Fetal therapies to target inflammation

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Declaration of interests

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Preterm birth is an inflammatory process
Burden of mortality and impairment for 15 million preterm babies born in 2010

- Neonatal deaths among preterm babies in 2010: 641,500
- 780,000 babies < 28 wk gestational age
- Survivors with neurodevelopmental disability in those born preterm in 2010: 72,400 children (52% of survivors)
- 1.6 million babies 28–31 wk gestational age
- 210,400 children (24.5% of survivors)
- 699,400
- Care for children with disability, family support
- 12.6 million babies 32–36 wk gestational age
- 629,900 children (5% of survivors)
- Other long-term effects, e.g., higher risk of non communicable disease
- Increased health and care load later in life

Blencowe H et al 2013 Pediatric Research 74:
Physiological Inflammation: Term Labour
Pathological Inflammation: Preterm Labour

Physiological Inflammation: Term Labour

Immune Cell Infiltration

Pro-Inflammatory Mediators
- IL-1, IL-6, IL-8, TNF-α
- MMPs
- Prostaglandins

Myometrial Contractions

Fetal Membrane Rupture

Cervical Ripening

Brain Injury

Inflammatory activation via IL-1

INFLAMMATION

Pathological Inflammation: Preterm Labour

Physiological Inflammation: Term Labour

Infection

Decay in progesterone function
Decidual Senescence
Hypoxia/Ischemia
Stress
Abruption/Haemorrhage
Overdistension
Cervical Incompetence

Evidence for the role of intrauterine infection and inflammation

Women who deliver preterm have:

- Higher incidence of chorioamnionitis than those delivering at term
- Greater bacterial load in fetal membranes in preterm labour
- Increased levels of pro-inflammatory mediators in amniotic fluid - e.g. TNF-α, IL-6 and MMP-8

Injection of bacteria or bacterial products increase inflammatory cytokines and effectively induce preterm labour in animal models
Fetal Inflammation and Injury

- Lung injury
- BPD
- White matter injury
- Cerebral palsy
- Autism

Adapted from Boyle, Rinaldi, Norman, Stock. Journal of Reproductive Immunology, 2017
Fetal Inflammatory Response Syndrome (FIRS)
Fetal Inflammatory Injury Can Occur without Preterm Labour

- Chorioamnionitis does not invariably lead to PTB

- Babies can be born with signs of systemic inflammation in the absence of preterm labour

- Changes in brain connectivity can be detected before preterm birth

In animal models, bacteria, and bacterial products which are not sufficient to cause preterm birth still result in fetal injury
Current Strategies to Prevent Preterm Birth and Fetal Injury

**Tocolysis:**
- Temporarily decrease myometrial contractions
- Does not improve neonatal outcome
  
  (Haas et al. BMJ. 2012 Oct 9;345)

**Progestogens:**
- Vaginal progesterone and 17-OHPC both reduced birth before 34 weeks’ gestation in high-risk singleton pregnancies (Relative risk [RR] 0.78, 95% CI 0.68–0.90)
  

**Aspirin:**
- 6-13+6 weeks, Nulliparous women
- PTB RR 0.89, CI 0.81–0.98
  
  (Hoffman MK et al Lancet 2020 Jan 25;395(10220):285-293.)

**Antibiotics:**
- Do not stop preterm birth
  
  (Kenyon et al Lancet. 2001 Mar 31;357(9261):989-94)
- Increase CP at age 7 years
  
Emerging preterm birth strategies:

• **Phase III**
  - Retosiban (GSK oxytocin receptor antagonist): terminated (no reduction in PTB)
  - Barusiban (Ferring oxytocine receptor antagonist): No reduction in PTB within 48 h
  - Lipocine (LPCN1107 [Oral 17-OHP]) vs IM 17-OHP – ongoing
  - Oral dydrogesterone (University of Hong Kong) – ongoing

• **Phase II**
  - OBE022 (Ebopiprant): prostaglandin F2 alpha receptor antagonist
  - Indomethacin: NSAID vs Nifedipine (proposed- Hadassah Medical Organization)
Preclinical: Targeting Cytokines, Chemokines & Signalling

<table>
<thead>
<tr>
<th>Substance</th>
<th>Animal</th>
<th>Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α antibody</td>
<td>Mouse</td>
<td>Decreases the rates of fetal death and preterm birth after LPS administration. Suppresses the expression of IL-6, IL-1β, TLR-2, CD14, and COX-1</td>
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<tr>
<td>IL-6R antibody</td>
<td>Mouse</td>
<td>Decreases the rate of LPS-induced PTD</td>
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<td></td>
<td>Human</td>
<td>Inhibits PGE2 production from human primary amniotic epithelial cells</td>
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<td></td>
<td>Rat</td>
<td>Increases the rate of pup mortality&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Increases IL-1 production in the serum&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IL-1R antagonist</td>
<td>Mouse</td>
<td>There is no significant difference in the rate of preterm birth</td>
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<td></td>
<td></td>
<td>Decreases fetal cortical brain injury</td>
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<tr>
<td></td>
<td>Mouse</td>
<td>IL-1 receptor-biased ligand specifically inhibits IL-1R downstream c-jun, and Rho GTPase/Rho-associated signaling delay IL-1β&lt;sub&gt;+&lt;/sub&gt;, TLR2&lt;sub&gt;+&lt;/sub&gt;, and TLR4-induced preterm birth</td>
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<tr>
<td>Cytokine-suppressive anti-inflammatory drugs (CSAILDs)</td>
<td>Ewe</td>
<td>Review</td>
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<tr>
<td></td>
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<td>Suppresses the production of PGE2 in amniotic fluid</td>
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<td>Decreases the IL-6 concentration of maternal plasma</td>
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<td>Inhibits the infiltration of polymorphonuclear cells into fetal lung</td>
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<tr>
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<td>Mouse</td>
<td>Decreases the rate of preterm birth</td>
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<td>Suppresses cytokine and/or chemokine levels of maternal plasma, liver, myometrium, and decidua</td>
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<td>Inhibits neutrophil infiltration into the myometrium</td>
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<tr>
<td>Antibiotics with dexamethasone and indomethacin</td>
<td>Macaque</td>
<td>Prolongs gestation following GBS-induced increases in uterine contractility</td>
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<td></td>
<td>Decreases IL-1β, TNF-α, PGE2, and PGF2α levels of amniotic fluids</td>
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<td>No changes in MMP-9 or -2 expression</td>
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<tr>
<td>COX-2 inhibitor (Celecoxib)</td>
<td>Mouse</td>
<td>Indomethacin and meloxicam but not diclofenac inhibit LPS-induced preterm birth</td>
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<td></td>
<td>Mouse</td>
<td>Reduces the rate of LPS-induced preterm birth</td>
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<td></td>
<td>Rat</td>
<td>Reduces the concentrations of PGE2 and PGF2α in uterine tissue</td>
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<td></td>
<td></td>
<td>Fetal ductal arteriosus is significantly limited&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>NF-κB inhibitors</td>
<td>Human</td>
<td>In an ex vivo model, NF-κB inhibitor suppresses LPS-induced IL-6 and TNF-α production by maternal and fetal compartments</td>
</tr>
<tr>
<td>IKK inhibitors</td>
<td>Human</td>
<td>Inhibits the level of LPS-inducing genes such as IL-6 and TNF-α in primary term choriodical cells</td>
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</tbody>
</table>
Preclinical: Targeting Cytokines, Chemokines & Signalling

• Interleukin-1 Receptor Antagonists:
  • Kineret, canakinumab, and rilonacept – Rx for inflammatory disorders
  • Reduce fetal inflammation (low dose) and preterm labour (intra-amniotic) in animal models
  • Side effects
  • Allosteric regulators

• Broad spectrum chemokine inhibitors (BSCI) Immunoliposome:
  • Block somatostatin receptor type 2 (SSTR2)
  • May directly inhibit uterine contraction
  • Blocks LPS induced PTB in moude model
  • Inhibits GBS induced preterm labour in primate model (but not microbial invasion of the fetus)

Presicce, P. JCI Insight 2018, 3.

## Preclinical: Suppressing Inflammation

### Lipid mediators (DAG resolution and resolvin E3)
- **ω3/resolvin E3**
  - **Mouse**
  - Decreases the rate of LPS-induced preterm birth
  - Inhibits IL-6, IL-1β, and TNF-α expression from peritoneal washes
  - EPA metabolite resolving E2 also exerts protective effects against LPS-induced preterm birth

### Lipoxin A4
- **Mouse**
- Does not delay LPS-induced preterm birth
- Decreases the mortality of prematurely delivered pups
- Regulates the local production and activity of prostaglandins

### 15d-PGJ2
- **Review**
- **Mouse**
- Delays LPS-induced preterm birth
- Suppresses NF-κB activity, cPLA2 expression, and c-Jun activity in uterine myometrium
- **Human**
  - Inhibits NF-κB activity of IL-1β-stimulated amnion epithelial and myometrial cells

### Others
- **Statin**
  - **Mouse**
  - Inhibits complement-activated uterine contraction
  - **Mouse**
  - Reduces IL-1β and IL-6 expression in the uterus and cervix and serum IL-1β and GM-CSF concentrations
  - Reduces IL-1β and IL-6 expression in the uterus, IL-6 and TNF-α in the cervix, and IL-1β, IL-2, IL-12p70, IL-13, TNF-α, GM-CSF, and IFN-γ concentrations in the serum and IL-6 in amniotic fluid

### Probiotics, *Lactobacillus rhamnosus* GR-1 supernatant
- **Mouse**
  - GR-1 decreases LPS-induced preterm birth
  - GR-1 SN decreases the LPS-induced IL-1β, IL-6, IL-12p40, TNF-α, CCL4, and CCL5 in maternal plasma; IL-6, IL-12p70, IL-17, IL-13, and TNF-α in myometrium; IL-6, IL-12p70, and IL-17 in placenta; and IL-6, TNF-α, CCL3, and CCL4 in amniotic fluid

### Folic acid
- **Mouse**
- Reduces LPS-induced preterm delivery and fetal death and IUGR

### Melatonin
- **Mouse**
- Suppresses LPS-induced NF-κB activation of mouse placenta in vivo and in vitro (JEG3)

### N-dimethylacetamide
- **Mouse**
- Decreases LPS-induced preterm birth and fetal death
- Reduces the LPS-induced rises in uterine PGE2, PGF2α, and COX-2

### Muscimol (GABA<sub>A</sub> agonist)
- **Mouse**
- Decreases LPS-induced inflammatory signaling and infiltration of inflammatory cells in placenta

### N-acetylcysteine (NAC)
- **Mouse**
- Decreases LPS-induced preterm birth
- Attenuates LPS-induced IL-6 expression of myometrium
- Attenuates LPS-induced cytokine expression of fetal brain and protects against brain injury
Preclinical: Suppressing Inflammation

• Lipid mediators:
  • 15d-PGJ2: Did delay LPS induced PTB and reduce inflammation in mice
  • 15-epi-lipoxin A4: Did not reduce LPS induced PTB but did reduce inflammation and pup mortality in mice

• Statins
  • Simvastatin: reduces PTB incidence in mice, and inhibits myometrial contractions, and exhibits key anti-inflammatory effects in ex vivo human tissue
    (Boyle et al FASEB J. 2019 Feb; 33(2): 2743–2758.)
  • Pravastatin: does not cross the placenta (Pippin)
Preclinical: Other strategies

• Antimicrobial peptides:
  • Cathelicidin-deficient (Camp⁻/⁻) mice are less susceptible to LPS induced PTB with decrease in IL-6.

Challenges

• Balancing pro and anti-inflammatory effects
• May need to treat infection and inflammation
• Mode of delivery of treatments

• Diagnosing preterm labour
• Detection of the fetus with inflammation

• Clinical trials are challenging
  • Feasibility of tocolysis trials
  • Need for long term follow up

• Recognition of risk for preterm birth – prevention
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