Naomi Scott B. Biomed Sci (Hon)

EFFECT OF MATERNAL ASTHMA DURING PREGNANCY ON ASPECTS OF PLACENTAL IMMUNE FUNCTION

A thesis submitted for the degree of Doctor of Philosophy

The University of Newcastle, Australia

May 2011

DECLARATION

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution This thesis contains no material which has been accepted for the award of any other degree or diploma in 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. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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Naomi M Scott

May 2011

ACKNOWLEDGEMENTS

Thank you to my supervisors Vicki Clifton and Roger Smith for your encouragement and support during my PhD. Thank you to all the staff and students of Mothers and Babies research centre for providing me with the type of research environment that stimulates and develops. Thank you to Vicki Clifton's research group, Annette, Pen, Michael, Jorge, Olivia, John, for your help and encouragement as well as some fun, and particularly Nicki, for statistics support, and taking time to read my drafts. I also particularly need to thank Vicki for her investment in me, and encouraging me to do my very best.

Thank you also to the women who volunteered to participate in the study, as well as the research midwives who did the recruiting and the team at the delivery suite.
Thank you to my family for encouraging me through all my years of schooling and university, and their endless support. My friends, I thank for understanding my inability to commit to anything 'just in case I get a placenta', and their encouragement. Thank you to Mike, my boyfriend, for your patience, belief in me, and your skills at document formatting.

PUBLICATION LIST

JOURNAL PUBLICATIONS

Scott N.M., Hodyl N.A., Murphy V.E., Osei-Kumah A., Wyper H., Hodgson D.M., Smith R., Clifton V.L.., *Placental cytokine expression covaries with maternal asthma severity and fetal sex.* J Immunol, 2009. **182**(3): p. 1411-20

Scott NM, Hodyl, NA, Osei-Kumah A, Stark MJ, Smith R, Clifton, VL, *The presence of maternal asthma during pregnancy suppresses the placental pro-inflammatory response to an immune challenge in vitro.* Placenta, 2011 Jun;32(6):454-61.

ABSTRACT PUBLICATIONS

Hodyl NA, **Scott N,** Osei-Kumah A, Path N, Stark MJ, Clifton VL. *Glucocorticoid receptor chaperone molecules and sex-specific sensitivity to cortisol in the human placenta*. 52nd Annual Scientific Meeting of the Endocrine Society of Australia, 23-26 August 2009.

Hodyl NA, Wyper H, **Scott N**, Osei-Kumah A, Murphy VE, Smith R, Clifton VL. *Relationship between placental glucocorticoid receptor expression and fetal growth in pregnancies complicated by asthma*. 51st Annual Scientific Meeting of the Endocrine Society of Australia, 25th-28th August 2008

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Clifton VL, Smith R, **Scott N**, Osei-Kumah A, Wyper H. *Alterations in the placental glucocorticoid receptor gene and protein function are associated with sex specific changes in neonatal birthweight*. Australian Health and Medical Research Congress 2006.

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Scott NM, Wyper HJ, Osei-Kumah A, Smith R, Murphy VE and Clifton VL *Sex specific differences in placental cytokine expression and their relationship to fetal*

glucocorticoid exposure. Annual Meeting of Society of Gynecologic Investigation, Toronto Canada, March 22-25 2006.

Wyper HJ, **Scott NM**, Osei-Kumah A, Smith R, Johnson RF, Clifton VL. *Placental glucocorticoid receptor expression in pregnancies complicated by asthma.* Endocrine Journal 2005 52(Supp):115.

Scott NM, Wyper HJ, Osei-Kumah A, Smith R, Hodgson D, Clifton VL. *Sex differences in placental cytokine expression and their relationship to fetal cortisol.* Endocrine Journal 2005 52(Supp):113

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11β HSD 2	11beta-hydroxysteroid dehydrogenase
CCL18	chemokine(C-C motif) ligand 18
CD	cluster of differentiation
cDNA	complimentary DNA
CISH	chromogenic in-situ hybridisation
CMRL-1066	media developed by Connaught Medical Research Laboratories
CSF	colony stimulating factor
Ct	*chlamydia trachomatis*
CXCL5	chemokine (C-X-C motif) ligand 5
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide-triphosphate
dsDNA	double stranded DNA
EGF	epidermal growth factor
ELISA	enzyme linked immunosorbent assay
ERK	extracellular signal-regulated kinas
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte-macrophage colony stimulating factor
GR	glucocorticoid receptor

hCG	human chorionic gonadotropin
HELLP	haemolysis, elevated liver enzyme and low platelet
HLA	human leukocyte antigens
HMGB1	high-mobility group box 1 also known as amphoterin
HRP	horseradish peroxidase
HSP	Heat shock protein
IDO	idolemine 2,3-dioxygenase
IFN	interferon
IKK	IκB kinase
IL	interleukin
IRAK	interleukin-1 receptor-associated kinase
IRF	interferon response factor
ISRE	interferon-sensitive response element
IUGR	intrauterine growth restriction
JNK	cJun N-terminal kinase
LBP	lipopolysaccharide binding protein
LDH	lacate dehydrogenase
LIF	leukemia inhibitor factor
LPS	lipopolysaccharide
Mal	MyD88 adapter like
MAPK	mitogen activated protein kinase
MHC	major histocompatibility complex
MKK	mitogen activated protein kinase kinase
mRNA	messenger ribonucleic acid

MyD88	myeloid differentiation primary response gene (88)
n	number of samples
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PBMC	peripheral blood mononuclear cell
PEFR	peak expiratory flow rate
pGR211	GR phosphorylated at serine at location 211
PVDF	polyvinylidene fluoride
RANTES	regulated on activation, normal T expressed and secreted
RBC	red blood cells
RIP	receptor interacting protein
ROS	reactive oxygen species
RT-PCR	reverse transcriptase-polymerase chain reaction
SEM	standard error of the mean
SGA	small for gestational age
SIGIRR	single immunoglobulin interleukin-1 receptor-related molecule
SLE	systemic lupus erythematosus
TAB	TAK-1 binding protein
TAK	transforming growth factor-β-activated kinase
TBK	TANK-binding kinase
TGF	transforming growth factor
Th	T lymphocyte helper
TLR	toll-like receptor
TNF-α	tumour necrosis factor alpha
TNFR	tumour necrosis factor receptor

TRAF6	tumour necrosis factor receptor-associated factor
TRAM	TRIF-related adapter molecule
TRIF	TIR-domain-containing adaptor-inducing interferon-β
VC	vital capacity
VEGF	vascular endothelial growth factor
VUE	villities of unknown aetiology
WHO	world health organisation

ABSTRACT

In the presence of maternal asthma, reduced placental blood flow, decreased cortisol metabolism, and reductions in fetal growth have been reported in response to maternal asthma and asthma exacerbations. The mechanisms that contribute to adverse outcomes for the neonate in pregnancies complicated by asthma may be mediated via changes in aspects of placental immune function.

The influence of maternal asthma and it's severity, maternal cigarette use, and fetal sex on placental cytokine mRNA expression was examined in a prospective cohort study of pregnant women with and without asthma. Placental expression of TNF-α, IL-1β, IL-6, IL-8 and IL-5 mRNA were all increased significantly in placenta of female fetuses whose mothers had mild asthma, but no changes were observed in placenta of male fetuses. The pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were negatively correlated with female cord blood cortisol, but there were no such correlations in placenta from males. Multivariate analysis indicated the strongest predictor of both cytokine mRNA expression in the placenta and birth weight was fetal cortisol, but only in females. Placental cytokine mRNA levels were not significantly altered by inhaled glucocorticoid use, moderate-severe asthma, or male sex. These data suggested that placental basal cytokine mRNA expression is sex specifically regulated in pregnancies complicated by asthma, and interestingly these changes are more prevalent in mild rather than severe asthma.

The placental cytokine response *in vitro* was examined in an additional prospective cohort study of women with asthma, and controls. Placentae were collected immediately following delivery, and placental explants were exposed to LPS immune stimulation, in the presence and absence of glucocorticoids *in vitro*. Cytokines, glucocorticoid receptor α (GR α) and p38 MAPKinase protein were measured. Placentae from pregnancies complicated by maternal asthma had a more rapid response to LPS than control placenta, regardless of fetal sex, with early production of the cytokines IL-6, IL-8 and IL-10, but did not sustain the enhanced cytokine response by 24 h relative to a control population. Cortisol inhibition of placental cytokine production was dependent on timing of exposure, fetal sex, and presence and absence of asthma. GRα and p38 MAPK protein expression did not appear to contribute to differences in response to endotoxin or cortisol. This data demonstrates that the placentae from pregnancies complicated by maternal asthma differ from control placentae in relation to the timing of the response to LPS stimulation, and the regulation of the response by cortisol.

This is the first study to examine the impact of maternal asthma during pregnancy on placental inflammatory pathways. The data has identified that asthma during pregnancy alters pro-inflammatory pathways in a sex specific manner. Female placentae readily control pro-inflammatory cytokine expression via the glucocorticoid pathway while male placentae appear glucocorticoid resistant. This data suggests that during a pro-inflammatory event such as an asthma exacerbation the female fetus may protect herself from the effects of this stress via cortisol. Males babies may be more at risk of a poor outcome due to an inability to regulate

inflammation via cortisol. Placentae from asthmatic pregnancies had an enhanced early cytokine response to LPS stimulation, suggesting previous exposure to inflammatory disease alters responsiveness of cytokines in the placenta to an LPS stimulation.