Differences in Regulatory Capacity of Umbilical Cord Blood Mononuclear Cells Based on Maternal Atopic Risk

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Abstract

Rationale: Maternal atopy is a known significant risk factor for the development of allergy in children, and may exert influence *in utero* on fetal immune cells. Atopy is generally viewed as a failure of immune tolerance, and we thus aimed to evaluate the effects of maternal atopy on umbilical cord blood (CB) mononuclear cell (MNC) production of regulatory cytokines following innate stimulation.

Methods: Pregnant women delivering via planned Caesarian Section at Kingston General Hospital were recruited for study participation, and maternal atopic status was recorded after written informed consent was obtained. CB MNCs were isolated from both atopic and non-atopic mothers (N=4 each) following delivery, and incubated with 1µg/mL of lipopolysaccaride (LPS) or peptidoglycan (PGN), for 6 and 24 hours. Cell supernatants were collected and assayed for regulatory cytokine production by ELISA.

Results: Production of Interleukin-10 (IL-10) and Transforming Growth Factor-Beta (TGF-β) was significantly lower in the CB MNC from atopic mothers compared to non-atopic mothers following innate stimulation. Specifically, after 24hrs incubation with PGN, IL-10 levels were 1.3 fold higher in MNCs supernatants from non-atopic mothers compared to their atopic counterparts ($p<0.05$) The level of TGF-β secreted by the MNCs of non-atopic mothers following 6hr stimulation with LPS was 3.5 fold higher than those from allergic mothers ($p<0.05$).

Conclusions: Cord blood MNCs from atopic mothers appear to have impaired regulatory capacity following innate stimulation compared to non-atopic controls, and may have relevance to the development of allergic disease in the offspring.

Background/Rationale

- Maternal atopy is a known significant risk factor for the development of allergy in children, and may exert influence *in utero* on fetal immune cells
- Atopy is generally viewed as a failure of immune tolerance, and we thus hypothesized that maternal atopy may alter the umbilical cord blood (CB) mononuclear cell (MNC) production of regulatory cytokines following innate immune stimulation

Objective

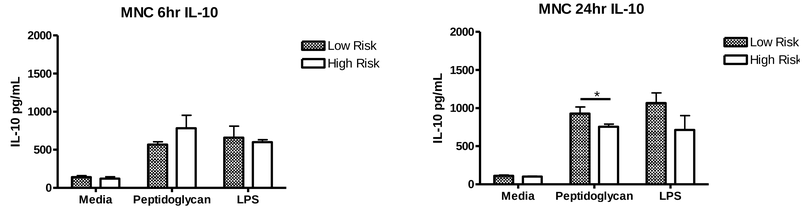
- We aimed to establish differences in regulatory cytokine responses of cord blood mononuclear cells derived from the umbilical cord blood of infants at higher risk of atopy compared to those of lower risk, based on maternal atopic status.

Methods

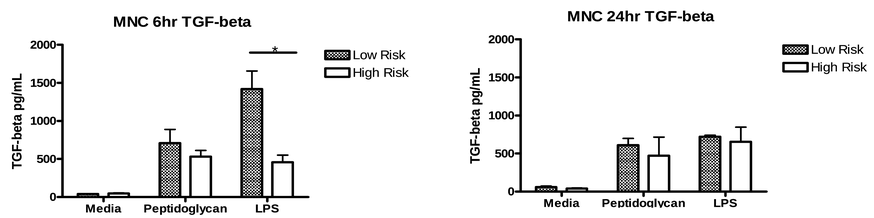
- Pregnant women delivering via planned Caesarian Section at Kingston General Hospital were recruited for study participation
- Maternal atopic status was recorded after written informed consent was obtained (subject self-report)
- CB MNCs were isolated from both atopic and non-atopic mothers (N=4 each) following delivery via Accuprep® density gradient
- MNCs were subsequently incubated with 1µg/mL of lipopolysaccharide (LPS) or peptidoglycan (PGN), for 6 and 24 hours
- Cell supernatants were collected and assayed for regulatory cytokine production (IL-10 and TGF-B) by ELISA (R&D systems)

Results

- Production of Interleukin-10 (IL-10) and Transforming Growth Factor-Beta (TGF-β) was significantly lower in the CB MNC from atopic mothers compared to non-atopic mothers following innate stimulation (See Figures)
- After 24hrs incubation with PGN, IL-10 levels were 1.3 fold higher in MNCs supernatants from non-atopic mothers compared to their atopic counterparts ($p<0.05$)
- The level of TGF-β secreted by the MNCs of non-atopic mothers following 6hr stimulation with LPS was 3.5 fold higher than those from allergic mothers ($p<0.05$)



Figures 1AB: Cord blood mononuclear regulatory cytokine production in response to innate immune stimulation. CB MNCs were incubated in 100mL of media with 1ug of LPS (Sigma), PGN (Sigma), or media alone. Supernatants were collected, and assayed by ELISA for IL-10. N = 4 samples per group, *p<0.05.



Figures 2AB: Cord blood mononuclear regulatory cytokine production in response to innate immune stimulation. CB MNCs were incubated in 100mL of media with 1ug of LPS, peptidoglycan, or media alone. Supernatants were collected, and assayed by ELISA for IL-10. N = 4 samples per group, *p<0.05.

Acknowledgments

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Discussion

- That maternal atopic status is a strong risk factor for atopy development (1) indicates that maternal-fetal interactions may well have influence over the developing immune system and hence promote atopy in the newborn
- Umbilical cord blood provides a unique window into the physiology of both the maternal and fetal environment prior to the commencement of the neonatal environment, and can be accessed without risk to either mother or infant.
- Others have shown that there is an association between paternal atopic status and decreased IL-10 and TGF-β in the cord blood of newborns. Additionally, caesarean section, a known risk factor for atopy, is also associated with reduced TGF-β in the cord blood of newborn infants (2)
- Our work expands on these previous research endeavors by documenting that even amongst a small cohort of pregnant women, all of whom were delivering via C-section, differences could be detected between the induced production of IL-10 and TGF-B by cord blood mononuclear cells undergoing TLR stimulation by LPS and PGN
- Ongoing research in our laboratory is underway evaluating a larger cohort of subjects in which the dendritic cell population has been isolated prior to innate stimulation, rather than the non-specific MNC population.

Conclusion

- **Cord blood MNCs from atopic mothers appear to have impaired regulatory capacity following innate stimulation compared to non-atopic controls, and this may have relevance to the development of allergic disease in the offspring.**

References

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