Expression of the Potent Inflammatory Cytokines, GM-CSF, IL6, and IL8, in Bronchial Epithelial Cells of Asthmatic Patients*

Sabrina Mattoli, M.D.; Ph.D.; Maurizio Marini, M.D.; and Angelo Rasoli, M.D.

Cultured human bronchial epithelial cells produce cytokines with potent proinflammatory properties upon exposure to immunologic and nonimmunologic stimuli or during recovery from injury, particularly GM-CSF and IL6. Because of this ability to release factors which affect the migration of granulocytes and lymphocytes and promote their local activation, airway epithelial cells can be viewed as cells that signal the inflammatory and immune system that penetration or disruption of the airway mucosa has occurred, and epithelial cell-derived cytokines may play an important role in the genesis and persistence of bronchial inflammation in some pathologic conditions, like asthma.

Indeed, we have previously demonstrated that the airway secretions from patients with symptomatic asthma contain increased amounts of GM-CSF and IL6, and these cytokines have been localized in the cytoplasm of nonciliated bronchial epithelial cells by immunospecific labeling. In addition, we have provided direct evidence of an upregulation of the expression of GM-CSF gene and protein in the bronchial epithelial cells of these patients. The GM-CSF released from asthmatic bronchial epithelial cells could prolong the survival of eosinophils, make them hypodense, and promote the release of mediators from those cells, thereby reproducing in vitro phenomena that are known to occur in asthma and that contribute to the pathogenesis of airway hyperresponsiveness.

In the present study, we have further evaluated the expression of cytokines with inflammatory properties in bronchial epithelial cells of asthmatic patients and tested the ability of corticosteroids and nedocromil sodium to downregulate their production.

Six asthmatic patients (4 women, mean age ± SD: 31.2 ± 9.8 years) and 6 healthy volunteers (3 women, mean age ± SD: 43.0 ± 15.5 years) completed the study. All subjects were nonsmokers and two in each group showed positive responses to skin prick testing with 12 common allergen extracts (Table 1).

The asthmatics were receiving treatment with inhaled bronchodilators alone, on demand, but their symptoms were not controlled, as they were elicited by nonspecific irritating stimuli, or required high doses of bronchodilators daily, or disturbed sleep. No patient had received corticosteroids in the last month. They had pulmonary function measurements and bronchoprovocation tests with methacholine to define their nonspecific bronchial hyperresponsiveness. Their mean baseline FEV1 (± SD) was 89.3 ± 14.5% of predicted value, and their geometric mean PC20 was 0.187 mg/ml (range from 0.04 to 0.844 mg/ml) (Table 1), the latter indicating that airway reactivity was moderately to severely increased in those patients.

Control subjects had no past history of asthma and no evidence of bronchial hyperresponsiveness (Table 1).

Asthmatics and control subjects underwent fiberoptic bronchoscopy according to a standard protocol, and 8 to 10 endobronchial biopsies were performed through the bronch...
choscope in randomly selected lobar and segmental bronchi of the lungs.\textsuperscript{5,7}

Appropriate informed consent was obtained from all studied subjects.

Bronchial epithelial cells were isolated from the biopsy specimens as previously described.\textsuperscript{6,7} Total RNA was extracted from the cells by the acid guanidinium thiocyanate-phenol-chloroform extraction procedure, and mRNA was purified by chromatography on oligothymidylic acid cellulose. Messenger RNA from samples of \texttimes{}10\textsuperscript{6} cells was reverse transcribed into cDNA and the expression of transcripts for an array of cytokines (GM-CSF, G-CSF, IL1\textalpha{}, IL1\textbeta{}, IL6, and IL8) was evaluated by message amplification phenotyping (MAPPing) using PCR and specific amplimer sets (Clontech Laboratories, Inc, Palo Alto, Cal). This method allows detection of cytokine transcripts in a few cells by yielding 10\textsuperscript{6} to 10\textsuperscript{9}-fold amplification of DNA.

The results of cytokine MAPPing by PCR are reported in Figure 1. Freshly isolated bronchial epithelial cells of asthmatic patients invariably expressed appreciable levels of GM-CSF and IL6 transcripts, and the cells from 5 patients also expressed IL8 transcripts. The epithelial cells of 2 control subjects expressed low levels of GM-CSF mRNA, and both the donors of those cells were atopic (Fig 1). Freshly isolated normal epithelial cells did not express the mRNA for any other cytokine tested.

To evaluate the ability of bronchial epithelial cells from asthmatic patients to release GM-CSF, IL6, and IL8, and to test the inhibitory effect of corticosteroids and nedocromil, replicate samples of epithelial cells were plated at high density (10\textsuperscript{6} cells/well) in collagen-treated 24-well culture plates and incubated for 4 days in IMDM medium without growth factors and fetal calf serum, in presence or in absence of different concentrations of hydrocortisone or nedocromil. In hormone-free medium, the cells attach to the culture surface but do not grow. The supernatants were harvested at 48-h intervals and replaced by fresh medium. The contents of immunoreactive GM-CSF, IL6, and IL8 were measured by ELISA.\textsuperscript{17,10} Fresh IMDM was used to dilute standards and as blank.

During the first 48 h of incubation, bronchial epithelial cells from asthmatic donors released high amounts of immunoreactive GM-CSF, IL6, and IL8 (Table 1). Only the supernatants of epithelial cells isolated from the 2 atopic control subjects contained measurable amounts of GM-CSF-like protein. Apparently, normal epithelial cells did not produce any IL6 or IL8 (Table 1).

The cytokine release from asthmatic bronchial epithelial cells spontaneously decreased during the last 2 days of incubation: the contents of immunoreactive GM-CSF and IL6 in the supernatants declined (mean ± SD) 1.45 ± 0.69 to 0.57 ± 0.49 ng/ml/10\textsuperscript{6} cells (n = 6, p<0.05) and from 1.19 ± 0.46 to 0.38 ± 0.28 ng/ml/10\textsuperscript{6} cells (n = 4), respectively. This indicates that the upregulation of the expression of cytokines with inflammatory properties in asthmatic airway epithelium is reversible, and it disappears when epithelium is removed from the diseased tissue.

The incubation of asthmatic bronchial epithelial cells with hydrocortisone, at concentrations as low as 10\textsuperscript{-7} M, completely abolished the release of immunoreactive GM-CSF during the first 48 h after cell isolation, whereas nedocromil, at concentrations up to 10\textsuperscript{-4} M, did not reduce the production of this cytokine (mean content of immunoreactive GM-CSF ± SD in the supernatants of cells treated with 10\textsuperscript{-4} M nedocromil 1.27 ± 0.65 ng/ml/10\textsuperscript{6} cells/48 h vs 1.45 ± 0.69 ng/ml/10\textsuperscript{6} cells/48 h in supernatants of untreated cells, n = 6, p>0.05).

Our results provide direct evidence that the synthesis and release of some cytokines with inflammatory properties are upregulated in bronchial epithelium of patients with symptomatic asthma and that corticosteroids, which are the most effective drugs in inducing remission of asthmatic symptoms, also abolish this upregulation.

The GM-CSF can induce the expression of adhesion molecules on endothelial and epithelial cells and primes granulocytes to release increased amounts of mediators upon stimulation, thereby promoting the infiltration of airway mucosa with inflammatory cells and their local activation.\textsuperscript{14,15} The IL6 induces T-cell activation and proliferation,\textsuperscript{12,19} and IL8 is chemotactic for neutrophils and T-cells.\textsuperscript{11} Since bronchial epithelium represents the first cell barrier encountered by agents known to induce asthma and airway inflammation,\textsuperscript{19} we believe that the local effect of these epithelial cell-derived factors can explain many of the pathologic alterations observed in the bronchial mucosa of asthmatic patients, including eosinophil and T-cell activation,\textsuperscript{16} and can make the inflammatory response in asthma different from that of any other lung disease.
ACKNOWLEDGMENT: The authors acknowledge the expert assistance of Dr. J. Hollemberg for PCR and Mrs. E. Vittori for bronchoprovocation tests and skin prick tests.

REFERENCES

1 Mattoli S, Miante S, Calabrò F, Mezzetti M, Allegra L. Human bronchial epithelial cells exposed to isocyanates potentiate the activation and proliferation of T-cells induced by antigen receptor triggering through the release of IL-1 and IL-6. In: Johansson SGO, ed. Cellular communication in allergic asthma. Uppsala: Pharmacia Allergy Foundation, 1990: 25-35
6 Mattoli S, Marini M, De Franchis R, Fasoli A. GM-CSF synthesis and release are upregulated in asthmatic bronchial epithelium and contribute to eosinophil activation. Am Rev Respir Dis 1991; 143(suppl):144

7 Soloperto M, Mattoso VL, Fasoli A, Mattoli S. A bronchial epithelial cell-derived factor in asthma which promotes eosinophil activation and survival as GM-CSF. Am J Physiol (Lung Cell Mol Physiol) 1991; 260:L530-38
9 Holsti MH, Rautel DH. IL-6 and IL-1 synergize to stimulate IL-2 production and proliferation of peripheral T cells. J Immunol 1989; 143:2514-19
11 Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. J Allergy Clin Immunol 1989; 83:1013-26
14 Larsen GC, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T-lymphocytes. Science 1989; 243:1464-67