E-1S showed a significantly raised neutrophil count compared to L-1S (n = 18, p = 0.02) but no difference in total cell count; all other cell types, including epithelial and squamous cells, were not significantly different between the two count collections. E-1S and BAL eosinophils were significantly correlated (n = 20, p = 0.0004) in all groups and in asthma alone (n = 10, p = 0.08, p = 0.04); L-1S showed weaker correlations for percentage of eosinophils with BAL (p = 0.51, p = 0.05 in all groups; p = 0.51, p = 0.049 in asthma). There were no significant correlations between either E-1S or L-1S with BAL for other cell types, and no significant correlations of E-1S or L-1S inflammatory cells with any submucosal cell types (lymphocytes-CD3, macrophages-CD68, neutrophil, or activated eosinophils-EG2+).

E-1S and to a lesser extent L-1S were highly correlated with BAL for eosinophils in all groups and in asthma, but did not correlate with endobronchial biopsy submucosal inflammatory cell counts. This may be attributed to the fact that sputum and BAL sample the airway lumen, whereas the cellular inflammation in the airway submucosa may be distinct. IS could be used to monitor luminal eosinophilic inflammation but probably not mucosal inflammation in asthma.

**REFERENCE**

1 Gershman NH, Lin H, Wong HH, et al. Fractional analysis of sequential induced sputum samples during sputum induction: evidence that different lung compartments are sampled at different time points. J Allergy Clin Immunol 1999; 104:322–328

Eosinophil Cationic Protein Concentration in Saliva Does Not Correlate With Eosinophil Cationic Protein Concentration in Sputum*

David L. Bowton, MD, FCCP; Michael C. Seeds, MD; and David A. Bass, MD, DPhil, FCCP

(CHEST 2003; 123:372S)

**Abbreviation:** ECP = eosinophil cationic protein

Eosinophilic infiltration is a prominent component of the inflammation characteristic of asthma. Measurement of eosinophils and eosinophil cationic protein (ECP) in induced sputum is a noninvasive indicator of changes in airways inflammation. Recently, Schmekel and colleagues reported that salivary ECP was elevated in subjects with asthma and was correlated with disease activity. The measurement of salivary ECP as a surrogate for sputum ECP is attractive because it can be done rapidly, requires less cooperation on the part of the subject, and is devoid of the potential adverse effects of sputum induction. We examined the correlation of sputum ECP and salivary ECP in 26 paired samples from 14 nonsmoking asthmatic subjects not receiving corticosteroids (12 subjects had specimens collected 4 weeks apart). Asthma severity ranged from mild intermittent to moderate persistent. After mouth rinsing, 3 mL of saliva was collected by repetitive expectoration. Sputum was then induced by inhalation of 3% saline solution and collected over 20 min. Specimens were collected on ice and processed within 60 min. ECP in sputum and saliva was measured by radioimmunoassay (Kabi Pharmacia Diagnostics; Uppsala, Sweden). Mean (± SD) values for sputum and salivary ECP were 85 ± 141 μg/L and 43 ± 20 μg/L, respectively. Following acid acetate extraction, as reported by Schmekel and colleagues, there was an increase in salivary ECP to 201 ± 189, likely reflecting lysis of cells. There was no correlation between sputum and saliva ECP (r² = 0.00). After acetate extraction, the correlation was better (r² = 0.26, p = 0.029) though not strong. Hence, while salivary ECP may be elevated in subjects with asthma compared to normal subjects, it is not useful as a surrogate marker for sputum ECP.

**REFERENCE**


Interferon-γ Reduces Interleukin-4– and Interleukin-13–Augmented Transforming Growth Factor-β2 Production in Human Bronchial Epithelial Cells by Targeting Smads*

Fu-Qiang Wen, MD, PhD; Xiangde D. Liu, MD; Yusuke Terasaki, Quinhong H. Yang, MD, PhD; Tetsu Kobayashi, MD, PhD; Shuji Abe, MD, PhD; and Stephen I. Rennard, MD, FCCP

(CHEST 2003; 123:372S–373S)

**Abbreviations:** HBEC = human bronchial epithelial cell; HFL = human fetal lung; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IFN = interferon; IL = interleukin; TGF = transforming growth factor; Th2 = T-helper type 2
The T-helper type 2 (Th2) cytokines, interleukin (IL)-4 and IL-13, and the T-helper type 1 cytokine interferon (IFN)-γ act differently in regulating airway inflammation and subepithelial fibrosis. The production of transforming growth factor (TGF)-β by airway epithelium and lung mesenchymal cells may be an important event in airway inflammation and remodeling. We hypothesized, therefore, that Th2 and T-helper type 1 cytokines may differentially regulate TGF-β production and may target TGF-β signaling.

Human bronchial epithelial cells (HBECS) and human fetal lung (HFL)-1 fibroblasts were incubated for 6 to 48 h with varying concentrations of IL-4 or IL-13 in the absence or presence of IFN-γ. Postculture media were assayed for TGF-β. TGF-β messenger RNA was evaluated by quantitative real-time polymerase chain reaction. Cellular localization of Smads 2, 3, 4, 7, Stat1α p91 and Stat1p54/p91, and Stat6 were evaluated by immunostaining.

In HBECS, IL-4 and IL-13 alone significantly enhanced the production of both active and latent forms of TGF-β2 as detected by enzyme-linked immunosorbent assay in a time- and dose-dependent manner (p < 0.01) and augmented messenger RNA expression of TGF-β2: 970 ± 20 × 10^3 vs 4700 ± 500 × 10^3 GAPDH units for IL-4; 970 ± 20 × 10^3 vs 3900 ± 230 × 10^3 GAPDH units for IL-13 (p < 0.01). In contrast, release of TGF-β by HFL-1 fibroblasts was not affected. IFN-γ dose-dependently inhibited spontaneous TGF-β2 release as well as IL-4- or IL-13-augmented production of TGF-β2 in HBECS (p < 0.01). Correspondingly, IFN-γ significantly reduced both basal (970 ± 20 × 10^3 vs 360 ± 30 × 10^3 GAPDH units) and IL-4- or IL-13-augmented messenger RNA expression of TGF-β2 (4,700 ± 500 × 10^3 vs 570 ± 20 × 10^3 GAPDH units) for IL-4; 3900 ± 230 × 10^3 vs 560 ± 20 × 10^3 GAPDH units for IL-13) in HBECS (p < 0.01), but had little effect on TGF-β messenger RNA expression in HFL-1 fibroblasts. A clear translocation of Stat1 and Stat6 from cytoplasm into the nucleus were observed in both HBECs and HFL-1 fibroblasts treated with IFN-γ or IL-4 and IL-13, respectively. However, only in HBECs did IFN-γ induce a clear paranuclear immunofluorescence for Smad 7. This was associated with significantly reduced nuclear localization of Stat1, Stat3, and Stat6, all of which were induced by IL-4 and IL-13. In contrast, IFN-γ had little effect on nuclear localization of Stat6.

IFN-γ may attenuate IL-4- and IL-13-enhanced TGF-β2 production in bronchial epithelial cells by targeting Smads signaling. Moreover, by inducing production of the inhibitory Smad, Smad 7, IFN-γ may interfere with TGF-β signaling. The inhibitory effect of IFN-γ on TGF-β2 production by HBECS stimulated by Th2 cytokines may play an important role in modulating airway repair.

Interleukin-13 Alters Mucociliary Differentiation of Human Nasal Epithelial Cells*

Marie Skowron, PhD(c); Eric Perret, PhD; Francelyne Marano, PhD; Daniel Caput, PhD; and Frédéric Tournier, PhD

(CHEST 2003; 123:3738–3745)

Lesions of the airway epithelium are frequently observed in asthma patients. Therefore, inflammatory mediators such as chemokines or cytokines may influence airway remodeling. Among these cytokines, interleukin (IL)-13 recently has emerged as a pivotal molecule with which to promote airway hyperresponsiveness.1 We have investigated the effect of IL-13 on the mucociliary differentiation of human nasal epithelial cells in primary culture. IL-13 alters ciliated cell differentiation and, in parallel, largely increases the proportion of secretory cells in a dose-dependent and time-dependent manner, and impairs lateral cell contacts. Treatment with IL-13 and increasing amounts of an IL-4 mutant (Y124D), which is a selective antagonist of the IL-4/IL-13 shared receptor, abolishes the effects of IL-13.2 Using differential screening of treated and nontreated epithelial cells during differentiation, we showed that transforming growth factor-β1 gene expression is increased in the presence of the cytokine, reinforcing the idea that IL-13 could induce tissue fibrosis by selectively activating transforming growth factor-β1, as was recently shown in mice.3 Because multiple IL-13 effects contribute to the asthma phenotype, inhibiting the cytokine or its receptors in vivo may be relevant in patients with chronic lung diseases such as asthma.

References

*From the Laboratoire de Cytophysiologie et Toxicologie Cellulaire (Drs. Skowron, Tournier, and Marano), Université Paris, Paris, France; and Sanofi-Synthelabo Recherche (Drs. Perret and Caput), Lábége, France.

Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (e-mail: permissions@chestnet.org).

Correspondence to: Stephen I. Remillard, MD, FCCP, Pulmonary and Critical Care Medicine Section, University of Nebraska Medical Center, 985125 Nebraska Medical Center, Omaha, NE 68198-5125; e-mail: remillard@unmc.edu

www.chestjournal.org CHEST / 123 / 3 / MARCH, 2003 SUPPLEMENT 373S

Downloaded From: http://journal.publications.chestnet.org/pdfaccess.ashx?url=data/journals/chest/21991/ on 06/26/2017