O
verexpression of interleukin (IL-13) in the murine lung causes eosinophil-predominant and macrophage-predominant inflammation, increased airway resistance, and airway hyperresponsiveness. These mice also manifest increased production and accumulation of surfactant apoproteins and alterations in surfactant phospholipid composition. However, the effects of IL-13 on surfactant function have not been defined. We hypothesized that IL-13 alters surfactant function as well as production.

To test this hypothesis, we evaluated surfactant function, phospholipid concentration, the total amount of proteins, and potential inhibitory factors in the BAL fluid from CC10-IL-13 transgene-positive (+) mice and transgene-negative (−) littermate controls. A capillary surfactometer was used to assess the ability of the surfactant to maintain the openness of a capillary glass tube modeling a terminal-conducting airway. IL-13(+) mice had increased phospholipid concentrations but showed significantly lower surfactant activity compared to those of littermate controls (p < 0.05), when calibrated to an equal amount of phospholipids. Purified surfactants from IL-13(+) and IL-13(−) mice were equally active in maintaining patency, indicating that the surfactants were functionally normal. We then determined whether the BAL protein fractions altered the function of a calf lung surfactant extract (CLSE) positive control. The protein fraction in BAL fluid samples from IL-13(−) mice showed no adverse effect on CLSE function. In contrast, the protein fraction from IL-13(+) mice suppressed CLSE function by > 80%. Furthermore, the inhibitory effect of this fraction on the CLSE function correlated with increased total protein concentrations in BAL fluid samples of IL-13(+) mice (p < 0.001).

Significantly increased levels of fibrinogen and serum albumin, both known inhibitors of surfactant function, were present in the BAL fluid samples from IL-13(+) mice, indicating either a leakage of serum proteins into the airways or an abnormal production of these inhibitors in the lungs of these mice. In conclusion, airway surfactants were increased in IL-13(+) mice, and purified surfactants were functionally normal. However, highly potent factors are present in the lungs of IL-13 transgenic mice that inhibit surfactant function. Surfactant alterations may contribute to the pathogenesis of IL-13-induced pathologic and physiologic abnormalities. Blockade or elimination of the factors that inhibit surfactant function may have therapeutic implications for IL-13-induced diseases.

References

Human Eosinophil Group IID Secretory Phospholipase A2 Causes Surfactant Dysfunction*

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We have reported that secretory phospholipase A2 (sPLA2) enzymes are elevated in the BAL fluid of asthmatic patients following inhaled allergen challenges, but the sources and functional impact of the sPLA2 enzymes are unknown. Eosinophils infiltrate lung mucosa during allergic asthma episodes, and eosinophils secrete inflammatory proteins, including sPLA2. We and others have found that eosinophils contain 14-fold to 50-fold more sPLA2 protein than neutrophils. We found that eosinophils secrete sPLA2 in vitro rapidly (peak in 2 min), while neutrophil secretion of similar enzymatic activity is continuous for > 15 min. We have now specifically identified the group IID messenger RNA, isolated from peripheral blood eosinophils using reverse-transcriptase polymerase chain reaction.

The complementary DNA was sequenced and was confirmed as the group IID gene product. The preliminary real-time polymerase chain reaction data indicate that the IID sPLA2 in eosinophils is increased by interleukin-5. In situ hybridization using the cloned complementary DNA showed that airway epithelial cells and infiltrating eosinophils (in nasal polyps) express the IID sPLA2 messenger RNA.

Studies of surfactant function, by others and by us, have found that surfactant from the BAL fluid of challenged

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asthmatic patients is dysfunctional relative to surfactant from healthy control subjects or from asthmatic patients prior to allergen challenge. Recombinant group IID sPLA₂ caused surfactant dysfunction. The group IID has specificity for phosphatidylglycerol (PG) (rather than phosphatidylcholine). Although phosphatidylcholine is the predominant phospholipid in surfactant, the group IID sPLA₂ potently induced surfactant dysfunction in vitro, and that dysfunction paralleled hydrolysis of the PG component in the surfactant. Thus, eosinophils may contribute to surfactant dysfunction in asthma patients by causing hydrolysis of the PG component of the surfactant.

Modulation of Vasodilator-Stimulated Phosphoprotein In Vivo in Human Epithelial Cells by Segmental Allergen Challenge and β₂-Agonist Therapy*

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Abbreviation: VASP = vasodilator-stimulated phosphoprotein

The attachment and migration of airway epithelial cells is an important aspect of the repair of antigen-induced inflammatory injury in patients with asthma. Cytoskeletal reorganization is required for the altered attachment and migration of epithelial cells. Vasodilator-stimulated phosphoprotein (VASP) mediates focal adhesion and actin filament binding in a variety of cells and acts as an inhibitor of cell movement. Phosphorylation of VASP via cyclic adenosine monophosphate-dependent and cyclic guanosine monophosphate-dependent protein kinases releases this “brake” on cell motility.

Segmental allergen (Ag) challenge increases phosphorylation of VASP in the airway epithelium. In addition, β₂-agonist use increases epithelial VASP phosphorylation and, thereby, alters cell adhesion and motility.

Materials and Methods

Brush biopsy specimens from the human bronchial epithelium from asthmatic subjects were obtained in the following two separate protocols: (1) before (day 1) and after segmental allergen challenges (days 2, 9, and 16) in a recovery-from-injury protocol (nine patients); and (2) before (day 1) and 24 h after segmental allergen challenge (day 2) at baseline, after a 2-week period of regular therapy with an inhaled β₂-agonist agent or placebo, and after 1 additional week of withdrawal from therapy with a β₂-agonist agent or placebo (five patients [two patients receiving β₂-agonist agent and three patients receiving placebo]). Cell lysates were assessed for VASP (46 kd) and phosphorylation of VASP (50 kd) by Western blot analyses.

Results

The ratio of 50 kd/46 kd VASP in epithelial cells increased 1 to 2 weeks after Ag challenge but not significantly (p = 0.12). Asthmatic epithelial cells have a significantly lower ratio of 50 kd/46 kd VASP than do nonasthmatic, nonasthmatic epithelial cells (eight patients) [p < 0.001]. After inhaled β₂-agonist use, the 50 kd/46 kd VASP ratio significantly increased in both control and challenged segment epithelium of asthmatic patients (p = 0.003), but not after placebo use (p = 0.79). Moreover, significantly increased numbers of Alcian blue-stained, nucin-secreting epithelial cells were observed in the BAL fluid of unchallenged segments of asthmatic patients receiving β₂-agonist therapy, but not in that of patients receiving placebo (p < 0.001 for group; p = 0.014 for day; and p = 0.004 for group vs day interaction). Accordingly, significantly increased total numbers of epithelial cells in BAL fluid (ie, Alcian blue-stained, ciliated, and other columnar epithelial cells) were observed for β₂-agonist agents but not placebo (p < 0.001 for group; and p = 0.014 for group vs day interaction).

Conclusions

Segmental Ag challenge does not significantly increase VASP phosphorylation, and asthmatic patients have significantly less VASP phosphorylation compared to healthy subjects, suggesting reduced epithelial cell motility in asthmatic patients. Inhaled β₂-agonists increase VASP phosphorylation in vitro and alter epithelial cell adhesion, possibly affecting migration.

Developing the Epithelial, Viral, and Allergic Paradigm for Asthma*

Giles F. Filley Lecture

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Abbreviations: epi–vir–all = epithelial, viral, and allergic; ICAM = intercellular adhesion molecule; IFN = interferon; IL = interleukin; RANTES = regulated and normal T cell expressed and secreted; ScV = Sendai virus; Th = T helper

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