Seminal Fluid and Cytokine Control of Regulatory T-Cells in Murine Pregnancy

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ABSTRACT

For successful pregnancy, the maternal immune system must tolerate the presence of a fetus that expresses alloantigens. The appropriate and timely acquisition of this state of tolerance is critical and emerging evidence suggests that it needs to be present from the time the embryo implants into the uterus. Recently it has been demonstrated that a subpopulation of lymphocytes termed $CD4^+CD25^+$ regulatory T cells (Treg cells) are required for immune tolerance of the fetus during pregnancy. Despite their importance the factors that control regulatory T cells during pregnancy, and in particular in the peri-implantation period, are poorly understood. Using mouse models we have assessed the role of the ejaculate and its components (sperm and seminal plasma) in coordinating Treg cells in the period prior to embryo implantation. We have also used mice with a null mutation in the interleukin 10 (IL-10) gene to assess the role of this cytokine in coordination of Treg cell populations in later pregnancy.

Experiments in the peri-implantation period just prior to implantation (day 3.5 post-coitum) showed that there was a significant increase (approximately 2-fold; $p<0.05$) in the total number of ($CD4^+Foxp3^+$) Treg cells in the iliac lymph nodes (LNs) that drain the uterus, but not in the distal inguinal LNs. This appeared not to be the result of a selective expansion in Treg cells but due to expansion of the entire $CD4^+$ cell pool, since the percent of $CD4^+$ cells expressing Foxp3 in any of the lymphoid tissues studied did not increase in response to mating. In addition, there was a similar increase in the density of these cells in the uterus just prior to implantation at day 3.5pc ($p<0.05$). By using males deficient in the sperm or seminal plasma components of the ejaculate we could show that the increase in both the lymph node and uterine Treg cell populations occurred in response to seminal plasma.

The role of seminal plasma in regulating expression of mRNAs encoding migratory molecules in the peri-implantation uterus, and the involvement of these genes in recruiting Treg cells following mating, was then assessed. We analysed the mRNAs for the chemokines *Ccl4, Ccl5, Ccl19*, *Ccl22*, the chemokine receptors *Ccr4, Ccr5, Ccr7* and the integrin *Cd103* using qRT-PCR. We showed a significant elevation in *Ccl19*

and *Ccr5* mRNA at day 3.5pc following mating to intact males. However the increase in mRNA was independent of factors associated with seminal fluid and might instead be regulated by ovarian steroid hormones.

Using *IL-10* null mutant (IL-10-/-) mice it was then shown that the cytokine IL-10 is involved in controlling Treg cell numbers in mid gestation. At gestational day (gd) 9.5, in IL-10-/- mice, there was an approximate 40% elevation in the proportion of $CD4^+$ cells expressing Foxp3 compared with wild-type control mice ($p<0.01$). This was seen in both the iliac LNs and inguinal LNs. In addition, there was a greater than 10-fold increase ($p<0.0001$) in the total number of Treg cells in the uterine-draining iliac LNs of IL-10-/- mice compared to wild-type mice. This was not seen in the inguinal LNs. Experiments comparing allogeneic and syngeneic mated mice showed that the proportional changes seen in the $CD4^+$ cell population was dependent on fetal alloantigens, although the elevation in total numbers still occurred in the absence of fetal alloantigens.

This study begins to unravel the process by which Treg cell populations are expanded and recruited into the uterus prior to embryo implantation and later in gestation. A greater understanding of this process may aid in the diagnosis and prevention of a range of pregnancy pathologies associated with immune dysregulation, such as pre-eclampsia and recurrent spontaneous abortion.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Leigh Guerin 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

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CONFERENCE ABSTRACTS ARISING FROM THIS THESIS

2009

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ABBREVIATIONS

^{3}HTdR	Tritiated thymidine
ANOVA	analysis of variance
APC	Antigen presenting cell
B6	C57BL/6
cDNA	Complementary deoxyribonucleic acid
CG	Choriogonadotroptin
CSIF	Cytokine synthesis inhibitory factor
Ct	Cycle threshold
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CV	Coefficient of variation
DC	Dendritic cell
E	Embryonic day
E2	Estradiol
Est	estrus
EtOH	Ethanol
FACS	Fluorescence-activated cell sorting
FasL	Fas lignad
FITC	Flurescein isothiocyanate
Foxp3	Forkhead box P3
gd	Gestational day
GFP	Green fluoresent protein
GITR	Glucocorticoid-induced tumor necrosis factor receptor
GM-CSF	Granulocyte-macrophage colony-stimulating factor

hCG	Human chronic gonadotropin
HLA	Human leukocyte antigen
HRP	Horseradish peroxidase
hrs	hours
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IHC	Immunohistochemistry
IL	Interleukin
Int	intact
IPEX	immune dysregulation polyendocrinopathy, entropathy, X-linked
LAG-3	Lymphocyte-activation gene 3
LH	leutinizign hormone
LIF	Leukemia inhibitory factor
LN	Lymph node
LPS	Lipopolysaccharide
MHC	Major histocompatibility complexe
mins	minutes
mRNA	messenger ribonucleic acid
NK	Natural killer
Nrp1	Neuropilin-1
PBL	Peripheral blood leukocyte
PBS	Phosphate-buffered saline
PBST	PBS Tween-20
pc	Post-coitum
PD1	Programmed death-1
PE	Phycoerthrin

PGE2	Prostaglandin E2
qRT-PCR	Quantitative real-time polymerase chain reaction
RA	Retinoic acid
ROR	Retinoic acid-related orphan receptor
SEM	Standard error of mean
STAT5	Signal transducer and activator of transcription 5
SV-	vesiculectomised
TCR	T-cell receptors
TGF	Transforming growth factor
Th	T helper
TNF	Tumour necrosis factor
Tr1	T regulator 1
Treg	T regulatory Cell
uNK	Uterine natural killer
VAS-	vasectomised