The role of macrophages in early pregnancy success

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Abstract

Macrophages are abundant in the ovary and uterus, with their densities and distribution changing throughout the estrous cycle in response to the sex steroid hormones, estrogen (E_2) and progesterone (P_4). In the uterus, macrophages are present in the endometrium, where they are located in close spatial proximity with uterine epithelial cells, and the myometrium. In the endometrium, macrophages are thought to have key roles in tissue remodelling in preparation for embryo implantation and trophoblast invasion. In the ovary, macrophages are present in the thecal cell layer where they surround developing follicles, and are also present in functional corpora lutea. They are thought to have a role in ovulation and in the extensive remodelling that occurs during formation of the corpus luteum. Other studies have suggested that macrophages enhance the output of P_4 from luteal cells. Their accumulation in the corpus luteum during luteolysis, as well as in atretic follicles, is suggestive of a role in tissue remodelling and removal of cellular debris.

The specific role of macrophages during the pre- and peri-implantation period remains to be fully elucidated. Previous studies in mice lacking important macrophage-supporting cytokines suggest the role of these cells in endometrial receptivity and ovarian function may not be essential. However, interpretation of the importance of macrophages using these models is made difficult due to changes in macrophage phenotype, or incomplete macrophage depletion. The studies conducted herein make use of the *Cd11b-Dtr* transgenic mouse. The expression of the simian diphtheria toxin (DT) receptor (R) on CD11B-expressing macrophages enables transient and systemic depletion of these cells. The murine form of the DTR binds DT poorly and as such, injection of DT to wild-type mice has no effect. Immunohistochemistry and flow cytometry showed that using this model an 85% depletion of uterine macrophages and a 90% depletion of ovarian macrophages is achieved 24 h following DT injection to *Cd11b-Dtr* mice at estrus.

Macrophage depletion in *Cd11b-Dtr* mice during the pre- and peri-implantation period caused complete pregnancy failure with loss during the peri-implantation phase. A direct adverse impact of DT on developing embryos was excluded as a contributing factor. Macrophage depletion did not affect uterine epithelial cell proliferation that occurs in response to E_2. Stromal cell proliferation that occurs in response to E_2 and P_4 in early pregnancy, prior to differentiation into decidual cells, also occurs normally following DT-elicited macrophage depletion.

Macrophage depletion caused decreased expression of glycosylated structures involved in embryo attachment to uterine epithelial cells. There was a 25% reduction in the intensity of the lectin UEA-1 immunostaining (detects fucosylated structures on the uterine epithelium), and a 67% reduction in LewisX immunostaining. However, the process of artificially induced decidualisation was not impacted by macrophage depletion.

Investigation into the impact of macrophage depletion on ovarian function showed that corpus luteum function was compromised, as evidenced by a 77% reduction in circulating plasma P_4 levels on day 4.5 post coitum (pc). Importantly, pregnancy could be rescued by the exogenous injection of P_4 and could be sustained into late gestation when P_4 support was maintained. The impact of macrophage depletion of the expression of mRNAs encoding enzymes responsible for the synthesis of P_4 was investigated using qRT-PCR. There was no change in mRNA expression of *Star* or *Cyp11a1* following DT-elicited macrophage depletion, but there was a significant 29% decrease in *Hsd3b1* mRNA expression. *Hsd3b1* is the gene that encodes the protein HSD3B1, responsible for the conversion of pregnenolone to P_4.

Following macrophage depletion, ovaries from *Cd11b-Dtr* mice were hemorrhagic at autopsy, and histology of ovarian tissue revealed evidence of impaired structural integrity of the corpora lutea. Staining for endothelial cell specific markers showed that endothelial cells were also depleted from some corpora lutea; the extent of endothelial cell depletion varied between corpora lutea within the same ovary. The lymphatic vasculature surrounding corpora lutea appeared not to be affected by macrophage depletion.

In early pregnancy (day 1.5 and 2.5 pc), during the formation of the corpus luteum, the impact of macrophage depletion on genes involved in endothelial cell angiogenesis and survival was investigated. There was more than a 3-fold increase in the mRNA expression of *Vegfa*, but a significant decrease in the mRNA expression of *Vegfc, Vegfd*, *Flt-1* and *Kdr* at both of these time-points following macrophage depletion.

This study shows that macrophages are essential for the establishment of early pregnancy. In the endometrium, macrophages appear to influence uterine epithelial cell remodelling to increase the expression of markers of receptivity, and may facilitate the process of implantation. In the ovary, macrophages act to regulate the structure and function of the corpus luteum, potentially through

regulating the expression of VEGFs and their receptors. These studies reveal new functions for macrophages in reproductive events and indicate that macrophage involvement in ovarian angiogenesis and support of steroidogenesis warrants further investigation.

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.

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Publications arising from this thesis

1. Jasper MJ, Care AS, Sullivan B, Ingman WV, Aplin JD, Robertson SA. *Macrophage-derived LIF and IL1B regulate α(1,2)fucosyltransferase (Fut2) expression in mouse uterine epithelial cells during early pregnancy.* Biology of Reproduction, 2011 84(1); 179-88. Epub 2010 Sep 23.
2. Care AS, Jasper MJ, Ingman WV, Robertson SA. (In preparation). *Macrophages are essential for corpus luteum progesterone synthesis and establishing early pregnancy in mice.*
3. Care AS, Ingman WV, Jasper MJ, Robertson SA. (In preparation). *Macrophages are not required for the uterine proliferative response to steroid hormones or deciduoma formation in mice.*

Abstracts arising from this thesis

2011

Care AS, Jasper MJ, Ingman WV, Robertson SA. *Macrophage regulation of corpus luteum angiogenesis in early pregnancy*. Oral presentation at the Society for Reproductive Biology, Cairns, Australia. October 2011.

2010

Care AS, Jasper MJ, Ingman WV, Robertson SA. *An essential role for macrophages in corpus luteum function and early pregnancy maintenance.* Poster presentation at the International Congress for Reproductive Immunology, Cairns, Australia. August 2010.

Care AS, Jasper MJ, Ingman WV, Robertson SA. *Macrophages are essential for maintenance of corpus luteum function in the mouse.* Oral presentation at the Society for the Study of Reproduction, Milwaukee, USA. August 2010

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2009

Care AS, Jasper MJ, Ingman WV, Robertson SA. *Why the immune system counts during pregnancy.* Oral presentation at the Young Investigator Award finals, Royal Institution of Australia, Adelaide, Australia. October 2009.

Care AS, Jasper MJ, Ingman WV, Robertson SA. *Macrophages are essential for maintenance of early pregnancy through regulation of corpus luteum function.* Oral presentation at the Young Investigator Award semi-finals, Women's and Children's Hospital, Adelaide, Australia. September 2009.

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Ingman WV, Care AS, Robertson SA, JW Pollard. *An acute macrophage depletion model reveals a role for macrophages in regulation of testicular steroidogenesis in vivo.* Oral presentation at the Society for Reproductive Biology, Christchurch, New Zealand. September 2007.

Table of Contents

List of Figures

List of Tables

Abbreviations

ADAMTS1	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1
ATP	adenosine triphosphate
bFGF	basic fibroblastic growth factor
BM	bone marrow
bp	base pair
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
CCL	chemokine (C-C) motif
Cd11b-Dtr	Tg(ITGAM-DTR/EGFP)34Lan, transgene insertion 34, Richard A Lang
CL	corpus luteum
COX-2	prostaglandin-endoperoxide synthase-2
CSF	colony-stimulating factor
Ct	cycle threshold
CXC	chemokine (C-X-C) motif
CYP11A1	cytochrome P450, family 11, subfamily a, polypeptide 1
CYP17A1	cytochrome P450, family 17, subfamily a, polypeptide 1
DAB	3,3 diaminobenzadine
DAMPs	damage-associated molecular patterns
DAPI	4',6-Diamidino-2-phenylindole dihydrochloride
DC	dendritic cell
DT	diphtheria toxin
DTR	diphtheria toxin receptor
E_2	estrogen; estradiol; 17β-estradiol
eCG	equine chorionic gonadotropin
ECM	extracellular matrix
EGF	epidermal growth factor
EP	ectopic pregnancy
ER	estrogen receptor
FACS	fluorescence-activated cell sorting

FIGF	c-fos induced growth factor
FLT1	FMS-like tyrosine kinase 1
FSH	follicle-stimulating hormone
FUT	fucosyltransferase
GnRH	gonadotropin-releasing hormone
GROA	growth-related oncogene-alpha
hCG	human chorionic gonadotropin
HGF	hepatocyte growth factor
hMG	human menopausal gonadotropin
HMGB1	high-mobility group box 1
HPG	hypothalamic-pituitary-gonadal
HRP	horseradish peroxidase
HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
HSP	heat shock protein
i.p.	intraperitoneal
i.v.	intravenous
IFN	interferon
IGF	insulin-like growth factor
IL	interleukin
IL1R t1	interleukin-1 receptor type-1
IUGR	intrauterine growth restriction
IVF	*in vitro* fertilisation
KDR	kinase insert domain protein receptor
LH	luteinising hormone
LHR	luteinising hormone receptor
LIF	leukaemia inhibitory factor
LPS	lipopolysaccharide
LTP	*Lotus tetragonolobus purpureas*
LYVE1	lymphatic vessel endothelial hyaluronan receptor-1
mAb	monoclonal antibody
MCM	macrophage-conditioned media
MD	macrophage depleted
MFI	mean fluorescence intensity

MHC	major histocompatibility complex
MMP	matrix metalloproteinases
MPS	mononuclear phagocyte system
MT-MMP	membrane-type-1-MMP
MUC	mucin
NK	natural killer
NMS	normal mouse serum
O/N	overnight
op	osteopetrotic
P_4	progesterone
PA	plasminogen activator
PAF	platelet activating factor
PAMPs	pathogen-associated molecular patterns
pc	post-coitum
PDGF	platelet-derived growth factor
PE	pre-eclampsia
PEC	peritoneal exudate cell
PECAM1	platelet/endothelial cell adhesion molecule 1
PG	prostaglandin
PIGF	progesterone-induced blocking factor
PMN	polymorphonuclear leukocyte
PR	progesterone receptor
PRL	prolactin
PRLR	prolactin receptor
qRT-RCR	quantitative real-time polymerase chain reaction
RBC	red blood cell
RT	reverse transcription
RT-PCR	real-time polymerase chain reaction
s.c.	subcutaneous
SEM	standard error of mean
SP	seminal plasma
STAR	steroidogenic acute regulatory protein
SV-	seminal vesicle deficient

TGF	transforming growth factor
TIMP	tissue inhibitors of matrix metalloproteinases
TLR	toll-like receptor
TNF	tumour necrosis factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UEA-1	*Ulex europaeus*
VEGF	vascular endothelial growth factor