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### Serum Amyloid A in airway cells

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**Introduction:** Acute-phase serum amyloid A (A-SAA) molecules, encoded for by SAA1 and SAA2 genes, are cytokine-inducible acute phase proteins. Increased A-SAA is implicated in various chronic inflammatory diseases including rheumatoid arthritis, asthma and COPD. Besides its major hepatic secretory source, extrahepatic A-SAA has been identified in bronchoalveolar lavage fluid, and has been claimed to be a potentially useful biomarker for airway inflammation. The cellular origin of airway-released A-SAA, however remains unknown.

**Aims:** This project aimed to (i) study cytokine-induced SAA transcriptional regulation in human airway related cell lines; (ii) develop a mature eosinophil cellular model by differentiation of EoL-1 cells; (iii) study expression profiles in cytokine-stimulated differentiated EoL-1 cells; (iv) compare putative transcription factor motif maps of the human A-SAA gene promoters.

**Methodology:** The well characterised SAA2 promoter was used as a template for study. A pGL4.10-SAA2 luciferase reporter construct was generated, transfected into A549 (alveolar epithelial) and EoL-1 (eosinophilic) cells and stimulated with different concentrations of IL1 $\beta$ +IL6. Promoter activity was measured using dual luciferase reporter assays. EoL-1 differentiation was studied using a panel of cell densities and concentrations of apicidin or sodium butyrate, and was followed through morphological and qPCR-based CCR3 biomarker expression studies. An expression microarray approach combined with Ingenuity® pathway analysis was used to study IL1 $\beta$ /IL6-dependent gene regulation while cytokine-induced cellular A-SAA secretion was investigated using ELISA assays. Transcription factor motif maps were generated in silico using Transplorer®.

**Results:** SAA2 promoter activity was upregulated following IL1 $\beta$ +IL6 stimulation in A549 and EoL-1 cells, with maximal activity at 2ng/ml IL1 $\beta$ +200ng/ml IL6, and 4ng/ml IL1 $\beta$ +400ng/ml IL6 respectively. The greatest morphological changes in EoL-1 cellular differentiation occurred at 1x10<sup>6</sup> cells/ml exposed to 300nM apicidin for 9 days, while the greatest increase in CCR3 expression (2.53 fold), occurred with 100nM apicidin. Expression profiling showed that stimulation of differentiated EoL-1 cells with 1ng/ml IL1 $\beta$ +100ng/ml IL6 induced no differential A-SAA expression. This was corroborated by the absence of EoL-1-secreted A-SAA and the lack of promoter activity at the same cytokine concentrations. Pathway analysis revealed a network of differentially expressed genes related to airway inflammation. Transcription factor maps suggested that A-SAA genes are transcriptionally regulated by similar putative transcription factor profiles, also having reported roles in eosinophilic differentiation, airway calibre, mediation of inflammatory responses and extrahepatic SAA production.

**Conclusion:** Cytokine-induced SAA2 promoter activation occurs in alveolar epithelial cells and eosinophils, but the concentrations required suggest that this may be potentially only relevant in severe inflammation.