

**Role of Toll-Like Receptor (TLR) Activation in Asthma Exacerbation: Experiments with in vitro models of human airway epithelial cells (EpiAirway™) and epithelial cell/fibroblast co-cultures (EpiAirway-FT™).**

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**INTRODUCTION:** Respiratory infections are a major cause of asthma exacerbations. The airway epithelium expresses innate responses to infectious agents via TLRs. We investigated effects of TLR stimulation in well differentiated in vitro models of human airway epithelium consisting of normal airway epithelial cells (AEC) (EpiAirway™) and AECs co-cultured with airway fibroblasts (EpiAirway-FT™).

**METHODS:** TLR expression was evaluated by RT-PCR. Cytokines, chemokines and growth factors were evaluated by bead based multiplex assays and/or ELISA assays. Histologic evaluation and caspase inhibitors were utilized to evaluate apoptotic effects.

**RESULTS:** RT-PCR confirmed expression of TLR 1, 2, 3, 5, 6, and TOLLIP by the models. Stimulation with TLR agonists caused apoptosis of epithelial cells and secretion of numerous cytokines and chemokines. High levels of fractalkine, G-CSF, IL-1 $\alpha$ , IL-1ra, IL-6, IL-8, IP-10, MIP-1a, MIP-1 $\beta$ , MIP-3a, RANTES, TNF $\alpha$  and VEGF were observed. Minor amounts of eotaxin-1 were induced in the absence of fibroblasts. However epithelial cell/fibroblast co-cultures produced high levels of eotaxin-1 after TLR stimulation. The most potent inducer of chemokine secretion was poly(I:C) (TLR3 ligand). TH2 cytokines associated with asthmatic disease (e.g. IL-13) synergized with Poly(I:C) in production of IL-8 and eotaxin-1. Caspase inhibition decreased apoptosis and chemokine secretion.

**CONCLUSIONS:** These data provide additional insight into mechanisms by which activation of epithelial/fibroblast TLR synergizes with TH2 conditions to induce apoptosis and secretion of chemokines, thereby promoting influx of neutrophils and eosinophils into the airway, hallmark features of asthmatic disease.