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Proteomic profiling to define pro-inflammatory cytokines IL-17 and TNF mediated synergistic responses in airways

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Background:

Airway inflammation is a characteristic feature of asthma which affects 13% of Canadian children. Cytokines IL-17 and TNF promote airway inflammation and are elevated in response to environmental exposures such as inhaled allergen and air pollution. IL-17 co-operatively enhances airway inflammation in combination with acute pro-inflammatory cytokines namely TNF. However, proteins altered by the combination of IL-17 and TNF are not completely defined.

Objective:

To identify proteins enhanced by the combination of IL-17 and TNF in the lungs.

Methods:

Human Bronchial Epithelial Cells (HBEC) were stimulated with IL-17A/F (50 ng/mL) and/or TNF (20 ng/mL) for 24h. Cell lysates (n=5) were probed using Slow Off-rate Modified Aptamer (SOMAmer®)-based proteomic array. Pairwise differential analysis was performed on normalized log2 protein expression values, along with Welch's t-test ($p<0.05$) to identify differentially abundant proteins. Selected proteins were independently validated *in-vitro* in HBECs using ELISA and western blots, and *in-vivo* using a house dust mite (HDM)-challenged murine model of airway inflammation.

Results:

SOMAmer array identified 70 proteins that were enhanced in response to the combination of IL-17 and TNF, compared to either cytokine**.** Proteomic profiling and independent validation showed that antimicrobial proteins associated neutrophilic airway inflammation such as lipocalin-2 and elafin, chemokines CXCL1 and CXCL8, and airway remodeling factor MMP13 were enhanced (>2-fold) in response to IL-17 and TNF co-exposure, compared to either cytokine alone in HBEC. Enhanced expression of IL-17A, IL-17A/F and TNF, as well as synergistically enhanced proteins identified *in-vitro* such as KC/GROα and lipocalin-2 were also confirmed *in-vivo* in lungs of HDM-challenged murine model.

Conclusion:

Combination of IL-17 and TNF enhances specific proteins associated with neutrophilic inflammation and airway remodeling. The protein biosignature identified in this study will facilitate interrogation of new intervention strategies for airway inflammation.