Impact of Prenatal Alcohol Exposure on Immune Function Throughout the Life Course

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Presenter Disclosure

• Relationship with commercial interests: None

Managing Potential Bias

• Not required
Presentation Overview

How does prenatal alcohol affect immune function throughout the life course?
Presentation Overview: Key Questions

1) Are the rates of autoimmune diseases (e.g. rheumatoid arthritis) higher in individuals with FASD?
2) Are the immune changes associated with alcohol consumption present during early postnatal life?
3) Does alcohol consumption during pregnancy impact the maternal immune environment?
4) Does prenatal alcohol exposure impact immune function during early childhood?
5) Are the rates of autoimmune diseases, including rheumatoid arthritis, higher in individuals with FASD?
Immune system immature at birth; capable of responding to an immune challenge.

**Rodent** – Gestation ~21 days

- **Gestation**
  - 1st Trimester Equivalent: days 1-10
  - 2nd Trimester Equivalent: days 11-21
  - 3rd Trimester Equivalent: postnatal days 0-10

**Human** – Gestation ~40 weeks

- **Gestation**
  - 1st Trimester: weeks 1-12
  - 2nd Trimester: weeks 13-27
  - 3rd Trimester: weeks 28-42

**Birth**

**Weaning** PND 21

**Adulthood**

**Immune competent at birth; capable of responding to an immune challenge**
Background: Overall functions of the immune system
The immune system and brain development

• The immune and neuroimmune systems are critically important for brain development

• Modulate:
  • Neurogenesis
  • Neuronal migration
  • Synaptogenesis
  • Synaptic pruning

Knuesel et al., 2014
Key immune system components

- **Cytokines**: Signaling molecules of the immune system the “hormones” of the immune system.
  - **Immunomodulating agents**: Their release impacts the *behaviour* of the cells around them.

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**Figure:**
- **Pro-inflammatory**
  - IL-1β
  - IL-2
  - TNF-α
  - IFN-γ
  - KC/GRO

- **Anti-Inflammatory**
  - IL-10
  - IL-4
  - IL-5
  - IL-13

**Legend:**
- Prenatal alcohol exposure
- Estrus cycle (cycling estradiol and progesterone) in females
- Circulating testosterone in males
- Circulating corticosterone produced by an acute stressor

Source: Drawing by authors.
Wide ranging impacts on immune function

Increased susceptibility to infections
Increased risk (15 fold) of early-life sepsis in very low-birth weight infants
Increased rates of asthma, nasal airway inflammation, persistent skin rashes
Decreased numbers of immune cells, impaired response to stimulation
Increased incidence of cancers

Increased susceptibility to infections
Deficits in adaptive immunity
Impaired immune organ development (thymus)
Deficits in development of immunological memory
Increased susceptibility to cancers
Key Questions

1) Are the rates of autoimmune diseases (e.g. rheumatoid arthritis) higher in individuals with FASD?
Model of Prenatal Alcohol Exposure

Pregnant Sprague Dawley dams, placed on diet throughout gestation:

Ethanol

Control

Image credit: Kasia Stepień
Effects of prenatal alcohol exposure on the response to a chronic immune challenge

**Chronic Mild Stress**
- Elevated Platform
- Restraint
- Cage Tilt
- Novel Cage
- Soiled Cage
- Social Isolation
- Water Deprivation
- Blood Sampling

**Adjuvant-Induced Arthritis (AA) Model:**
- Complete Freund’s Adjuvant (CFA)

Adolescence (P31 – 41)
Key Questions:

1) Are the rates of autoimmune diseases (e.g. rheumatoid arthritis) higher in individuals with FASD?
   - Alcohol-exposed animals show increased incidence and severity of adjuvant-induced arthritis.
   - Alcohol-exposed animals show impaired recovery from adjuvant-induced arthritis.
   - The postnatal environment has an impact on adjuvant-induced arthritis outcomes: the combination of alcohol-exposure and adolescent stress resulted in the greatest damage at the joint level.

2) Are the immune changes associated with alcohol consumption present during early postnatal life
Impacts of PAE on immune system development

Birth: $P_0$

Early-Life: $P_8$

Weaning: $P_{22}$
Impacts of PAE on immune system development

**prenatal treatment**, the asterisk (*) indicates a significant effect of KC/GRO was not detected at any age and data were omitted from all graphs.

**Table 1**

3.1. Pregnancy outcome

Differences were considered significant at p < 0.05 (for clarity. Note: Significant effects of KC/GRO were not post hoc comparisons, according to our hypotheses. Non-significant p values were reported in post hoc hypotheses. Non-significant

3.2. Offspring body, brain, and spleen weight

On P1, both PAE and PF pups had lower body weight than C pups [F(2, 26) = 8.76, p < 0.01] (Fig. 1A). Similarly, there were no prenatal group effects on maternal weight throughout gestation revealed, as expected, a significant interaction between prenatal group and **p < 0.05** (Fig. 2E); p = 0.05 > p > 0.01 (Fig. 2F).

Analysis of maternal weight throughout gestation revealed, as expected, a significant interaction between prenatal group and post hoc comparisons, according to the following range: ***p < 0.001***; **p < 0.01**; *p < 0.05*, for clarity. Note: Significant effects of KC/GRO were not post hoc comparisons, according to our hypotheses. Non-significant

Total intake of liquid ethanol diet is shown in Fig. 1 (S1). There were no prenatal group effects on maternal gestation length, number of pups per litter, or number of pup deaths (**p < 0.001**), with PAE and PF dams weighing less than C dams from GD 7 through GD 21 (**p < 0.001**). Of note, when corrected for body weight, brain weight was not different among (**p < 0.05**). Of note, when corrected for body weight, brain weight was increased in PAE and PF compared to C pups (**p < 0.05**). Of note, when corrected for body weight, brain weight was increased in PAE compared to (**p < 0.05**, and trends (**p < 0.05**, and trends **p < 0.05**, and trends with the comparison being made to the control group, unless otherwise indicated.

Differences were considered significant at p < 0.05, and trends **p < 0.05**, and trends **p < 0.05**, and trends with the comparison being made to the control group, unless otherwise indicated.

Spleen weight was significantly increased in PAE compared to (**p < 0.05**, and trends **p < 0.05**, and trends **p < 0.05**, and trends with the comparison being made to the control group, unless otherwise indicated.

Post hoc:
Impacts of PAE on immune system development

### Functions of the spleen:
- Storage of red blood cells
- Rich in cells of the adaptive immune system
- Implications for immune cell populations

#### Figures

**G** P1: Spleen weight

**H** P8: Spleen weight

**I** P22: Spleen weight

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Bodnar et al., 2016
Impacts of PAE on immune system development

Birth: **P0**

Early-Life: **P8**

Weaning: **P22**

10-plex: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, TNF-α, IFN-γ, KC/GRO (CXCL1)
Cytokine Profile on P8

IL-1β: Hippocampus

- C
- PF
- PAE

TNF-α: Hypothalamus

- C
- PF
- PAE

IL-6: PFC

- C
- PF
- PAE

IL-10: Spleen

- C
- PF
- PAE
Key Questions:

2) Are the immune changes associated with alcohol consumption present during early postnatal life
   - Prenatal alcohol-exposure results in increased spleen size.
   - Alcohol-exposed animals show changes to the cytokine balance in key brain areas during the critical early postnatal period.
   - Immune changes associated with alcohol are present from birth and likely underlie the well described alterations in adult immune function.

3) Does alcohol consumption during pregnancy impact the maternal immune environment?
Evaluation of the impact of alcohol consumption on the maternal immune profile

- Samples collected as part of Dr. Chambers’ longitudinal study in Western Ukraine (funded by CIFASD)
- Blood samples collected during the second and third trimesters of pregnancy from alcohol-consuming women and women reporting low/no alcohol-consumption.
- Measurement of levels of 40 cytokines and related factors

Collaboration with Dr. Christina Chambers and her team at UCSD
Evaluation of the impact of alcohol consumption on the maternal immune profile

- Expect cytokine levels to be increased with alcohol consumption based on previous work (Crews et al., 2006, Ahluwalia et al., 2000).
- **Approach:**
  - Investigate whether maternal immune profiles differ based on child outcome (Bayley assessment)
Overall cytokine profiles

Second Trimester

Low/no alcohol-consumption; typical neurodevelopment (n = 60)

Low/no alcohol-consumption; neurodevelopmental delay (n = 35)

Alcohol-consumption; typical neurodevelopment (n = 22)

Alcohol-consumption; neurodevelopmental delay (n = 35)

Bodnar et al., 2018
Overall cytokine profiles

Third Trimester

Low/no alcohol-exposure; typical neurodevelopment (n = 60)
Low/no alcohol-exposure; neurodevelopmental delay (n = 35)
Alcohol-consumption; typical neurodevelopment (n = 22)
Alcohol-consumption; neurodevelopmental delay (n = 35)

Bodnar et al., 2018
### How is the maternal immune profile affected in other neurodevelopmental disorders?

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Cytokine Pattern</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>↑ IL-6, IFN-γ, IL-1α, ↓ IL-8, MCP-1</td>
<td>Jones et al., 2017</td>
</tr>
<tr>
<td>Autism</td>
<td>↑ TNF-α, TNF-β, IL-4, IL-10</td>
<td>Abdallah et al., 2013</td>
</tr>
<tr>
<td>Autism</td>
<td>↓ CRP</td>
<td>Zerbo et al., 2016</td>
</tr>
<tr>
<td>Autism</td>
<td>↑ CRP</td>
<td>Brown et al., 2014</td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td>↑ TNF-α, IL-1β, IL-6</td>
<td>Yoon et al., 1997</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>↑ TNF-α</td>
<td>Buka et al., 2001</td>
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<tr>
<td>Schizophrenia</td>
<td>↑ CRP</td>
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Differential activation/inhibition of cytokine networks

Alcohol-Exposure Network

Exposure/Neurodevelopmental Delay Network

Vulnerability Network

Bodnar et al., 2018
Key Questions:

3) *Does alcohol consumption during pregnancy impact the maternal immune environment?*
   - Alcohol consumption has an impact on the cytokine profile during pregnancy.
   - More than “a few key cytokines” were involved.
   - Maternal cytokine profiles could be used to predict child outcomes (risk vs. resilience).
   - *More work is needed to explore whether these networks hold true for other populations, exposure levels, etc.*

4) *Does prenatal alcohol exposure impact immune function during early childhood?*
Evaluation of immune function following prenatal alcohol exposure

- Samples collected as part of Dr. Chambers’ longitudinal study.
- Blood samples collected at 2 – 3.5 years of age
- Levels of 40 key cytokines/chemokines and related factors
- Experimental questions:
  1) Is prenatal alcohol exposure associated with a differential immune profile?
  2) Are immune profiles different based on child outcome (Bayley assessment)?

Collaboration with Dr. Christina Chambers and her team at UCSD
How is the childhood cytokine profile affected in other neurodevelopmental disorders?

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<td>Cerebral palsy</td>
<td>↑ TNF-α, IL-1β, IL-8</td>
<td>Varner et al., 2015</td>
</tr>
<tr>
<td>Autism</td>
<td>↑ IL-1β, IL-4</td>
<td>Krakowiak et al., 2015</td>
</tr>
<tr>
<td>Autism</td>
<td>↑ IL-1β, IL-8, IL-5, IL-12p40</td>
<td>Ashwood et al., 2011</td>
</tr>
<tr>
<td>Autism</td>
<td>↑ IL-1β, IL-6, IL-12, IL-23, TNF-α</td>
<td>Businaro et al., 2013</td>
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<tr>
<td>Autism</td>
<td>↓ IFN-γ, IL-4, IL-10</td>
<td>Abdallah et al., 2012</td>
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</table>
Presentation Overview: Key Questions

4) Does prenatal alcohol exposure impact immune function during early childhood?
   - Differential cytokine profiles were identified based on prenatal alcohol exposure and child neurodevelopmental outcomes.
   - Network approach may be more powerful in differentiating the immune profile associated with prenatal alcohol exposure from other neurodevelopmental disorders.
   - More work is needed to explore whether these networks hold true for other populations, exposure levels, age etc.

5) Are the rates of autoimmune diseases, including rheumatoid arthritis, higher in individuals with FASD?
The Lay of the Land: Preliminary results of a health survey of adults with FASD

- The first to show that the rates of autoimmune disorders may be 4 – 6 times higher in adult with FASD.
- Estimated prevalence of rheumatoid arthritis in adults with FASD: 6.6% (global prevalence ~0.24%)
- These data helped to inspire and shape our ongoing CIFASD study on adult health in individuals with FASD.

Authors: Myles Himmelreich, CJ Lutke, Emily Travis

http://interprofessional.ubc.ca/webcasts/fasd2017/
ADULT HEALTH STUDY

A Collaborative study led by Drs. J. Weinberg, T. Oberlander, and C. Loock

Investigating the role of the immune system on health in adults with FASD

ADULTHEALTH.FASD@UBC.CA | 604-809-5574 | VANCOUVER, BC
Ongoing Adult Health Study in Vancouver

Wide range of health-related measures being collected:

- Health Survey (collaboration with Drs. Coles and Grant)
- Particular emphasis on autoimmune diseases including pre-clinical markers of rheumatoid arthritis
- Immune measures (immune cell counts, levels of key cytokines/chemokines and related factors), health records etc
Acknowledgments

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