Evaluation of IL-10 in Primary Cultures of Dendritic Cells as Predictors of Atopy

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Background

Maternal atopic status is a primary influence to the later development of allergic disorders in children[1,2]. It is predicted that dendritic cells (DC) isolated from umbilical cord blood will express different levels of cytokines based on maternal atopy. The cytokine IL-10 is known to play key roles in the regulation of the immune response and we thus aim to evaluate its use as a potential biomarker and possible predictor for the later development of atopy in the child.

Methods

Cord Blood Collection:
Informed consent was obtained from mothers undergoing planned caesarean section. An optional questionnaire, regarding the parental atopic status, was also performed at this time. Umbilical cord blood was collected using a heparin-containing syringe.

Mono-nuclear Cell (MNC) Isolation:
Isolation of mononuclear cells (MNCs) was completed with the addition of Dextran followed by layering onto an Accuprep® gradient and collection of the resulting buffy coat. MNCs were frozen in freezing media for temporary storage at -80 °C until stimulations could be performed.

Dendritic Cell (DC) Selection:
After flash thawing, the MNCs underwent negative DC selection using EasySep® Human Pan-DC Pre-Enrichment Kit and EasySep® magnetic particle selection techniques (Stem Cell Technologies). Two different stimulation protocols have been implemented.

Pilot Study (8 cord blood samples)
- stimulated with Lipopolysaccharide (LPS) or Peptidoglycan (PG) for a period of 6 or 24 hours
- Cell supernatants were collected and examined for IL-10 levels via ELISA (R&D Systems)

Current Study (5 cord blood samples)
- DCs were plated 5 hours prior to stimulation with Control Standard Endotoxin (CSE) from *Escherichia coli* (Cape Cod inc.), or plain RPMI complete media.
- Supernatants were collected 24 hours post-stimulation.
- ELISA (eBioscience) was used to determine the amounts of IL-10 produced.

Results

Pilot Study

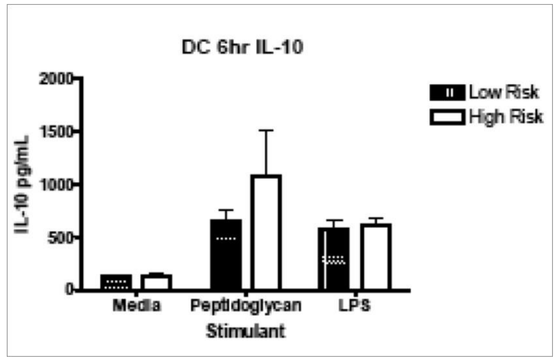

Figure 1: Pilot dendritic cell assay for IL-10 production. Dendritic cells were isolated and plated at 30,000 cells per well. The cells were incubated in 100 mL of media with 1 ug of LPS or peptidoglycan, or media alone. Supernatants were collected, and assayed by ELISA for IL-10. 4 samples per group.

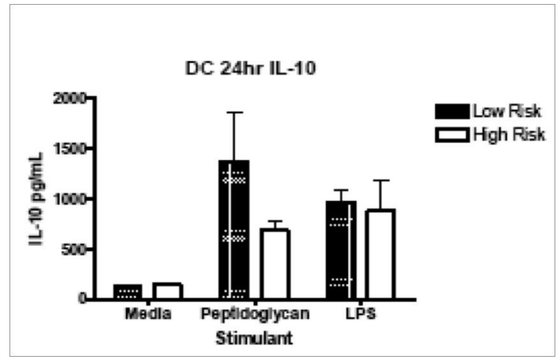

Figure 2: Pilot dendritic cell assay for IL-10 production. Dendritic cells were isolated and plated at 30,000 cells per well. The cells were incubated in 100 mL of media with 1 ug of LPS or peptidoglycan, or media alone. Supernatants were collected, and assayed by ELISA for IL-10. 4 samples per group.

Revised Protocol

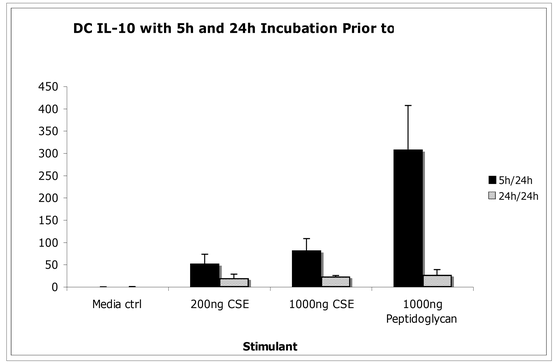

Figure 3: Reformated protocol for dendritic cell assay of IL-10 production. Dendritic cells were isolated and plated at 30,000 cells per well. Stimulation occurred either 5 hours or 24 hours after plating. The cells were incubated in 100 uL of RPMIc media with either 0.2 ug, 1 ug of CSE, 1 ug of PG or RPMIc media alone. Supernatants were collected, and assayed by ELISA for IL-10.

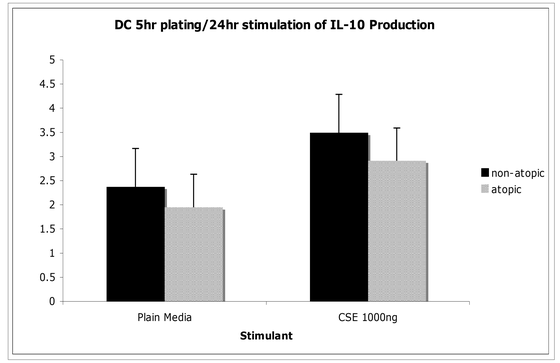

Figure 4: Dendritic cell assay for IL-10 production. Dendritic cells were isolated and plated at 30,000 cells per well. The cells were incubated in 100 uL of RPMIc media with 1 ug of CSE, or media alone. Supernatants were collected, and assayed by ELISA for IL-10. 2 samples per group.

Discussion

Our pilot study analyzed 8 cord blood samples of known atopic risk. Dendritic cells were isolated, and incubated for 6 and 24 hours with LPS, peptidoglycan or media only. Levels of IL-10 were assessed by ELISA. Decreased levels of IL-10 were observed after 24 hours of incubation with peptidoglycan, from the dendritic cells from allergic mothers.

It was later decided that the process of plating the dendritic cells itself may illicit subsequent activation and cytokine production. This could in turn influence any resulting cytokine levels. We therefore proceeded to incubate the cells in RPMI complete media for 5 or 24 hours prior to stimulation. Incubation for 5 hours prior to stimulation proved to generate a greater IL-10 response when compared to the 24 hour incubation period (Figure 3). Peptidoglycan was removed from the protocol due to solubility concerns. The implementation of the new protocol focused on stimulation using plain media compared to control standard endotoxin (CSE; Associates of Cape Cod Inc.) instead of LPS.

Applying the revised protocol to samples of atopic and non-atopic status, we observed slight differences in IL-10 production. It should be noted that IL-10 expression after CSE stimulation was reduced in atopic mothers compared to non-atopic mothers as predicted. However, fluctuations and inaccuracies throughout the stimulation could also explain this difference.

Limitations of the current study are that all samples were obtained from C-sections and did not employ diagnostic testing for allergies such as skin prick tests, but relied instead on subject self-report.

Future Directions

- Presently, there are insufficient samples in order to draw significant conclusions. We therefore aim to continue collecting and processing umbilical cord blood DCs in the hopes of producing a reasonable sample size.
- TGF-ß is another cytokine known to be important in the regulation of immune response. Analysis of DC production of this cytokine based on maternal atopic status is also currently underway.
- Only maternal atopic status was considered in this study. Paternal atopic status could be considered in the future.

References and Acknowledgements

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