# Cytokines and programming the pre-implantation embryo

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# Abstract

As the pre-implantation embryo traverses the female reproductive tract, it experiences fluctuations in the composition of the surrounding maternal environment, including the availability of nutrients, growth factors and cytokines. In particular, the cytokine milieu surrounding the early embryo is pivotal in programming optimal embryo development. The pre-implantation embryo is sensitive to a range of perturbations such as maternal diet or *in vitro* culture. These and other insults influencing the maternal environment including infection, stress and environmental toxins may in part act via impact on oviduct and uterine cytokine synthesis. However the effect of maternal perturbation to inflammation or infection, on the embryo and the role of cytokines in mediating this is not fully elucidated. The studies described in this thesis employed an *in vivo* mouse model of maternal systemic inflammation with the pro-inflammatory bacterial lipopolysaccharide (LPS), where a pro-inflammatory cytokine response was elicited on days 2.5 and 3.5 post coitum (pc), prior to implantation. This model was studied in wildtype C57Bl/6 (*Il10* $^{+/+}$) mice and mice with a null mutation in the *Il10* gene (*Il10* $^{-/-}$) were studied to investigate the effects of maternal deficiency in the anti-inflammatory cytokine IL-10 during LPS treatment.

We demonstrated that the altered cytokine signals resulting from a low level pro-inflammatory LPS challenge (0.5 μg/mouse) in the pre-implantation period elicit changes in the embryo developmental trajectory that in turn alter fetal growth and delay postnatal growth in the male progeny from LPS-treated mothers. As LPS did not directly impact development of the embryo at low and moderate doses, this result appears to reflect indirect effects of LPS mediated via the maternal tract. This is consistent with data from day 3.5 pc oviduct and uterus tissues which revealed increased mRNA expression of pro-inflammatory cytokines including *Il6*, *Tnfa* and *Il12b* following maternal LPS treatment.

Peri-conceptional low dose LPS treatment in *Il10* $^{+/+}$ and *Il10* $^{-/-}$ mice revealed that the number of viable fetuses and fetal weight were both significantly reduced after LPS treatment, particularly in the *Il10* $^{-/-}$ mice. Embryo transfer was then utilised to investigate the mechanism by which LPS acts on the embryo, where day 3.5 pc embryos from donors treated with 0.5 μg LPS or PBS on days 2.5 and 3.5pc were transferred into day 2.5 pc pseudopregnant Swiss female recipients. The effect of maternal LPS treatment on fetal and placental development was seen to be maintained even after embryo transfer, suggesting that any effects of altered cytokine expression in embryos are exerted during cleavage stages before embryo recovery from donors.

In addition, postnatal investigation of male and female progeny derived from control PBS and LPS-treated *Il10* $^{+/+}$ and *Il10* $^{-/-}$ females from birth until 19 weeks of age showed that maternal LPS treatment constrains postnatal growth in male progeny regardless of maternal *Il10* genotype, compared to male progeny from PBS-treated mothers. While the adult male progeny from LPS-treated *Il10* $^{+/+}$ and *Il10* $^{-/-}$ mothers did not display changes in fat mass compared to their PBS-treated control counterparts, the combination of maternal LPS treatment and maternal IL-10 deficiency resulted in greater fat mass accumulation in the adult male progeny from LPS-treated *Il10* $^{-/-}$ mothers compared to adult male progeny from LPS-treated *Il10* $^{+/+}$ mothers.

In addition, we investigated the effects of maternal systemic inflammation during the pre-implantation period on the response to LPS challenge during adulthood. Male progeny from LPS-treated *Il10* $^{-/-}$ mothers had a dampened response in LIF cytokine following a 100μg/kg LPS challenge at 18 weeks of age.

This study implies a role for cytokines as mediators of programming the embryo during the pre-implantation period, with altered responses in the event of maternal systemic inflammation impacting on later fetal and postnatal development. The anti-inflammatory cytokine IL-10 acts to protect the embryo from the adverse programming effects of exposure to LPS during the pre-implantation period, with absence of IL-10 resulting in altered postnatal phenotype and particularly fat mass accumulation in the male progeny during adulthood. It appears likely that the absence of IL-10 in the maternal environment delays the clearance of adverse pro-inflammatory cytokines induced during an inflammatory challenge, resulting in prolonged exposure of the embryo to circulating pro-inflammatory cytokines in the maternal tract, supporting a cytokine-mediated mechanism. These studies provide additional evidence for a role of cytokines in embryo sensing of environmental conditions, and indicate that IL-10 is a key regulator of this communication pathway.

# Declaration

This work contains no material which has been accepted for the award of any other degree or diploma at any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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# Publications arising from this thesis

1. Robertson SA, Chin PY, Glynn DJ, Thompson JG. *Peri-conceptual cytokines – Setting the trajectory for embryo implantation, pregnancy and beyond.* American Journal of Reproductive Immunology, 2011, 66, 2-10.
2. Chin PY, Thompson JG, Robertson SA. (In preparation). *IL-10 regulates oviduct cytokine expression and embryo sensitivity to maternal LPS challenge during early pregnancy.*
3. Chin PY, Thompson JG, Robertson SA. (In preparation). *Maternal IL-10 deficiency elevates sensitivity to the programming effects of a low dose LPS insult in the pre-implantation period.*

# Abstracts arising from this thesis

2012

**Chin PY**, Thompson JG, Robertson SA. *Low dose LPS insult during pre-implantation period programs fetal development.* Oral presentation at the Society for Reproductive Biology. 44th Annual Conference, Gold Coast Australia. August 2012.

2011

**Chin PY**, Thompson JG, Robertson SA. *Maternal Interleukin-10 Deficiency Increases Sensitivity to Adverse Programming Effects of a Low Dose LPS Insult in the Pre-Implantation Period.* Poster presentation at the ANZSCDB meeting, Adelaide Australia. November 2011.

**Chin PY**, Thompson JG, Robertson SA. *Maternal Interleukin-10 Deficiency Increases Sensitivity to Adverse Programming Effects of a Low Dose LPS Insult in the Pre-Implantation Period.* Oral presentation at the Society for the Study of Reproduction. 44th Annual Meeting, Portland Oregon USA, September 2011.

2010

**Chin PY**, Thompson JG, Robertson SA. *A modest inflammatory insult in the pre-implantation period alters oviduct cytokine expression and programs fetal development.* Oral presentation at the Society for Reproductive Biology. 42nd Annual Conference, Sydney Australia. August 2010.

**Chin PY**, Thompson JG, Robertson SA. *Programming embryo development with a pre-implantation inflammatory insult.* Poster presentation at the International Congress of Reproductive Biology, Cairns Australia. August 2010.

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# Abbreviations

| | |
|---|---|
| ANOVA | analysis of variance |
| ART | assisted reproductive technology |
| β-actin | beta actin |
| βc | beta common |
| BMI | body mass index |
| BMK1 | big MAPK1 |
| BMP | bone morphogenetic protein |
| BSA | bovine serum albumin |
| CD4+ | cluster of differentiation 4 positive T cell |
| CD8+ | cluster of differentiation 8 positive T cell |
| DAMPs | damage-associated molecular patterns |
| DEXA | dual-energy X-ray absorptiometry |
| DNA | deoxyribonucleic acid |
| DNMT | DNA methyl transferases |
| DOHaD | developmental origins of health and disease |
| *E.coli* | *Escherichia coli* |
| EGF | epidermal growth factor |
| ErbB1 | epidermal growth factor receptor |
| ErbB4 | v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian) |
| ERK | extra-cellular-signal-regulated kinase |
| F1 | first generation |
| F2 | second generation |
| FAS | apoptosis stimulating fragment |
| FGF | fibroblast growth factor |
| FOAD | developmental origins of adult diseases |
| G-CSF | granulocyte colony-stimulating factor |
| GD | gestational day |
| GH | growth hormone |
| GM-CSF | granulocyte-macrophage colony-stimulating factor |
| GM-Rα | GM-CSF specific alpha subunit |

| | |
|---|---|
| HAT | histone acetyltransferase |
| $H_2O_2$ | hydrogen peroxide |
| *Hegf1* | human epidermal growth factor 1 |
| HMGB1 | high-mobility group protein B1 |
| HSP | heat shock protein |
| i.p. | intra-peritoneal |
| ICM | inner cell mass |
| ICSI | intracytoplasmic sperm injection |
| IFNγ | interferon gamma |
| IGFBP1 | insulin-like growth factor binding protein 1 |
| IGFBP2 | insulin-like growth factor binding protein 2 |
| IGFBP3 | insulin-like growth factor binding protein 3 |
| IGF-I | insulin-like growth factor 1 |
| IGF-II | insulin-like growth factor 2 |
| IGF-IR | insulin-like growth factor 1 receptor |
| IgG1 | immunoglobulin G, subclass 1 |
| IGR-IIR | insulin-like growth factor 2 receptor |
| IL-10 | interleukin 10 |
| *Il10* $^{-/-}$ | interleukin 10 deficient |
| IL-10R | interleukin 10 receptor |
| IL-12α | interleukin 12, alpha |
| IL-12β | interleukin 12, beta |
| IL-15 | interleukin 15 |
| *Il15* $^{-/-}$ | interleukin 15 deficient |
| IL-1α | interleukin 1, alpha |
| IL-1β | interleukin 1, beta |
| IL-6 | interleukin 6 |
| IL-6Rα | interleukin 6 receptor alpha |
| IP-10 | interferon gamma-induced protein 10 |
| IUFD | intrauterine fetal death |
| IUGR | intrauterine growth restriction |
| IVF | *in vitro* fertilisation |

| | |
|---|---|
| KC | keratinocyte chemo-attractant |
| kDa | kilo Dalton |
| LE | luminal epithelium |
| LIF | leukocyte inhibitory factor |
| *Lif*$^{-/-}$ | leukocyte inhibitory factor deficient |
| LPS | lipopolysaccharide |
| MAPK | mitogen-activated protein kinase |
| MCP-1 | monocyte chemotactic protein-1 |
| M-CSF | macrophage colony-stimulating factor |
| MIP-1$\alpha$ | macrophage inflammatory protein 1 alpha |
| MIP-1$\beta$ | macrophage inflammatory protein 1 beta |
| MS | multiple sclerosis |
| mRNA | messenger RNA |
| miRNA | microRNA |
| mtDNA | mitochondrial DNA |
| MyD88 | myeloid differentiation primary response 88 |
| NFKB | nuclear factor kappa-light-chain-enhancer of activated B cells |
| NO | nitric oxide |
| $O_2^-$ | superoxide |
| PBS | phosphate buffered saline |
| pc | post coitum |
| PCR | polymerase chain reaction |
| PI3K | phosphoinositide 3-kinase |
| piRNA | Piwi-interacting RNA |
| qPCR | quantitative polymerase chain reaction |
| RA | rheumatoid arthritis |
| RANTES | regulated on activation, normal T cell expressed and secreted |
| rmIL-10 | recombinant mouse interleukin 10 |
| ROI | region of interest |
| ROS | reactive oxygen species |
| SAPK | stress-activated protein kinase |
| SEM | standard error of the mean |

| | |
|---|---|
| siRNA | small interfering RNA |
| STI | sexually transmitted infection |
| TE | trophectoderm |
| TGFβ | transforming growth factor beta |
| Th1 | type 1 T helper |
| Th17 | type 17 T helper |
| Th2 | type 2 T helper |
| TIRAP | toll-like receptor adapter protein |
| TLR | toll-like receptor |
| TLR2 | toll-like receptor 2 |
| TLR4 | toll-like receptor 4 |
| TNFα | tumour necrosis factor alpha |
| TNFαRc | tumour necrosis factor alpha receptor |
| Tollip | toll interacting protein |
| TRAIL | TNF-related apoptosis-inducing ligand |
| $T_{reg}$ | regulatory T cell |
| VAS | vasectomised |
| X-ray | X-radiation |