Biol* 2004;31:358-64.
11. Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3- and Th2 cytokines-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol* 2007;179:1080-7.
12. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of TH2-attracting chemokines and disease severity. *J Immunol* 2005;174:8183-90.
13. Liu YJ. Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation. *J Allergy Clin Immunol* 2007;120:238-44.