Moderate Prenatal Alcohol Exposure Increases Toll-Like Receptors Activity Detectable in Umbilical Cord Blood at Birth

Jessie R. Maxwell, MD[1,2], Shahani Noor, PhD[2], Nathaniel Pavlik, BS[1], Dominique Rodriguez[3], Lidia Enriquez Marquez[3], Jared DiDomenico[3], Ludmila N. Bakhireva, MD, PhD, MPH[3]

[1]Department of Pediatrics, [2]Department of Neurosciences,
[3]College of Pharmacy at The University of New Mexico, Albuquerque, New Mexico, USA

INTRODUCTION

- More than 10% of pregnant women consume alcohol during the pregnancy
- 1-5% of infants are diagnosed with Fetal Alcohol Spectrum Disorders (FASD)
- Prenatal alcohol exposure (PAE) increases the risk of infection in the newborn by altering development with resultant changes in immune function including cytokine expression
- Toll-like receptors (TLRs) are expressed on multiple types of cells
- TLRs play a vital role in modulation of the innate immune system and the release of inflammatory mediators
- **We hypothesized that PAE would result in dysregulated cytokine expression following stimulation with TLR agonists in umbilical cord blood samples**

METHODS

Ethanol, Neurodevelopment, Infant, and Child Health (ENRICH) - 2

- The ENRICH-2 cohort at the University of New Mexico (UNM) was utilized
- ENRICH-2 is a prospective cohort study with the following assessment time points: 2nd Trimester Visit, 3rd Trimester Visit, Birth Hospitalization, 6-month follow-up assessment
- Pregnancy alcohol use is ascertained by four Timeline Follow-Back (TLFB) interviews and comprehensive ethanol biomarker panels
- Alcohol use was moderate (AA/day[SD]=0.2[0.2])
- Women without alcohol use are controls

Peripheral Blood Mononuclear Cells (PBMC) Isolation

- Blood was removed from an umbilical cord artery
- PBMCs were isolated by Ficoll-isopaque density gradient centrifugation
  - Washed free of platelets and Ficoll
  - Stored in fetal calf serum (FCS) and Dimethyl Sulfoxide in liquid nitrogen
  - PBMCs were washed in RPMI 1640 and diluted in complete medium

Cell Stimulation and Cytokine Measurements

- Cells were plated with 0.2 million cells per well of 500 uL of medium
- Well then had one of the following added: control (complete medium), lipopolysaccharide (LPS) 1 ug/mL
  - LPS = TLR 4 Agonist
- Media isolated after stimulation (3 hours and 24 hours) was analyzed using an electrochemiluminescence assay
- Cytokines typically produced from innate immune cells were measured including: IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8 and TNF-α

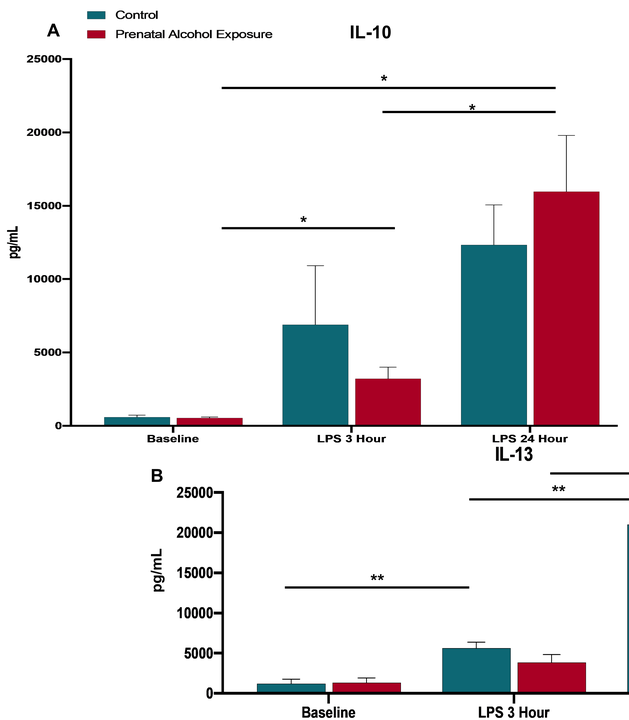
Statistical Analysis

- Data was compared with pooled variances t-test, Mann-Whitney test and Fisher's exact test when appropriate (as defined in Table 1)
- Cytokine protein expressions were compared by student's t-test
- Statistical significance was defined as p <0.05
- Sample size to date: 10 controls and 8 alcohol exposed infants

RESULTS

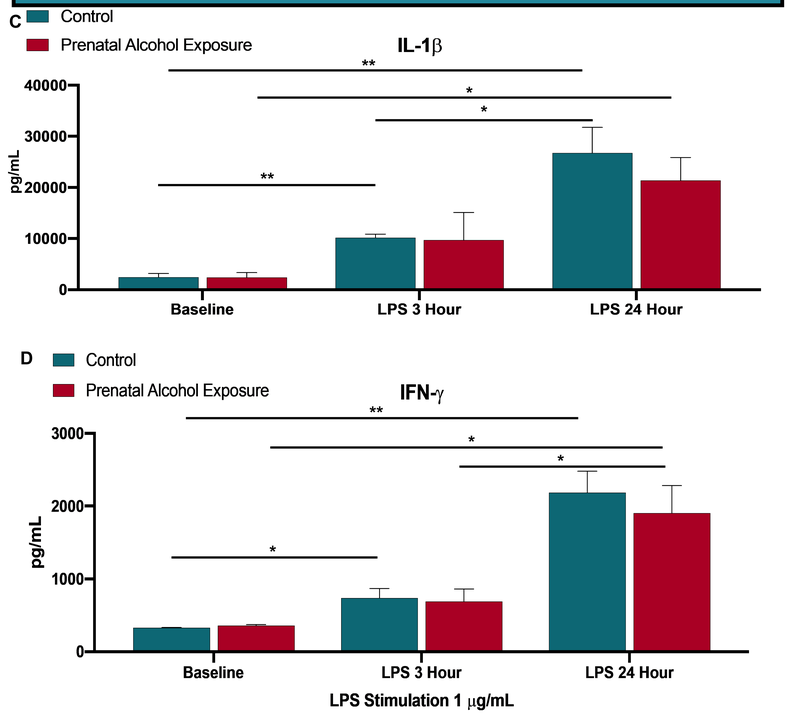
Variable	Healthy Control (N=10)	Alcohol Exposed (N=8)	P-Value
Age (Mean ± SD)	27.0 ± 6.1	27.5 ± 4.9	0.85°
Years of Education (Mean ± SD)	13.3 ± 1.3	14.3 ± 2.7	0.65¹
Gestational Age (Mean ± SD)	38.4 ± 1.1	40.1 ± 1.0	0.0032°
Birthweight in grams (Mean ± SD)	3028.5 ± 547.8	3382.3 ± 286.8	0.12°
Marital Status			
Single/ Separated/ Divorced	1 (10.0%)	5 (62.5%)	0.043³
Married/ Cohabitating	9 (90.0%)	3 (37.5%)	
Race			
White	5 (50.0%)	4 (50.0%)	0.55³
Black or African American	1 (10.0%)	0 (0.0%)	
American Indian or Alaskan Native	3 (30.0%)	1 (12.5%)	
Other	1 (10.0%)	3 (37.5%)	

Table 1 The alcohol exposed infants were born significantly later at 40.1 weeks gestation compared to 38.4 weeks gestation in the control group (p<0.01). Additionally, more women were married/cohabitating in the control group compared to the alcohol exposed group (p<0.05).
⁰Pooled Variances t-test; ¹Mann-Whitney test; ³Fisher's exact test



Graphs A and B show significant increases in the expression of IL-10 and IL-13 from baseline to 3-hour and 24-hour simulation with LPS (*p<0.05, **p<0.01). IL-10 and IL-13 are both anti-inflammatory cytokines. PAE resulted in a more robust expression of IL-10 compared to controls.

RESULTS



Graphs C and D shows significant increases in the expression of IL-1β and IFN-γ from baseline to 3-hour and 24-hour stimulation with LPS (*p<0.05, **p<0.01). IL-1β and IFN-γ are both pro-inflammatory cytokines. The control group had a more robust expression of both cytokines compared to the PAE group.

CONCLUSIONS

- No significant differences in baseline measures of cytokine expression in medium observed between control and PAE groups
- Significant increases in cytokine expression in both groups in response to LPS stimulation at 3 hours and 24 hours
- These data suggest that PAE may alter the pattern of cytokine expression in response to TLR agonists
- Variable drinking patterns by mothers may have contributed to the variation in cytokine expression after TLR agonist stimulation

ACKNOWLEDGEMENTS

This project is supported by the NIAAA grant 2R01AA021771-06 (PI: Bakhireva) and the Department of Pediatrics.