Multiplexed Analysis of Primary Cord Blood Adherent-Mononuclear Cell Supernatants Related to Maternal Atopy

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Abstract

Rationale: We have previously reported decreased levels of interleukin(IL)-10 in primary cord blood adherent-mononuclear cell cultures (AMMCs) from atopic versus non-atopic mothers following Control Standard Endotoxin (CSE) stimulation. In the current study we examined a multiplexed panel of 12 cytokines in these cultures. This may potentially identify a set of atopic-risk biomarkers that can be employed at birth.

Methods: Cord blood samples were obtained from mothers undergoing elective C-sections and atopic or non-atopic status was self-reported. AMMCs were isolated and cultured with or without Interleukin(IL)-10 gamma and/or CSE for 5.5 hours, following which supernatants were collected and frozen. ELISA and Luminex multiplex analysis using the High Sensitivity Human Cytokine Magnetic Bead Kit (EMD Millipore) were utilized to determine the following cytokine concentrations: IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12, IL-13, GM-CSF and TNF-α.

Results: Stimulation with IL-10 and/or CSE, as well as IL-10 alone, significantly upregulated IL-10, IL-2, IL-4, IL-12, IL-13, IL-10, IL-13, GM-CSF and TNF-α concentrations (P<0.05, compared to media control). Also, IL-10 was significantly elevated (P<0.05) in atopic supernatants when stimulated with IL-10 and IPN together compared to CSE alone in the non-atopic controls. Furthermore, we observed a trend towards upregulated levels of IL-2, IL-4, IL-8, IL-10, IL-12, IL-13 and GM-CSF as well as a lower IL-10 levels in the atopic samples when compared to non-atopics.

Conclusions: Using a small set of atopic- and non-atopic samples, preliminary differences were noted in a specific set of cytokines between atopic and non-atopic mothers following CSE treatment.

Results

Analytes involved in Th2 responses

Analytes involved in Th1 responses

Analytes involved in chemotaxis

Method

Controls

ADCC

Cord Blood

AMMC

AMMC supernatants following 5.5 hours of incubation

MMR Culture supernatants of 4 atopic and 5 non-atopic patients

MULTIPLEX for High Sensitivity Human Cytokine Panel: IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12, IL-13, GM-CSF and TNF-α

Informed consent was obtained from mothers undergoing elective Caesarian-sections at Kingston General Hospital for the procurement of cord blood samples. Atopic or non-atopic status was self-reported. AMMCs were isolated and cultured with or without a Bead purified IL-10 gamma and/or CSE. From these samples, mononuclear cells (MNCs) were isolated via density gradient centrifugation and subsequently cultured in triplicates.

Upon thawing, adherent-mononuclear cells (AMNCs) were separated by culturing 5×10^5 MNCs per condition for 2 hours (2% FCS and 5% CO2) in RPMI Life Technologies™ complete media containing 10 % FBS [see table], 1% Pen/Strep/Neomycin [Life Technologies™] and 2.5% HEPES (Hepes Buffer Solution) using BD Primaria™ culture flasks (via VWV). The MNCs were incubated in three conditions; plain RPMI complete media (IFN at 1 ng/ml, Tgama, Adderly) or IFN (at 1 ng/ml) together with CSE at 10 ng/ml (via MBS Biologics). The cells were incubated under these conditions at 37°C and 5% CO2, for 5.5 hours. Afterwards, supernatants were collected and aliquots were frozen at -80°C.

The MILLIPLEX® Human Sensitivity Human Cytokine Magnetic Bead Kit (EMD Millipore) was utilized to determine IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12, IL-13, GM-CSF and TNF-α levels.

The samples used in this project are as follows:

<table>
<thead>
<tr>
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Table 1: Summary of cord blood samples (self-reported atopic status) used per condition.

Summary and Directions

In this preliminary study we investigated supernatants of primary cord blood adherent-mononuclear cell (AMNC) populations derived from four atopic and five non-atopic cord blood samples. This stimulation protocol of these cells with IFN (at 1 ng/ml), CSE (at 10 ng/ml), or IFN and CSE (at 1 ng/ml and 10 ng/ml, respectively) along with CSE (1 ng/ml) resulted in a significant upregulation of IL-10, IL-2, IL-4, IL-8, IL-10, IL-12, IL-13, GM-CSF and TNF-α concentrations (P<0.05, compared to media control). Interestingly, IL-2 and IFN (levels were significantly elevated (P<0.05) in the supernatants from mothers with self-declared atopic status when stimulated with IFN and CSE compared to non-atopics. No differences could be noted between atopic and non-atopic cytokine supernatants in the untreated (untreated) controls, mainly due to cytokine concentrations below the limit of detection.

The elevated levels of IL-10 and IFN (levels were significantly elevated (P<0.05) in the supernatants from mothers with self-declared atopic status when stimulated with IFN and CSE compared to non-atopics. No differences could be noted between atopic and non-atopic cytokine supernatants in the untreated (untreated) controls, mainly due to cytokine concentrations below the limit of detection. The median IL-10 concentration was significantly elevated in the atopic group compared to the non-atopic group (P<0.05).

Furthermore, the accuracy required to reliably identify the atopic status in this cohort remains an ongoing challenge. Verification of the self-reported atopic or non-atopic status by a more consistent technique, like skin-prick testing, could improve the intra-group variation observed, especially in the self-reported non-atopic group.

References

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