Cell-Specific Regulation of TNF-Induced Activation of the NF-κB Signaling Pathway by IL-10.

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Introduction: Activation of macrophages in Acute Respiratory Distress Syndrome (ARDS) leads to increased production of TNF-α, a pro-inflammatory cytokine. In turn, TNF-α triggers chemokine expression in both lung macrophages and lung epithelial cells through NF-κB activation. Though IL-10 attenuates acute lung inflammation via inhibition of the NF-κB pathway, the mechanism by which this occurs remains incompletely understood. Furthermore, it is unknown whether IL-10 specifically modulates TNF-α signaling in these cells.

Hypothesis: We hypothesized that IL-10 would attenuate TNF-induced NF-κB signaling in both monocytes and epithelial cells via a similar mechanism.

Methods: The human monocyte cell line, THP-1, and the human lung epithelial cell line, A549 were used. IL-10 treatment was achieved by overexpression using transient transfection with a pcDNA-huIL-10 vector. Post-transfection, cells were treated with TNF-α (2ng/ml). NF-κB-driven gene expression (measured using 3x-κB-luciferase expression), IκKinase activation (kinase assay), and IκB-α degradation (Western) were determined at times indicated following TNF-α stimulation. The effect of extracellular IL-10 was measured by exposure to exogenous IL-10 prior to TNF-α treatment.

Results: In both THP-1 and A549 cells, intracellular IL-10 decreased TNF-induced, NF-κB-driven luciferase expression (62% and 57%, respectively; $p<0.01$). In THP-1 cells, this effect appeared to be mediated by intracellular IL-10 expression and was associated with inhibition of IκKinase activity (at 5 mins) and IκB-α degradation (at 20 mins). In contrast, in A549 cells, IκKinase activity and IκB-α degradation were unaffected by intracellular IL-10 expression. In both cell lines, the inhibitory effect of IL-10 on TNF-induced, NF-κB-driven luciferase expression could not be replicated by treatment with exogenous IL-10, or supernatant transfer studies.

Conclusions: IL-10 regulates TNF-induced NF-κB activation in monocytes and epithelial cells, but by different mechanisms. While both cell lines required intracellular IL-10 expression, inhibition in epithelial cells appeared to be independent of IκKinase. These results suggest a potential novel pathway of NF-κB regulation by IL-10.