

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-conceptual 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

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Table of contents

Abstract	ii
Declaration	iv
Acknowledgements	v
Publications arising from this thesis	vi
Abstracts arising from this thesis	vii
Table of contents	viii
List of Figures	xiv
List of Tables	xvi
Abbreviations	xviii
Chapter 1	1
1.1. INTRODUCTION.....	2
1.2. MOUSE PRE-IMPLANTATION EMBRYO DEVELOPMENT.....	3
1.3. CYTOKINE RECEPTOR SIGNALLING PATHWAYS IN PRE-IMPLANTATION EMBRYO.....	4
1.3.1. Mitogen-activated protein kinase (MAPK) pathway	4
1.3.2. Janus kinase/Signal transducer and activator of transcription (JAK/STAT) pathway	5
1.3.3. Phosphatidylinositol-3 kinase (PI3K)/Protein Kinase B (AKT) pathway.....	7
1.4. CYTOKINES.....	7
1.4.1. Anti-inflammatory cytokines and pro-inflammatory cytokines.....	8
1.4.2. Cytokines promoting pre-implantation embryo development.....	9
1.4.2.1. Granulocyte-macrophage colony-stimulating factor (GM-CSF).....	9
1.4.2.2. Leukaemia inhibitory factor (LIF).....	10
1.4.2.3. Insulin-like growth factor I and II (IGF-I and IGF-II)	11
<i>Chin</i>	viii

1.4.2.4.	Heparin-binding EGF-like growth factor (HB-EGF).....	12
1.4.3.	Cytokines inhibiting pre-implantation embryo development.....	13
1.4.3.1.	Tumour necrosis factor alpha (TNF α).....	13
1.4.3.2.	Interferon gamma (IFN γ).....	14
1.4.4.	Other important cytokines	15
1.4.4.1.	Interleukin-10 (IL-10).....	15
1.4.4.2.	Interleukin-6 (IL-6).....	16
1.4.5.	Cytokines as immune modulators in protecting pregnancy	17
1.5.	CYTOKINE ACTION IN CYCLING AND PREGNANT UTERUS.....	17
1.6.	ENVIRONMENTAL REGULATION OF EMBRYO DEVELOPMENT.....	18
1.6.1.	Factors regulating cytokine production.....	19
1.6.1.1.	Assisted Reproductive Technology (ART).....	20
1.6.1.2.	Infection and inflammation.....	20
1.6.1.3.	Diet.....	21
1.6.1.4.	Stress	22
1.6.1.5.	Other factors.....	23
1.7.	LIPOPOLYSACCHARIDE (LPS).....	23
1.7.1.	LPS effect on cytokines.....	24
1.7.2.	Inflammatory response to LPS in pregnancy.....	25
1.8.	PREGNANCY COMPLICATIONS.....	26
1.9.	EARLY ORIGINS OF ADULT DISEASE AND FETAL PROGRAMMING.....	26
1.9.1.	Mechanisms of programming	27
1.9.1.1.	Epigenetic changes	27
1.9.1.2.	Mitochondria.....	28
1.9.1.3.	Reactive oxygen species.....	29
1.9.2.	Consequences of programming	29

1.9.3.	The role of cytokines in embryo programming.....	30
1.10.	SUMMARY	31
1.11.	HYPOTHESES.....	32
1.12.	AIMS	32

Chapter 2 34

2.1.	ANIMALS AND SURGERIES	35
2.1.1.	Mice.....	35
2.1.2.	General procedures.....	36
2.1.3.	Matings.....	36
2.1.4.	Lipopolysaccharide (LPS) treatment	36
2.1.5.	Vasectomies and Seminal Vesicle Removal	37
2.1.6.	Embryo transfer.....	37
2.2.	IN VITRO EMBRYO CULTURE	38
2.2.1.	Superovulation	38
2.2.2.	Growth media	38
2.2.3.	Embryo collection and culture	38
2.2.4.	Differential staining.....	39
2.2.5.	Mouse blastocyst collection, differential staining and tissue freezing	39
2.2.6.	Assessment of day 17.5 pc pregnancy outcomes	39
2.3.	OFFSPRING ASSESSMENT	40
2.3.1.	Offspring cohort.....	40
2.3.2.	Dual-energy X-ray absorptiometry (DEXA).....	40
2.3.3.	Offspring inflammatory response to LPS	40
2.3.4.	Full body post mortem.....	41
2.4.	QUANTITATIVE REAL TIME – POLYMERASE CHAIN REACTION	41
2.4.1.	RNA Isolation	41

2.4.2.	Reverse Transcription, cDNA generation and qRT-PCR.....	42
2.4.3.	Oligonucleotide primer design	42
2.5.	ASSAYS.....	44
2.5.1.	Luminex.....	44
2.6.	IMMUNOHISTOCHEMISTRY	45
2.6.1.	Tissue collection, paraffin embedding and sectioning	45
2.6.2.	Tissue staining	45
2.6.3.	Image analysis and quantification of fat cell size.....	45
2.7.	STATISTICAL ANALYSIS	46
Chapter 3	47
3.1.	INTRODUCTION.....	48
3.2.	EFFECT OF MATERNAL INFLAMMATORY RESPONSE TO LPS DURING THE PRE- IMPLANTATION PERIOD ON <i>IN VIVO</i> EMBRYO DEVELOPMENT	50
3.3.	EFFECT OF LPS IN CULTURE MEDIA ON PRE-IMPLANTATION EMBRYO DEVELOPMENT <i>IN VITRO</i>	53
3.4.	EFFECT OF LPS AND/OR IL-10 CYTOKINE ADMINISTRATION TO CULTURE MEDIA ON PRE-IMPLANTATION EMBRYO DEVELOPMENT <i>IN VITRO</i>	56
3.5.	EFFECT OF ADMINISTRATION OF LPS DURING THE PRE-IMPLANTATION PERIOD ON FETAL AND PLACENTAL DEVELOPMENT.....	58
3.6.	DISCUSSION	62
Chapter 4	66
4.1.	INTRODUCTION.....	67
4.2.	EFFECT OF LPS ADMINISTRATION DURING THE PRE-IMPLANTATION PERIOD ON CYTOKINE ENVIRONMENT IN THE REPRODUCTIVE TRACT.....	70
4.2.1.	Cytokine gene expression in day 3.5 pc oviduct tissue	71
4.2.2.	Cytokine gene expression in day 3.5 pc uterus tissue.....	75

4.3.	EFFECT OF TNF α ANTAGONIST ETANERCEPT (Enbrel®) ON FETAL AND PLACENTAL DEVELOPMENT FOLLOWING LPS ADMINISTRATION DURING THE PRE-IMPLANTATION PERIOD	78
4.4.	EFFECT OF LPS ADMINISTRATION DURING THE PRE-IMPLANTATION PERIOD ON FETAL AND PLACENTAL DEVELOPMENT FOLLOWING EMBRYO TRANSFER.....	81
4.5.	EFFECTS OF MATERNAL LPS TREATMENT DURING PRE-IMPLANTATION PERIOD ON FETAL AND PLACENTAL DEVELOPMENT IN NATURAL MATING AND EMBRYO TRANSFER.....	84
4.6.	DISCUSSION.....	86
Chapter 5	91
5.1.	INTRODUCTION.....	92
5.2.	EFFECT OF MATERNAL SYSTEMIC INFLAMMATION DURING PRE-IMPLANTATION PERIOD ON POSTNATAL GROWTH AND DEVELOPMENT	95
5.3.	EFFECT OF MATERNAL LPS TREATMENT DURING PRE-IMPLANTATION ON BONE AND BODY COMPOSITION OF PROGENY.....	102
5.3.1.	Bone composition.....	103
5.3.2.	Body composition.....	105
5.4.	EFFECT OF MATERNAL SYSTEMIC INFLAMMATION DURING PRE-IMPLANTATION PERIOD ON BODY COMPOSITION OF PROGENY.....	109
5.5.	VALIDATION OF THE ACCURACY AND PRECISION OF THE DEXA APPROACH IN BODY FAT MEASUREMENT.....	116
5.6.	EFFECT OF MATERNAL SYSTEMIC INFLAMMATION DURING PRE-IMPLANTATION PERIOD ON FAT CELL SIZE OF MALE AND FEMALE PROGENY OF <i>Il10</i> ^{+/+} AND <i>Il10</i> ^{-/-} MOTHERS	119
5.7.	EFFECT OF MATERNAL SYSTEMIC INFLAMMATION DURING PRE-IMPLANTATION PERIOD ON PROGENY IMMUNE RESPONSE TO LPS CHALLENGE.....	122
5.8.	DISCUSSION.....	127
Chapter 6	132

6.1.	DISCUSSION AND CONCLUSION.....	133
	Chapter 7	144
	Chapter 8	148
8.1.	REFERENCES.....	149

List of Figures

Figure 1.1	Development of pre-implantation embryo in mice from embryonic day 0 (E0) to embryonic day 5 (E5) (NIH, 2001).	3
Figure 1.2	Schematic illustration of the proposed role of cytokines as mediators of environmental insult during the pre-implantation period.....	33
Figure 2.1	Gel image of PCR genotyping.	35
Figure 3.1	The effect of low dose LPS on fetal and placental development in <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} females at day 17.5 pc.....	61
Figure 4.1	Cytokine gene expression profile in day 3.5 pc oviduct tissue following maternal LPS treatment during the pre-implantation period.....	73
Figure 4.2	Cytokine gene expression profile in day 3.5 pc uterus tissue following maternal LPS treatment during the pre-implantation period.....	76
Figure 4.3	The effect of maternal treatment with LPS and etanercept (Enbrel) during the pre-implantation period on day 17.5 pc fetal and placental development.	80
Figure 4.4	The effect of LPS treatment in donor females during the pre-implantation period on fetal and placental development at day 17.5 pc following embryo transfer.	83
Figure 5.1	Effect of low dose LPS at days 2.5 and 3.5 pc on pregnancy parameters at term.	97
Figure 5.2	Growth trajectory of male progeny of <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} mothers from birth till 19 weeks..	100
Figure 5.3	Growth trajectory of female progeny of <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} mothers from birth till 19 weeks.	101
Figure 5.4	Example of DEXA analysis output of an individual mouse.....	102
Figure 5.5	Bone composition of adult male and female progeny of <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} mothers at week 19.	104
Figure 5.6	Body composition of adult male and female progeny of <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} at week 19.	107
Figure 5.7	The relation between excised and DEXA measured fat tissue.....	118
Figure 5.8	Effect of maternal systemic inflammation during pre-implantation period on fat cell size of male and female progeny of <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} mothers.....	120

Figure 5.9 Serum cytokine concentrations (pg/ml) of adult male progeny from <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} females 3.5 hours after LPS challenge.....	124
Figure 5.10 Serum cytokine concentrations (pg/ml) of adult female progeny from <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} females 3.5 hours after LPS challenge.....	125
Figure 5.11 Serum cytokine concentrations (pg/ml) of adult male and female progeny from <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} females 3.5 hrs after LPS challenge.....	126
Figure 6.1 Schematic illustration of mechanism of maternal systemic inflammation during the pre-implantation period.....	141

List of Tables

Table 1.1	Cytokines and JAK/STAT pathways	6
Table 2.1	PCR primers for RT-PCR analysis.....	43
Table 3.1	The effect of maternal LPS treatment and dose on incidence of viable embryos and number of blastocyst stage embryos flushed per female.....	52
Table 3.2	The effect of maternal LPS treatment and dose on cell numbers and allocation to the inner cell mass (ICM) and trophectoderm (TE) in blastocyst stage embryos.	52
Table 3.3	The effect of LPS concentration in culture media on embryo development <i>in vitro</i>	55
Table 3.4	The effect of LPS concentration in culture media on blastocyst stage cell numbers, allocation to ICM and TE in blastocyst stage embryos <i>in vitro</i> .*	55
Table 3.5	The effect of LPS and/or IL-10 cytokine addition to culture media on embryo development <i>in vitro</i>	57
Table 3.6	The effect of LPS and/or IL-10 cytokine addition to culture media on cell numbers, allocation to ICM and TE in blastocyst stage embryos <i>in vitro</i>	57
Table 4.1	Effect of donor LPS treatment on pregnancy parameters on day 17.5 pc following embryo transfer.....	82
Table 4.2	Effect maternal LPS treatment on fetal and placental development in natural mating and embryo transfer.....	85
Table 5.1	Effect of maternal LPS treatment during the pre-implantation period on progeny sex ratio.	98
Table 5.2	Effect of maternal LPS treatment during pre-implantation period and <i>I110</i> genotype on body composition (absolute weight) of male adult progeny.....	111
Table 5.3	Effect of maternal LPS treatment during pre-implantation period and <i>I110</i> genotype on body composition (relative weight) of male adult progeny.....	112
Table 5.4	Effect of maternal LPS treatment during pre-implantation period and <i>I110</i> genotype on body composition (absolute weight) of female adult progeny.....	114
Table 5.5	Effect of maternal LPS treatment during pre-implantation period and <i>I110</i> genotype on body composition (relative weight) of female adult progeny.....	115

Table 5.6	Correlations and differences between DEXA versus excised data in progeny from <i>Il10</i> ^{+/+} and <i>Il10</i> ^{-/-} mothers.	117
Table 7.1	Composition of G1.2 and G2.2 embryo culture media	145
Table 7.2	Formulation of MOPS buffer for embryo handling.....	146
Table 7.3	Concentration of amino acids used in G1, G2 and MOPS medium	147

Abbreviations

ANOVA	analysis of variance
ART	assisted reproductive technology
β -actin	beta actin
β c	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
<i>E.coli</i>	<i>Escherichia coli</i>
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-R α	GM-CSF specific alpha subunit
<i>Chin</i>	

HAT	histone acetyltransferase
H ₂ O ₂	hydrogen peroxide
<i>Hegf1</i>	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
IFN γ	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
IIGR-IIR	insulin-like growth factor 2 receptor
IL-10	interleukin 10
<i>Il10</i> ^{-/-}	interleukin 10 deficient
IL-10R	interleukin 10 receptor
IL-12 α	interleukin 12, alpha
IL-12 β	interleukin 12, beta
IL-15	interleukin 15
<i>Il15</i> ^{-/-}	interleukin 15 deficient
IL-1 α	interleukin 1, alpha
IL-1 β	interleukin 1, beta
IL-6	interleukin 6
IL-6R α	interleukin 6 receptor alpha
IP-10	interferon gamma-induced protein 10
IUFD	intrauterine fetal death
IUGR	intrauterine growth restriction
IVF	<i>in vitro</i> fertilisation

KC	keratinocyte chemo-attractant
kDa	kilo Dalton
LE	luminal epithelium
LIF	leukocyte inhibitory factor
<i>Lif</i> ^{-/-}	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 α	macrophage inflammatory protein 1 alpha
MIP-1 β	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
TGF β	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
TNF α	tumour necrosis factor alpha
TNF α Rc	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