

Pathways of paternal antigen presentation to initiate antigen-specific immune responses in pregnancy

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Abstract

The fetus and its placenta, collectively called the conceptus, are semi-allogeneic to the mother, as they express transplantation antigens of paternal origin. Foreign tissues generally experience immunological rejection by the host immune system; however in a normal healthy pregnancy the conceptus does not undergo immune attack. Emerging evidence indicates the conceptus avoids rejection through a number of mechanisms including the induction of active maternal immune tolerance specific for paternal antigens. However, the mechanisms responsible for establishing this tolerance remain undefined, including the timing of the first encounter with paternal antigen and the cellular processes by which paternal antigen is presented to the maternal immune system. Exposure to paternal transplantation antigens occurs in two waves: initially in the context of male seminal fluid at conception, and secondly after placental trophoblast invasion of maternal tissues in mid-gestation pregnancy. Therefore the aim of this research was to evaluate the female immune response to paternal antigens in seminal fluid and those associated with the conceptus. The mechanisms of antigen presentation, the impact of the cytokine environment and the consequences of T cell activation on pregnancy were also investigated.

A transgenic system using ovalbumin (OVA) as the model paternal antigen was established. The transgenic Act-mOVA mouse expresses OVA constitutively and ubiquitously under a β -actin promoter and OVA was shown to be present in seminal fluid and in the fetal and placental tissue of sired progeny. The OVA-reactive CD8⁺ OT-I and CD4⁺ OT-II T cells were employed to gauge the relative amount of OVA antigen presented, with the strength of the maternal immune response quantified based upon the extent of T cell proliferation, as assessed by CFSE dye-dilution.

Utilising bone marrow chimeric mice, it was demonstrated that upon insemination by an Act-mOVA male, seminal fluid-derived OVA was processed and indirectly presented by maternal bone marrow-derived antigen presenting cells to induce activation and proliferation of the CD8⁺ OT-I T cells within the uterine-draining para-aortic lymph nodes of the female. Likewise, OT-II T cells were responsive to MHC class II-restricted presentation of seminal fluid OVA. Post-implantation conceptus-derived OVA was detected within peripheral lymph nodes and the spleen where it was presented via the MHC class I and class II-restricted pathways to induce systemic proliferation of both OT-I and OT-II T cells. Furthermore, as gestation

advanced the extent of OVA presentation and hence T cell proliferation intensified. Conceptus-derived OVA was still presented systemically until 20 days pp.

The impact of the uterine cytokine environment was assessed to determine its influence on seminal OVA antigen processing and presentation. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a key factor in regulating the leukocyte population of the female reproductive tract. GM-CSF-deficient female mice were unable to process and present seminal fluid OVA as effectively or efficiently as their wildtype counterparts, as assessed by their reduced capacity to drive OT-I and OT-II T cell proliferation following insemination by an Act-mOVA male.

Finally, with highly-reactive OVA-specific T cells activated in response to seminal and conceptus OVA antigen, it was of interest to determine the effect of OT-I T cell activation on fetal survival and pregnancy success. It was found that OT-I T cells activated *in vivo* to paternal OVA antigen in the context of seminal fluid and pregnancy were not deleterious to pregnancy outcomes. However the transfer of cytotoxic OT-I T cells generated *in vitro* in the presence of an IL-2 into female mice carrying OVA-expressing conceptuses was detrimental to fetal survival.

Collectively these experiments demonstrated that the initial exposure to paternal antigen, and hence the first opportunity to develop paternal antigen-specific tolerance, occurs at insemination. Paternal antigen is presented to the maternal T cell repertoire throughout gestation and may play a role in maintaining immune tolerance during pregnancy. The processing and presentation of paternal-derived antigen is chiefly performed by female bone marrow-derived antigen presenting cells. The cytokine environment of the mated female reproductive tract is critical in allowing optimal antigen processing and presentation, to generate an immune response consistent with maternal immune tolerance of the conceptus.

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.

I further grant my consent to the University of Adelaide to make this thesis available in all forms of media, now or hereafter known.

Lachlan Millhouse Moldenhauer

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Table of Contents

Abstract	i
Declaration	iii
Acknowledgements	iv
Publications arising from these and related studies	v
Abstracts arising from these and related studies	vi
Table of contents	xi
List of figures	xvii
List of tables	xix
Abbreviations	xx

CHAPTER 1; LITERATURE REVIEW	1
1.1 INTRODUCTION	2
1.2 THE ESTABLISHMENT OF PREGNANCY	3
1.2.1 Fertilisation	3
1.2.2 Pre-implantation embryo development	4
1.2.3 Embryo implantation	4
1.2.4 Trophoblast invasion and placental development	5
1.3 AN OVERVIEW OF THE IMMUNE SYSTEM	6
1.3.1 Introduction	6
1.3.2 Innate immunity	6
1.3.3 Adaptive immunity	7
1.3.4 Major histocompatibility complex	8
1.3.4.1 Antigen presenting role of the major histocompatibility complex	8
1.3.4.2 Polymorphisms of the major histocompatibility complex	9
1.4 THE IMMUNOLOGY OF PREGNANCY	11
1.4.1 Medawar's hypotheses on the immune paradox of pregnancy	11
1.4.2 Antigenicity of the conceptus	11
1.4.3 The uterus is not an immune-privileged site	13
1.4.4 Mechanisms of maternal immune tolerance in pregnancy	14
1.4.4.1 Antigen and partner-specific tolerance	14
1.4.4.2 Tolerance of paternal-antigen reactive T cells	14
1.4.4.3 Cytokine expression within the mated female reproductive tract	15
1.4.4.4 Multiple mechanisms of maternal immune tolerance	16
1.4.5 The immune-activating and immune-deviated properties of seminal fluid	17
1.4.5.1 Introduction	17
1.4.5.2 The antigenicity of semen	18
1.4.5.3 Seminal fluid initiates the post-mating inflammatory response	19
1.4.5.4 The immune-deviating and suppressive capabilities of seminal fluid	21
1.4.5.5 Seminal plasma and pathologies of pregnancy	22
1.4.6 Leukocyte populations of the female reproductive tract	23
1.4.6.1 Introduction	23
1.4.6.2 Macrophages	23
1.4.6.3 CD4+ T cells	24
1.4.6.4 CD8+ T cells	25
1.4.6.5 Natural killer cells	26
1.4.6.6 B cells and antibody production	27
1.5 DENDRITIC CELLS, CROSS-PRESENTATION AND IMMUNE TOLERANCE	27
1.5.1 Dendritic cell subtypes	27
1.5.2 The mechanism of cross-presentation	28
1.5.3 Dendritic cells within the female reproductive tract	29

1.5.4	Dendritic cells and cross-tolerance	30
1.6	OVA T CELL TRANSGENIC MODELS	32
1.7	SUMMARY	33
1.8	REASERCH QUESTIONS	34
1.9	HYPOTHESES	34
1.10	AIMS	35
CHAPTER 2; MATERIALS AND METHODS		36
2.1	ANIMALS AND SURGERIES	37
2.1.1	Mouse Strains	37
2.1.2	Mouse Surgery	38
2.1.2.1	Vasectomy and seminal vesicle removal	38
2.1.2.2	Uterine ligation	39
2.1.2.3	Retro-orbital bleeds	39
2.2	QUANTIFICATION OF OVA EXPRESSION IN THE EJACULATE AND FETAL AND PLACENTAL TISSUE	39
2.2.1	Harvesting and preparation of ejaculate and conceptus tissue samples	39
2.2.2	OVA-specific ELISA	40
2.3	IDENTIFICATION OF LYMPH NODES DRAINING THE UTERUS OF FEMALE MICE	40
2.4	ADMINISTRATION OF OVA PROTEIN AND PEPTIDE TO FEMALE RECIPIENT MICE	40
2.5	OT-I AND OT-II T CELL RECEPTOR TRANSGENIC T CELL ASSAYS	41
2.5.1	Adoptive transfer of OVA-reactive OT-I and OT-II T cells	41
2.5.2	Flow cytometric analysis of OT-I and OT-II T cell responses to OVA antigen	41
2.5.3	Quantification of OT-I and OT-II T cell responses to OVA antigen	42
2.6	B3Z T CELL HYBRIDOMA ACTIVATION ASSAY	45
2.7	BONE MARROW CHIMERA PROTOCOLS	45
2.8	THE EFFECT OF GM-CSF ON SEMINAL FLUID OVA PROCESSING AND PRESENTATION	46
2.9	THE EFFECT OF CYTOTOXIC OT-I T CELLS ON PREGNANCY OUTCOME	47
2.9.1	Generation of cytotoxic OT-I T cells	47
2.9.2	The effect of cytotoxic OT-I T cells on pregnancy	47
2.10	STATISTICAL ANALYSIS	47

CHAPTER 3; DEVELOPMENT AND VALIDATION OF THE OVA T CELL TRANSGENIC MODEL	48
3.1 INTRODUCTION	49
3.2 THE PARA-AORTIC LYMPH NODES DRAIN THE UTERUS OF PARTICULATE AND SOLUBLE ANTIGENIC MATERIAL	50
3.3 THE KINETICS AND DOSE DEPENDENT RESPONSE OF OVA-REACTIVE T CELLS	52
3.4 CHARACTERISATION OF THE NATURE AND QUANTITY OF OVA WITHIN THE EJACULATE OF THE TRANSGENIC ACT-MOVA MOUSE	55
3.5 QUANTIFICATION OF OVA WITHIN THE GESTATIONAL TISSUES	58
3.6 DISCUSSION	61
CHAPTER 4; THE KINETICS OF SEMINAL-FLUID OVA AND CONCEPTUS- ASSOCIATED OVA ANTIGEN PRESENTATION TO OT-I AND OT-II T CELLS	67
4.1 INTRODUCTION	68
4.2 SEMINAL FLUID OVA AND CONCEPTUS-DERIVED OVA ACTIVATE CD4+ OT-II AND CD8+ OT-I T CELLS	69
4.3 KINETICS OF THE LOCAL OT-I T CELL RESPONSE IN THE PARA-AORTIC LYMPH NODES AT INSEMINATION, DURING PREGNANCY AND POST-PARTUM	75
4.4 KINETICS OF THE SYSTEMIC OT-I T CELL RESPONSE AT INSEMINATION, DURING PREGNANCY AND POST-PARTUM	76
4.5 CONCEPTUS-DERIVED OVA IS PRESENTED SYSTEMIC WITHIN LYMPHOID TISSUE AND SPLEEN DISTAL TO THE UTERUS DURING PREGNANCY	81
4.6 DISCUSSION	83
CHAPTER 5; INDIRECT PRESENTATION OF SEMINAL FLUID OVA BY MATERNAL BONE MARROW-DERIVED ANTIGEN PRESENTING CELLS	88
5.1 INTRODUCTION	89
5.2 SEMINAL FLUID-DERIVED OVA ANTIGEN IS PRESENTED TO CD8+ OT-I T CELLS BY MATERNAL CELLS	91

5.3	CONCEPTUS-DERIVED OVA ANTIGEN IS PRESENTED TO CD8+ OT-I T CELLS BY MATERNAL CELLS	92
5.4	GENERATION OF BONE MARROW CHIMERAS	95
5.5	SIINFEKL PEPTIDE ADMINISTERED TO THE UTERUS IS INDIRECTLY PRESENTED TO CD8+ OT-I T CELLS BY BONE MARROW-DERIVED ANTIGEN PRESENTING CELLS	97
5.6	SOLUBLE OVA PROTEIN ADMINISTERED TO THE UTERUS IS INDIRECTLY PRESENTED TO CD8+ OT-I T CELLS BY BONE MARROW-DERIVED ANTIGEN PRESENTING CELLS	99
5.7	ACT-MOVA SEMINAL FLUID-DERIVED OVA IS INDIRECTLY PRESENTED TO CD8+ OT-I T CELLS BY BONE MARROW-DERIVED ANTIGEN PRESENTING CELLS	103
5.8	DISCUSSION	106
CHAPTER 6; GM-CSF REGULATION OF ANTIGEN PROCESSING AND PRESENTATION IN THE UTERUS		112
6.1	INTRODUCTION	113
6.2	GM-CSF IS REQUIRED FOR AN OPTIMAL CD4+ OT-II T CELL PROLIFERATION RESPONSE TO SEMINAL FLUID-DERIVED OVA ANTIGEN	115
6.3	GM-CSF IS REQUIRED FOR AN OPTIMAL CD8+ OT-I T CELL PROLIFERATION RESPONSE TO SEMINAL FLUID-DERIVED OVA ANTIGEN	115
6.4	GM-CSF IS REQUIRED FOR OPTIMAL MHC CLASS I-RESTRICTED PRESENTATION OF SEMINAL FLUID DERIVED OVA ANTIGEN TO CD8+ OVA-SPECIFIC T CELLS	118
6.5	DISCUSSION	120
CHAPTER 7; THE EFFECT OF OVA-REACTIVE T CELLS ON PREGNANCY OUTCOME		124
7.1	INTRODUCTION	125
7.2	METHODOLOGY TO INVESTIGATE THE EFFECT OF OT-I T CELLS ON PREGNANCY OUTCOME	126
7.3	THE EFFECT OF OT-I T CELL TRANSFER AT DAY 0.5 POST-COTIUM ON PREGNANCY	128
7.3.1	The effect of OT-I T cells on pregnancy rates and implantation rates	128
7.3.2	The effect of OT-I T cells on fetal viability	129
7.3.3	The effect of OT-I T cells on fetal and placental weights	129
7.4	THE EFFECT OF OT-I T CELL TRANSFER AT DAY 3.5 POST-COTIUM ON PREGNANCY	132
7.4.1	The effect of OT-I T cells on pregnancy and implantation rates	132

7.4.2	The effect of OT-I T cells on fetal viability	133
7.4.3	The effect of OT-I T cells on fetal and placental weights	136
7.5	DISCUSSION	139
	CHAPTER 8; GENERAL DISCUSSION AND CONCLUSIONS	145
	APPENDIX	159
	CHAPTER 9; BIBLIOGRAPHY	162

List of figures

Figure 1.1	Classical and cross-presentation pathways	10
Figure 2.1	Gating and analysis of OT-I T cells	43
Figure 3.1	Particulate antigen administered to the uterus drains to the PALN	51
Figure 3.2	OVA antigen administered to the uterus drains to the PALN	53
Figure 3.3	The kinetics of OVA-reactive T cell proliferation in the PALN after transcervical OVA immunisation	54
Figure 3.4	Paternal-derived OVA is present within the seminal fluid of the Act-mOVA mouse	57
Figure 3.5	Seminal vesicle-derived OVA activates a CD4 ⁺ OT-II T cell response	59
Figure 3.6	Seminal vesicle-derived OVA activates a CD8 ⁺ OT-I T cell response	60
Figure 3.7	Paternal-derived OVA is expressed by conceptus tissue	62
Figure 4.1	Act-mOVA seminal fluid initiates OT-II T cell proliferation in the PALN and conceptus-derived OVA initiates systemic OT-II T cell proliferation	70
Figure 4.2	OT-II T cell proliferation is OVA-dependent	71
Figure 4.3	Act-mOVA seminal fluid initiates OT-I T cell proliferation in the PALN and conceptus-derived OVA initiates systemic OT-I T cell proliferation	73
Figure 4.4	OT-I T cell proliferation is OVA-dependent	74
Figure 4.5	Representative flow cytometry data showing the kinetics of OT-I T cell activation and proliferation in the PALN of females mated to Act-mOVA males	77
Figure 4.6	Kinetics of OT-I T cell activation and proliferation post-mating, during pregnancy and post-partum	79
Figure 4.7	Systemic proliferation is driven by presentation of conceptus-derived OVA in distal lymphoid tissue	82
Figure 5.1	Maternal H-2K ^b -expressing cells are required for seminal fluid OVA antigen processing and presentation to OT-I T cells	93
Figure 5.2	Maternal H-2K ^b -expressing cells are required for conceptus-derived OVA antigen processing and presentation to OT-I T cells	94
Figure 5.3	Host bone marrow was ablated by radiation and donor bone marrow engraftment in the host was successful	98

Figure 5.4	Soluble SIINFEKL peptide administered to the uterus is indirectly-presented to OT-I T cells by bone marrow-derived APCs	100
Figure 5.5	Soluble OVA protein administered to the uterus is indirectly-presented to OT-I T cells by bone marrow-derived APCs	102
Figure 5.6	Act-mOVA seminal fluid-derived OVA administered to the uterus is indirectly-presented to OT-I T cells by bone marrow-derived APCs	105
Figure 6.1	GM-CSF is required for an optimal CD4+ OT-II T cell proliferation response to seminal fluid-derived OVA antigen	116
Figure 6.2	GM-CSF is required for an optimal CD8+ OT-I T cell proliferation response to seminal fluid-derived OVA antigen	117
Figure 6.3	GM-CSF is required for an optimal CD8+ OT-I T cell proliferation response to SIINFEKL peptide post-insemination	119
Figure 7.1	Effect of OT-I T cell administration at day 0.5 pc on pregnancy rates, implantation rates and fetal viability	130
Figure 7.2	Effect of OT-I T cell administration at day 0.5 pc on fetal and placental growth	131
Figure 7.3	Effect of OT-I T cell administration at day 3.5 pc on pregnancy rates, implantation rates and fetal viability	135
Figure 7.4	Effect of OT-I T cell administration at day 3.5 pc on fetal and placental growth	137
Figure 8.1	A model for the induction of maternal immune tolerance	157

List of tables

Table 7.1	Experimental groups to assess the effect of OT-I T cells on pregnancy outcomes	128
Table 7.2	Cytotoxic OT-I T cells tend to induce OVA-positive fetal loss in an antigen-specific manner	134
Table A.1	Administration of OT-I T cell at day 0.5 post-coitum does not interfere with normal pregnancy outcome	160
Table A.2	Administration of OT-I T cell at day 3.5 post-coitum does not interfere with normal pregnancy outcome	161

Abbreviations

♂	male
♀	female
°C	degrees Celsius
%CD25+	percentage of CD25 positive cells in the parent peak
%CD69+	percentage of CD69 positive cells in the parent peak
am	<i>ante meridiem</i>
APC	antigen presenting cell
B6	C57Bl/6 mouse strain
B6.SJL	B6.SJL-PtprcaPep3b/BoyJArc mouse strain
B6.SJL → B6.SJL	B6.SJL mouse donor bone marrow into B6.SJL host
B6.SJL → bm1	B6.SJL mouse donor bone marrow into bm1 host
bm1	bm1 mouse strain
bm1 → B6.SJL	bm1 mouse donor bone marrow into B6.SJL host
bm1 → bm1	bm1 mouse donor bone marrow into bm1 host
BSA	bovine serum albumin
cDNA	complementary deoxyribonucleic acid
CFSE	5,6 - carboxyfluorescein diacetate succinimidyl ester
CLN	cervical lymph node
CpG DNA	cytosine-guanine island deoxyribonucleic acid
CSF	colony stimulating factor
CSF-1R	colony stimulating factor 1 receptor
Crry	complement receptor related protein
CTL	cytotoxic lymphocyte
CTLA-4	cytotoxic T lymphocyte-associated protein 4
CTLA4-CR	cytotoxic T lymphocyte-associated protein 4 counter receptor
DC	dendritic cell
DES-TCR	Désiré T cell receptor transgenic mouse
dpc	days post-coitum

DSMO	dimethyl sulfoxide
dsRNA	double stranded ribonucleic acid
EAE	experimental autoimmune encephalomyelitis
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence-activated cell sorting
FASL	FAS ligand
FCS	fetal calf serum
Fig	figure
Gal	galactosidase
G-CSF	granulocyte-colony stimulating factor
GM-CSF	granulocyte macrophage colony-stimulating factor
GM-CSF ^{-/-}	granulocyte macrophage colony-stimulating factor null mutant mice
Gy	gray
h	hour
HCl	hydrochloric acid
HLA	human leukocyte antigen
HSV	herpes simplex virus
i.v.	intravenous
ICSI	intracytoplasmic sperm injection
IDO	indoleamine 2,3-dioxygenase
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IL-2R	interleukin 2 receptor
ING	inguinal lymph nodes
IUGR	intrauterine growth restriction
IVF	<i>in vitro</i> fertilisation
JAK	janus Kinase
KC	keratinocyte-derived chemokines

KHCO ₃	potassium bicarbonate
KLH	keyhole limpet hemocyanin
LCMV	lymphocytic choriomeningitis virus
LN	lymph node
LPS	lipopolysaccharide
μg	microgram
μl	microlitre
μM	micromolar
M	molar
MCP	monocyte chemoattractant protein
MES	mesenteric lymph nodes
MgCl ₂	magnesium chloride
MHC	major histocompatibility complex
min	minute
MIP	macrophage inflammatory protein
ml	millilitre
mm	millimetre
mM	millimolar
MMP	matrix metalloproteinases
mRNA	messenger ribonucleic acid
MUC	mucin
n	number
Na ₂ HPO ₄	disodium hydrogen phosphate
NF	nuclear factor
ng	nanograms
NH ₄ Cl	ammonium chloride
NK	natural killer
NLR	NOD-like receptors
nm	nanometres
NP-40	nonyl phenoxy polyethoxy ethanol

OVA	chicken ovalbumin
PALN	para-aortic lymph nodes
PBS	phosphate buffered saline
pc	post-coitum
PE	phycoerythrin
PE-Cy	phycoerythrin-cyanine
PGE	prostaglandin
PI	proliferation index
pm	post-meridiem
PMSF	phenylmethanesulphonylfluoride
pp	post-partum
PRR	pattern recognition receptor
RANTES	regulated upon activation normal T cell expressed and secreted
RBC	red blood cell
RER	rough endoplasmic reticulum
RLR	retinoid-inducible gene 1-like receptors
s.c.	subcutaneous
SEM	standard error of mean
SIINFEKL	ovalbumin peptide amino acids 258-265
SOCS	suppressor of cytokine signalling
SPL	spleen
ssRNA	single stranded ribonucleic acid
SV	simian virus
SVX	seminal vesicle-deficient
TAP	transporter associated with antigen processing
TCR	T cell receptor
TGF	transforming growth factor
Th	T helper
TIMP	tissue inhibitors of metalloproteinase
TLR	Toll-like receptor

Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
uNK	uterine natural killer
VAS	vasectomised
VEGF	vascular endothelial growth factor
VSV	vesicular stomatitis virus