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 Stat11 p84/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 × 10^5 vs 4,700 ± 500 × 10^5 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) units for IL-4; 970 ± 20 × 10^5 vs 3,900 ± 230 × 10^5 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-enhanced production of TGF-β2 in HBECs (p < 0.01). Correspondingly, IFN-γ significantly reduced both basal (970 ± 20 × 360 ± 30 × 10^5 GAPDH units) and IL-4- or IL-13-enhanced messenger RNA expression of TGF-β2 (4,700 ± 500 × 10^5 vs 570 ± 20 × 10^5 GAPDH units for IL-4; 3,900 ± 230 × 10^5 vs 560 ± 20 × 10^5 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 nuclear immunofluorescence for Smad 7. This was associated with significantly reduced nuclear localization of Smad 2, 3, and 4, 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*

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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. 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. 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. 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


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Identification of Genes and Proteins Regulated by Interleukin-5 in Human Eosinophils Using Microarrays and Two-Dimensional Electrophoresis/Mass Spectrometry*

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Abbreviation: IL = interleukin

The cellular events underlying the development and maintenance of allergic airway disease are complex. A number of different inflammatory cell types, including lymphocytes, mast cells, and eosinophils, are thought to play key roles in allergic airway disease. The accumulation of eosinophils in the airway is one of the hallmark signs of asthma, and a large body of work has focused on understanding the biology of eosinophils in patients with asthma. Interleukin (IL)-5 is a crucial factor in the biology of eosinophils and has been shown to regulate a number of eosinophil functions, including apoptosis, chemotaxis, and exocytosis. In order to identify genes and proteins that are regulated by IL-5, we have performed proteomic and genomic analyses of human eosinophils that had and had not been treated with IL-5. In one set of experiments, eosinophils were prepared from the peripheral blood of five healthy donors and were stimulated in vitro with IL-5 or vehicle. Protein extracts were resolved on two-dimensional gels, and differentially expressed proteins were excised, trypsinized, and identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. In parallel experiments, microarrays (U95A; Affymetrix; Santa Clara, CA) were run on pooled RNA from primary human eosinophils that had and had not been treated with IL-5. Genes expressed in nonstimulated eosinophils were identified, as were subsets of genes that had been either up-regulated or down-regulated by IL-5 treatment. The results from the genomics analysis were compared with the results from the proteomics analysis.

Lack of Association Between the Tumor Necrosis Factor-α Regulatory Region Genetic Polymorphisms Associated With Elevated Tumor Necrosis Factor-α Levels and Children With Asthma*

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Abbreviation: TNF = tumor necrosis factor

Cytokine response after the occurrence of various inflammatory stimuli. The pathophysiology of asthma involves, in part, the inflammation of the airway epithelium. Tumor necrosis factor (TNF)-α, a proinflammatory cytokine, is released into the airway and serum of patients with asthma. Indeed, higher serum levels of TNF-α have been demonstrated in children with active disease. We tested the hypothesis that children with asthma have genetic polymorphisms in the regulatory region of the gene coding for TNF-α that are associated with higher levels of TNF-α.

Materials and Methods

Blood samples were obtained from children with exacerbations of asthma. Genotypes were determined by polymerase chain reaction amplification and restriction enzyme digestion. Genotypic frequencies were determined and compared with samples from healthy control subjects (Table 1). Statistical analysis was performed using χ² analysis.

Results

Thirty-eight children with asthma were enrolled into the study. The genotypic frequencies of the polymorphic

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