Infection and inflammation in preterm birth

Sarah A. Robertson, PhD
Inflammatory pathways in preterm labour

• Positive amniotic fluid cultures in large proportion of women with spontaneous preterm birth 40-50% (Goncalves et al. 2002; Kemp 2014; Witkin 2015)

• Bacteria also present in women with term delivery (Goncalves et al. 2002)

• Standard culture techniques detect only~10% bacteria

• Reproductive tract / placental microbiome is complex – many bacteria not identified, ‘probiotic’ versus ‘pathogens’ not well defined
inflammatory stimulus (PAMPs) 

↓

Toll-like receptors 

↓

↑ pro-inflammatory cytokines (IL-1β ➔ TNFα, IL-8, chemokines ➔ IL-6, IL-10) 

↓

↑ neutrophils 

↓

↑ macrophages, T cells 

↓

↑ PGs, MMPs 

myometrial contractility 

rupture of membranes 

cervical ripening
animal models to identify genes and pathways that are limiting in timing of labour
effect of IL6 deficiency on LPS-induced preterm labour

Robertson et al, Endocrinol 2006
effect of TLR4 and MyD88 deficiency on LPS-induced preterm labour

**TLR4**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>TLR4-/−</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0</strong></td>
<td>(14)</td>
<td>(10)</td>
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**MyD88**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>MyD88-/−</th>
</tr>
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<tbody>
<tr>
<td><strong>0</strong></td>
<td>(14)</td>
<td>(15)</td>
</tr>
</tbody>
</table>

Robertson, Dorian et al, accepted for publication
effect of TLR4 or MyD88 null mutation on LPS-induced *Il6* mRNA expression

Robertson, Dorian et al., unpublished
effect of TLR4 or MyD88 null mutation on LPS-induced *Il1a* expression

*Il1a* UTERUS

WT | TLR4-/- | MyD88-/-
--- | --- | ---
(11) | (10) | (10) | (10) | (9) | (8) | (12)

*Il1a* PLACENTA

WT | TLR4-/- | MyD88-/-
--- | --- | ---
(11) | (9) | (7) | (6) | (6) | (5)

Robertson, Dorian et al, unpublished
effect of novel TLR4 antagonist on LPS-induced preterm labour

Dorian, Hutchinson et al, unpublished
effect of novel TLR4 antagonist on preterm birth after in utero heat-killed E.coli challenge

Chin, Hutchinson et al, unpublished
effect of novel TLR4 antagonist on pup survival after in utero heat-killed E. coli challenge

Chin, Hutchinson et al, unpublished
inflammatory stimulus (PAMPs)

Toll-like receptors

↑ pro-inflammatory cytokines (IL-1β ➔ TNFα, IL-8, chemokines ➔ IL-6, IL-10)

↑ neutrophils

↑ macrophages, T cells

↑ PGs, MMPs

myometrial contractility rupture of membranes cervical ripening

NEW TARGETS?
current tocolytic agents
NON-INFECTIONOUS INSULTS

- Genetics
- Stress
- Smoking & Alcohol
- Environmental Pollutants
- Obesity
- Stretch / Ischaemic Injury

INFLAMMATION

BIRTH
inflammatory stimulus (DAMPs)

Toll-like receptors

↑ pro-inflammatory cytokines (IL-1β → TNFα, IL-8, chemokines → IL-6, IL-10)

↑ neutrophils

↑ macrophages, T cells

↑ PGs, MMPs

myometrial contractility rupture of membranes cervical ripening
effect of TLR4 null mutation on timing of term delivery and perinatal outcomes

Gestation length (days)

viable pups at weaning

Robertson, Dorian et al, accepted for publication
effect of TLR4 antagonist on timing of normal delivery

Robertson, Dorian et al, unpublished
effect of TLR4 null mutation on expression of inflammatory cytokine gene $Tnf$

$Tnf$ UTERUS

$Tnf$ DECIDUA

Wahid et al, unpublished
effect of TLR4 null mutation on expression of uterine activation gene *Ptgfr*

*Ptgfr* UTERUS

*Ptgfr* DECIDUA

Wahid et al, unpublished
effect of TLR4 null mutation on expression of uterine activation gene *Oxtr*

**Oxtr UTERUS**

**Oxtr DECIDUA**

Wahid et al, unpublished
Summary

- TLR4 binds PAMPs to induce infection-associated preterm labour
- TLR4 binds DAMPs to induce normal on-time labour
- TLR4 may bind DAMPS in non-infection associated preterm labour
- TLR4 is upstream of IL6, IL1a, TNF, IL10 in LPS-induced inflammatory cascade
- Expression of inflammatory cytokines and uterine activation genes is delayed in term TLR4 null mice
Working hypothesis

infection-associated inflammation (PAMPs) → TLR4 activation → PRETERM LABOUR

sterile inflammation (DAMPs) → TLR4 activation → TERM LABOUR

physiological inflammation (DAMPs)
Labour is an inflammatory cascade – so must involve elevation in inflammatory agents.
Labour is an inflammatory cascade – so must involve overcoming protection of anti-inflammatory agents.
effect of IL10 deficiency on LPS-induced preterm labour

Robertson et al, J Immunol 2006
Experimental protocol: macrophage depletion

*Cd11b-Dtr* or FVB

FVB

**mating**

- **day 0.5 pc**
- **day 16.5 pc**
- **24 - 48 h**

perinatal and postnatal parameters

- *red light videotaping to document time of labour*
- *pup viability and weight*
## CD11b+ cell depletion at day 16.5 pc

<table>
<thead>
<tr>
<th></th>
<th>Cd11b-dtr</th>
<th>WT</th>
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</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td><strong>gestation length</strong></td>
<td>$17.9 \pm 0.5^*$</td>
<td>$18.8 \pm 0.4$</td>
</tr>
<tr>
<td><strong>duration of labour</strong></td>
<td>$3.9 \pm 1.6^*$</td>
<td>$1.1 \pm 0.2$</td>
</tr>
<tr>
<td><strong>litter size</strong></td>
<td>$8.1 \pm 1.6$</td>
<td>$9.4 \pm 2.2$</td>
</tr>
<tr>
<td><strong>viability at birth</strong></td>
<td>$26% (17/65)^*$</td>
<td>$100% (65/65)$</td>
</tr>
<tr>
<td><strong>viability at 24 h</strong></td>
<td>$100% (17/17)$</td>
<td>$99% (64/65)$</td>
</tr>
<tr>
<td><strong>viability at 3 weeks</strong></td>
<td>$100% (17/17)$</td>
<td>$98% (46/47)$</td>
</tr>
<tr>
<td><strong>weight at 3 weeks</strong></td>
<td>$6.4 \pm 2.2^*$</td>
<td>$8.0 \pm 1.3$</td>
</tr>
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Macrophage depletion and cytokine gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change</th>
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<tbody>
<tr>
<td>Tlr1</td>
<td>-3.36</td>
</tr>
<tr>
<td>Mapk8</td>
<td>-3.09</td>
</tr>
<tr>
<td>Ccr2</td>
<td>-3.58</td>
</tr>
<tr>
<td>Cd14</td>
<td>+3.09</td>
</tr>
<tr>
<td>Myd88</td>
<td>+4.36</td>
</tr>
<tr>
<td>Tnfa</td>
<td>+3.91</td>
</tr>
<tr>
<td>Il1β</td>
<td>+3.03</td>
</tr>
<tr>
<td>Il6</td>
<td>+11.95</td>
</tr>
<tr>
<td>Il9</td>
<td>+5.27</td>
</tr>
<tr>
<td>Cxcl3</td>
<td>+26.17</td>
</tr>
<tr>
<td>Cxcl9</td>
<td>+3.6</td>
</tr>
<tr>
<td>Cxcl10</td>
<td>+11.31</td>
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</tbody>
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Cd11b-Dtr +DT / Cd11b-Dtr +PBS

Gomez-Lopez et al, unpublished
Experimental protocol: macrophage replacement

Cd11b-Dtr or FVB

FVB

mating

day 0.5 pc

A. DT

B. PBS

24 - 48 h

perinatal and postnatal parameters

red light videotape

BM-derived macrophages + CSF-1
day 16.5 pc
day 17.5 pc
Macrophage replacement

Gomez-Lopez, Chin et al, unpublished
Working hypothesis

M2 macrophage

uteroine quiescence

uteroine activation
→ preterm birth
Treg cells are disrupted in IL10 deficient mice, with poor stability and suppressive function.

(Robertson et al., 2007; Prins et al., unpublished)
Working hypothesis

Th1 / Th9 / Th17
M1 macrophages

Treg cells
M2 macrophages

pro-inflammatory

anti-inflammatory
Th1/Th9/Th17 M1

Treg cells M2

Th1/Th9/Th17 M1

Treg cells M2

PAMPs

DAMPs

RESISTANT

SENSITIVE
Preterm birth can result from inflammatory insult
Preterm birth might also result from insufficient anti-inflammatory protection
Implications for predictive tools in women?

- Human data suggests same pro-inflammatory versus anti-inflammatory balance may exist
- Human studies suggest change in balance of may be present from early or mid-gestation
- Total Foxp3+ Treg cells, highly suppressive CD25hi Tregs, and Treg suppressive function are all reduced in PBMC of PTL women in midgestation.

Omega-3 LCPUFA and preterm delivery

- 690 women, from 5500+ ORIP study* randomised to LCPUFA or control
- PBMC collected at 12 wks, 25 wks and 37 wks gestation and frozen in DMSO at -70°C for retrospective analysis
- Comprehensive immune mediator analysis:
  1. plasma cytokines, resolvins, PGE2, phospholipids
  2. FACS for Treg cell number, activation phenotype + M1/M2 macrophages, DCs and T cells
  3. Treg cell Foxp3 TDSR methylation status to determine suppressive competence
- determine relationship with LCPUFA and time of birth

*with M Makrides, R Gibson and colleagues
Flow cytometry in human PBMCs

A. Treg cells
- CD25
- CD127

B. Treg phenotype
- HLA-DR
- CD45RA

C. Dendritic Cells
- CD209
- CD11C

D. Macrophages
- CD14
- CD68
Foxp3 TDSR methylation analysis

- Analysis of methylation status of FOXP3 promoter TSDR region
- Shows near complete demethylation of the locus in pure CD25++ Treg cells (left) but partial demethylation of the less pure CD25+ T cell pool (right) from 3 donors
PAMPs
DAMPs

M2 / Treg cell

omega3 fats
tvitamin D
short chain FA
microbiome
miRNA
epigenetics
genetics
progesterone

anti-inflammatory

pro-inflammatory

PAMPS
DAMPs

security

stress
infection
obesity
microbiome
miRNA
epigenetics
innate

birth
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Camilla Dorian
Alison Care
Loretta Chin
Lachlan Moldenhauer
David Olson