HUMAN ENDOGENOUS RETROVIRUSES AND IMMUNE TOLERANCE IN PREGNANCY

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Abstract

The human placenta expresses endogenous retroviral envelope proteins which have been postulated to play an important role in the physiology of pregnancy. Of these, syncytin-1 and syncytin-2 are highly expressed in the syncytiotrophoblast and cytotrophoblast respectively and are thought to be key factors in the regulation of syncytialisation due to their fusiogenic properties. In addition to their role in cell fusion, it has also been speculated that syncytin-1 and syncytin-2 may have a role in maternal immune tolerance due to the presence of the highly conserved immunosuppressive domain (ISD) within its sequence. However, no studies are yet to confirm this putative role. Another factor which has been speculated to have a role in maternal immune tolerance is Corticotropin Releasing Hormone (CRH) which has been shown to promote implantation and the maintenance of early pregnancy via the regulation of FasL. Interestingly, both syncytin-1 and FasL have been identified in immunosuppressive placental exosomes. Since CRH stimulates cyclic AMP (cAMP) production and syncytin-1, syncytin-2 and FasL are all stimulated by the cAMP second messenger pathway, it was hypothesised that syncytin-1 and syncytin-2 may be regulated by CRH. Further, it was hypothesised that syncytin-1 may contribute to the modulation of the maternal immune environment during pregnancy. To examine the regulation of syncytin-1 and syncytin-2 by CRH, a combined nucleic acid and protein extraction procedure was developed using column based nucleic acid extraction kits. Using 2D buffer, proteins extracted using this method were shown to have a comparable protein profile to conventionally extracted proteins. This method was then used to examine RNA and protein levels in CRH treated BeWo cells. Following CRH treatment of BeWo cells, a significant upregulation of syncytin-1, syncytin-2 and FasL mRNA was observed. CRH also increased the production of the syncytin-1 precursor in an exosomal fraction. To examine the immunosuppressive properties of syncytin-1, the recombinant ectodomains of human and mouse syncytins were produced and purified using a combination of affinity chromatography and gel filtration. The immunosuppressive properties of the syncytin-1 recombinant ectodomain were then tested using a whole blood culture model stimulated with LPS or PHA.

Syncytin-1 recombinant ectodomain at a concentration of 1μM inhibited the production of TNF-α by 50% and CXCL10 by 65% in whole blood cultures following maximal stimulation with LPS. Syncytin-1 recombinant ectodomain also inhibited the production of IFN-γ by 30% in PHA stimulated PBMC. These studies demonstrate for the first time that syncytin-1 has immunosuppressive properties. Further, these studies show that CRH has a role in the stimulation of syncytin-1 and its subsequent sorting into exosomes. Circulating placental exosomes containing syncytin-1 and other immunosuppressive factors including FasL may interact with maternal immune cells to prevent an immune response against the fetal-placental unit. This is a novel mechanism that may contribute to our understanding of how a genetically different fetus can be tolerated by the mother during pregnancy.

Statement of Originality

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.

John Even Schjenken

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Declaration

The work presented in chapter 2 was part of collaborations between John Schjenken and Jorge Tolosa. This work was also presented in the thesis of Jorge Tolosa as an appendice and has been published in the journal Biotechniques (1).
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Abbreviations

CHAPS -	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
ACTH -	Adrenocorticotropic hormone
AGRF -	Australian Genome Research Facility
bp -	Base pair
cDNA -	Complementary DNA
CRH -	Corticotropin Releasing Hormone
CRH-BP -	CRH binding protein
CRH-R -	CRH receptor
cAMP -	cyclic AMP
DISC -	Death Inducing Signalling Complex
DAF -	Decay Accelerating Factor
DTH -	Delayed type hypersensitivity
dH_2O -	Distilled water
ERV -	Endogenous retroviral/retrovirus
ERVs -	Endogenous retroviruses
env gene -	Envelope gene
ERK -	Extracellular signal-regulated kinase
EVT -	Extra villous trophoblast
FADD -	Fas-associated death domain
FasL -	Fas Ligand
FPLC -	Fast Protein Liquid Chromatography

FeLV -	Feline Leukaemia Virus
Gcma -	Glial cells missing a
GM-CSF -	Granulocyte macrophage colony stimulating factor
HBSS -	Hanks buffered salt solution
HPLC –	High Performance Liquid Chromatography
Hitrap IMAC -	Hitrap Immobilised Metal Affinity Chromatography
HRP -	Horse radish peroxidase
HERV -	Human Endogenous Retrovirus
HERVs -	Human Endogenous Retroviruses
HIV -	Human Immunodeficiency Virus
HLA -	Human Leukocyte Antigen
Ig -	Immunoglobulin
ISD -	Immunosuppressive domain
IDO -	Indoleamine 2,3,-dioxygenase
IFN -	Interferon
IL -	Interleukin
JAK3 -	Janus Kinase 3
IPTG -	Isopropyl β-D-1-thiogalactopyranoside
LDH -	Lactate Dehydrogenase
LAL -	Limulus Amebocyte Lysate
LPS -	Lipopolysaccharide
LCMS -	Liquid Chromatography mass spectrometry
LB –	Luria broth

MHC -	Major Histocompatibility Complex
MPMV -	Mason Phizer Monkey Virus \
MAC -	Membrane Attack Complex
MCP -	Membrane Co-factor Protein
mRNA -	Messenger RNA
µg -	Microgram
µM -	Micromolar
mg -	Milligram
mM -	Millimolar
MEK –	mitogen-activated protein kinase/ERK kinase
M -	Molar
MMulV -	Moloney Murine Leukaemia Virus
ng -	Nanogram
nm -	Nanometres
nM -	Nanomolar
NK cells -	Natural Killer cells
OD -	Optical density
1D -	One dimensional
PBMC -	Peripheral Blood Mononuclear Cells
PMA -	phorbol 12-myristate 13-acetate
PBS -	Phosphate buffered saline
Pi3-K -	phosphoinositide-3 kinase
PHA -	Phytohaemagglutinin

PCR -	Polymerase Chain Reaction
PIBF -	progesterone induced blocking factor
PD1 -	Programmed Death 1
PDL1 or PDL2 -	Programmed death Ligand 1 / 2
PKA -	Protein kinase A
RT-PCR -	Real-time Polymerase Chain Reaction
RCA -	Regulators of Complement Activation
T-reg cells -	Regulatory T cells
rpm -	Revolutions per minute
RT -	Room temperature
SDS-PAGE -	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
ASCT2 -	Solute Carrier Family 1 (neutral amino acid transporter),
member 5	
SEB -	Staphylococcal Enterotoxin B
SOB -	Super Optimal Broth
SOCS -	Suppressors of cytokine signaling
TB -	Transformation buffer
TGF -	Transforming Growth Factor
TAE buffer -	Tris-acetate-EDTA buffer
TBST -	Tris-buffered saline Tween 20
TE buffer -	Tris-EDTA buffer
TNF -	Tumour Necrosis Factor
2D -	Two dimensional

2D buffer -	Two dimensional electrophoresis lysis buffer
Th1 -	Type 1 helper T cells
Th2 -	Type 2 helper T cells
UV -	Ultra-violet