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.

Signed......................................

Naomi M Scott

May 2011
ACKNOWLEDGEMENTS

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**JOURNAL PUBLICATIONS**


**ABSTRACT PUBLICATIONS**


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


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LIST OF ABBREVIATIONS

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 kinases
FEV₁       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
<table>
<thead>
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<tr>
<td>hCG</td>
<td>human chorionic gonadotropin</td>
</tr>
<tr>
<td>HELLP</td>
<td>haemolysis, elevated liver enzyme and low platelet</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigens</td>
</tr>
<tr>
<td>HMGB1</td>
<td>high-mobility group box 1 also known as amphoterin</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>IDO</td>
<td>idolemine 2,3-dioxygenase</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IKK</td>
<td>IκB kinase</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IRAK</td>
<td>interleukin-1 receptor-associated kinase</td>
</tr>
<tr>
<td>IRF</td>
<td>interferon response factor</td>
</tr>
<tr>
<td>ISRE</td>
<td>interferon-sensitive response element</td>
</tr>
<tr>
<td>IUGR</td>
<td>intrauterine growth restriction</td>
</tr>
<tr>
<td>JNK</td>
<td>cJun N-terminal kinase</td>
</tr>
<tr>
<td>LBP</td>
<td>lipopolysaccharide binding protein</td>
</tr>
<tr>
<td>LDH</td>
<td>lacate dehydrogenase</td>
</tr>
<tr>
<td>LIF</td>
<td>leukemia inhibitor factor</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>Mal</td>
<td>MyD88 adapter like</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen activated protein kinase</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MKK</td>
<td>mitogen activated protein kinase kinase</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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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
<table>
<thead>
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<tr>
<td>TRAF6</td>
<td>tumour necrosis factor receptor-associated factor</td>
</tr>
<tr>
<td>TRAM</td>
<td>TRIF-related adapter molecule</td>
</tr>
<tr>
<td>TRIF</td>
<td>TIR-domain-containing adaptor-inducing interferon-β</td>
</tr>
<tr>
<td>VC</td>
<td>vital capacity</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
<tr>
<td>VUE</td>
<td>villities of unknown aetiology</td>
</tr>
<tr>
<td>WHO</td>
<td>world health organisation</td>
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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.