

MEETING ABSTRACT

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# Analysis of *indoleamine 2,3-dioxygenase 1 (IDO1)* expression of cultured cord blood adherent mononuclear cells as an indicator of atopic risk

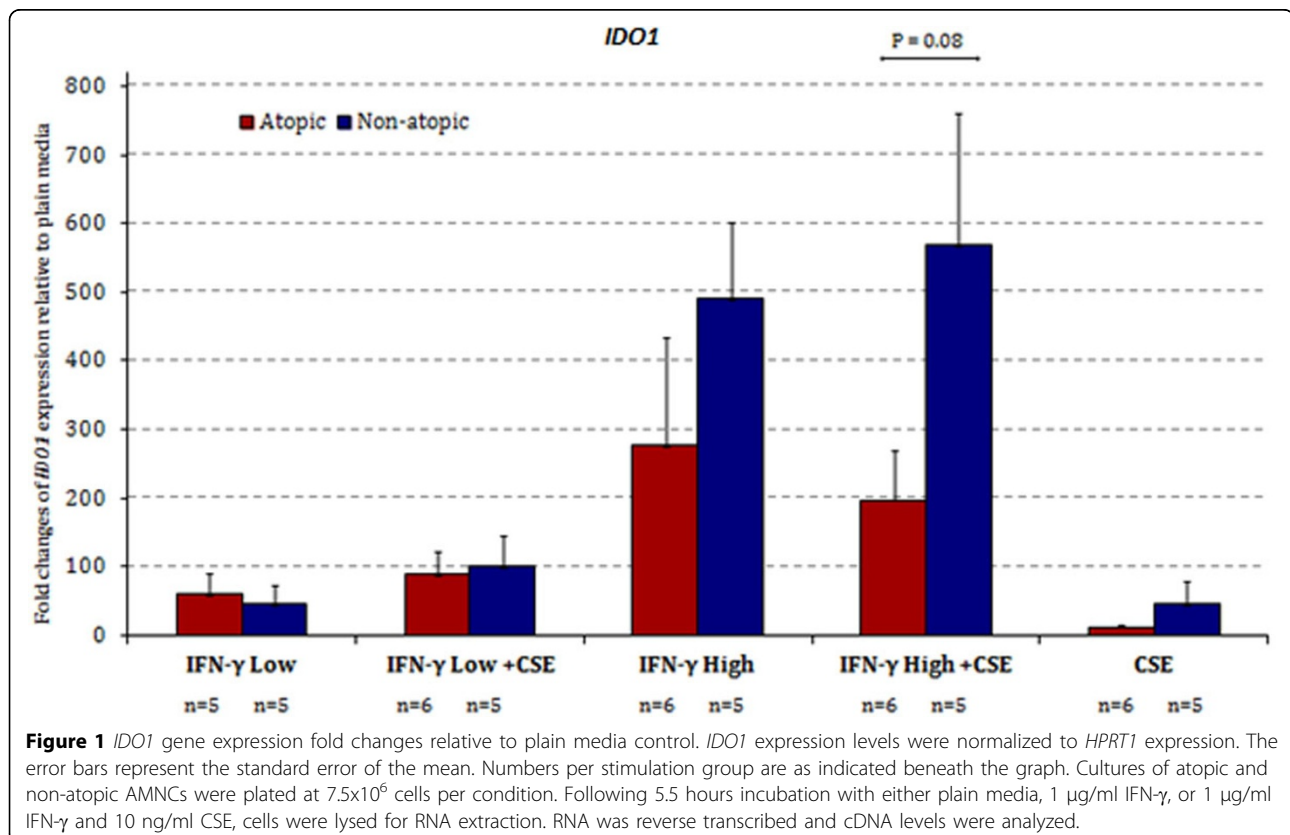
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## Background

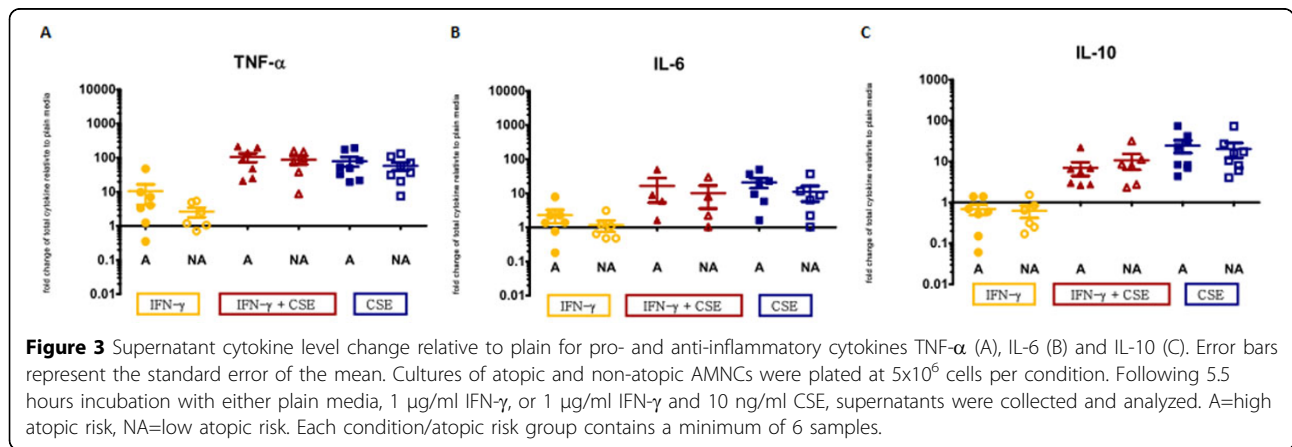
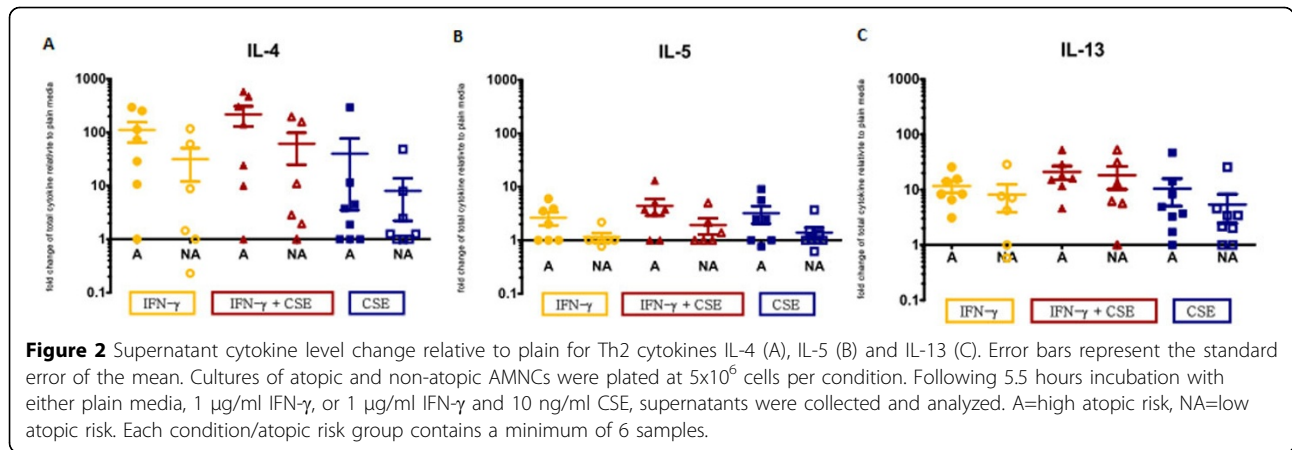
Maternal atopy is a known risk factor for allergy development in children. This link can be studied to find potential indicators of atopic risk by examining

umbilical cord blood. *Indoleamine 2,3-dioxygenase 1 (IDO1)*, the initiator of the IDO pathway, plays a regulatory role in the immune response and may differ in expression in the adherent mononuclear cells (AMNC)



**Figure 1** *IDO1* gene expression fold changes relative to plain media control. *IDO1* expression levels were normalized to *HPRT1* expression. The error bars represent the standard error of the mean. Numbers per stimulation group are as indicated beneath the graph. Cultures of atopic and non-atopic AMNCs were plated at  $7.5 \times 10^5$  cells per condition. Following 5.5 hours incubation with either plain media, 1  $\mu\text{g/ml}$  IFN- $\gamma$ , or 1  $\mu\text{g/ml}$  IFN- $\gamma$  and 10 ng/ml CSE, cells were lysed for RNA extraction. RNA was reverse transcribed and cDNA levels were analyzed.

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of atopic and non-atopic individuals. Supernatants of these AMNC cultures may also exhibit different cytokine profiles.

## Methods

Cord blood samples were collected from consenting women undergoing elective Caesarian-sections and atopic status was self-reported. Mononuclear cells were isolated and cryopreserved. Once thawed, AMNCs were cultured and stimulated with interferon-gamma (IFN- $\gamma$  1 $\mu\text{g/ml}$  or 1ng/ml) with or without control standard endotoxin (CSE 10ng/ml). In each condition,  $7.5 \times 10^6$  cells were seeded for gene analysis and  $5 \times 10^6$  cells were seeded for cytokine analysis. Cells were lysed for RNA isolation, reverse transcribed and cDNA levels were analyzed using qPCR. Supernatant cytokine levels were analyzed using the Luminex<sup>®</sup> xMAP<sup>™</sup> Technology.

## Results

*IDO1* expression was significantly increased in all stimulated conditions ( $P < 0.05$ ) except for the CSE only condition. The high atopic risk group displayed trend towards decreased *IDO1* expression, however, high and low atopic

risk groups did not show significant differences (Figure 1). Supernatant cytokine analysis show heightened levels of Th2 cytokines IL-4, IL-5, IL-13 (Figure 2). Similarly, heightened levels of TNF- $\alpha$  and IL-6 were observed, while levels of IL-10 were decreased in the high atopic risk samples in all stimulated conditions (Figure 3).

## Conclusions

Preliminary differences detected suggest that further research could elucidate a suitable biomarker to predict atopic risk. Due to the lack of significant differences between high and low atopic risk groups for *IDO1* expression and cytokine expression, a reliable biomarker was not determined in this study.

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