Insulin-Like Growth Factor-I Modulates Bcl-2 Expression in Hyperplastic Mucous Cells

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Bcl-2 expression sustains airway epithelial cell proliferation and mucous cell metaplasia following lipopolysaccharide (LPS)-inflammatory responses. The present study employed two approaches to identify the inflammatory mediators that modulate Bcl-2 expression in airway epithelial cells. In one approach, bronchoalveolar lavage fluid (BALF) collected from LPS-instilled rats at 3, 10, and 24 h post instillation were assessed in the naïve rat airway organ explant culture system. Bcl-2-positive mucous cells were highest in explants treated with BALF from 10 h post LPS instillation. Because Bcl-2 positivity among mucous cells was enhanced upon the depletion of polymorphonuclear neutrophils (PMNs) in previous in vivo studies, BALF was harvested from LPS-instilled rats treated with anti-PMN antibody or control IgG 10 h post LPS-instillation and analyzed in the airway organ culture system. Bcl-2-positive mucous cells were increased in explants treated with BALF from PMN-depleted compared to IgG-treated rats. Cytokine analysis showed that upon LPS instillation IL-6 and TNF-α were decreased, VEGF was increased, and IL-1β and IL-9 levels were unchanged in anti-PMN, compared to IgG-treated controls suggesting that IL-6, TNF-α, IL-1β, IL-9, and VEGF may be involved in modulating Bcl-2 expression. The second approach used microarray analysis of RNA isolated from epithelia microdissected by laser capture microscopy from rats 2 d post LPS-instillation and non-instilled controls. Of the 800 differentially expressed genes, a significant 6- to 7-fold induction in the IGF-1 and IL-1β mRNA levels was observed and validated by quantitative real-time RT-PCR. The inflammatory mediators selected by these two approaches were then tested in various airway epithelial cell lines for the affect on Bcl-2 expression. IL-6, TNF-α, IL-1β, IL-9, and VEGF increased Bcl-2 mRNA but not protein levels. In contrast, exogenous IGF-1 treatment consistently showed induction of Bcl-2 mRNA and protein levels. In addition, Bcl-2 mRNA half-life was increased in cells treated with IGF-1 and the P2 region within the 5′-UTR appeared to play a role in the IGF-1-induced slowing of the mRNA decay. Thus, the present data suggests that IGF-1 is one of the key inflammatory mediators that modulate Bcl-2 expression in the airway epithelium during LPS-induced mucus cell metaplasia. These findings may help to identify inflammatory factors that are responsible for Bcl-2-mediated increase in mucous hypersecretion in cystic fibrosis patients.