

**Modulation of Antigen Presenting Cell
Function to Affect Innate and Adaptive
Immune Responses:
Implications for Organ Transplantation**

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**Even now, I wrap what's most fragile
in the long gauze of science.
The more elusive the truth,
the more carefully it must be carried.**

- **Anne Michaels**

THESIS ABSTRACT

Transplantation is the best form of treatment for end-stage kidney disease, by improving quality of life, reducing mortality and lowering healthcare costs. However, the immunosuppressive medications required have non-selective mechanisms of action, affecting both patient and graft longevity. Tolerance, the acceptance of an allograft in the absence of immunosuppression, remains a major goal in clinical transplantation research. Dendritic cells (DC) are potent antigen-presenting cells (APC) capable of promoting anti-donor immunity and antigen-specific tolerance, and are a promising target for immunomodulation. Current tolerogenic techniques involve *ex vivo* DC manipulation which limits immediate clinical applicability. The scope of this thesis involves identification of a novel biologic agent, curcumin, to induce tolerogenic DC and the use of this immunomodulatory agent within a liposomal construct to target and modify DC function *in vivo*.

Chapter 1 discusses the context of this thesis and contains a comprehensive literature review.

Chapter 2 outlines methodology and materials utilised in this thesis.

Chapter 3 demonstrates the use of curcumin for *in vitro* generation of tolerogenic DC that promote expansion of functional FoxP3+ regulatory T-cells (Tregs). *In vivo* infusion of curcumin-treated DC was also able to induce subsequent immune hyporesponsiveness mediated by FoxP3+ Tregs, and represents a potential avenue for transplant recipient conditioning using donor (or recipient) -derived DC.

Chapter 4 demonstrates the use of liposomes to target APC *in vivo*. Liposomal incorporation of immunomodulatory agents facilitates targeted cellular delivery to tissue-resident APC and forms a basis for *in vivo* modulation of APC function. This work demonstrates that the *in vitro*

results demonstrated in Chapter 3 can be replicated *in vivo*, potentially eliminating the need for *ex vivo* DC manipulation in a transplant setting.

Chapter 5 demonstrates the utility of liposomal curcumin in ameliorating aspects of ischaemia-reperfusion injury (IRI), a consequence of transplant surgery that promotes graft immunogenicity and limits graft longevity. For the first time renal tubular epithelial and antigen-presenting cell endocytosis of liposomes is demonstrated, as is salvage of renal function which is mediated by reduced pro-inflammatory cytokine and chemokine production, and diminished oxidative stress. The results also identify thioredoxin-interacting protein (TXNIP) as a potential novel marker of tissue injury in IRI, and curcumin effectively reduces this aspect of cellular redox stress. These data represent a novel and effective delivery method for this immunomodulatory agent, preventing significant renal damage in a manner that has immediate clinical applicability.

Chapter 6 describes a refinement in liposomal targeting of DC, using a DC-specific liposome capable of binding to human monocyte-derived DC with high affinity via the receptor DC-SIGN. The gene for marmoset DC-SIGN was cloned and the cross-reactivity of a human-DC-targeted liposome to its marmoset counterpart was investigated *in vitro*. Additional attempts were made to synthesize a marmoset DC-targeted liposome through basic, non-specific, chemical modification of a monoclonal antibody to DC-SIGN known to be cross-reactive with both humans and marmosets, with the aim of creating a cell-free DC-targeted negative vaccine that could be tested in non-human primates.

Thus, the work presented in this thesis creates a platform for future studies from which DC-based cellular and cell-free immune tolerance therapies can be developed in a transplant model.

DECLARATIONS

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution to Natasha Mireille Rogers and, to the best of my knowledge, 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. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time. I acknowledge that the copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works.

HONOURS AND AWARDS

- 2010 Transplantation Society of Australia and New Zealand
Janssen-Cilag Travelling Fellowship
- 2010 AusBiotech-GlaxoSmithKline
Student Excellence Award, South Australian and National Winner
- 2010 Australian and New Zealand Society of Nephrology
ANZSN Travelling Fellowship
- 2010 Australian and New Zealand Society of Nephrology
Novartis Overseas Travelling Fellowship
- 2010 Australian and New Zealand Society of Nephrology
Finalist, Young Investigator Award
- 2010 Australian and New Zealand Society of Nephrology
Travel grant to attend the Annual Scientific Meeting
- 2010 Transplantation Society of Australia and New Zealand
Travel grant to attend XXII Congress of the Transplantation Society
- 2010 Transplantation Society of Australia and New Zealand
Young Investigators Award
- 2010 Transplantation Society of Australia and New Zealand
Winner, President's Prize for best invited oral presentation
- 2010 Australian Society for Medical Research, Adelaide
Winner, Ross Wishart Prize for best oral presentation
- 2007 National Health & Medical Research Council
Medical Postgraduate Scholarship
- 2007 Kidney Health Australia
Postgraduate Scholarship
- 2007 The University of Adelaide
Australian Postgraduate Award

PUBLICATIONS

Peer reviewed papers

Rogers NM, Matthews, TJ, Kitching, AR, Coates, PT. Kidney dendritic cells: their role in homeostasis, inflammation and transplantation. *Nephrology* 2009 14(7):620-35.

Rogers NM, Kireta S, Coates PTH. Curcumin generates maturation-resistant dendritic cells and T regulatory cells *in vitro*. *Clin Exp Immunol*, Accepted June 2010.

Rogers NM, Stephenson M, Kitching AR, Horowitz JD, Coates PT. Amelioration of renal ischemia-reperfusion injury by liposomal delivery of an NFκB inhibitor to renal tubular epithelial and antigen presenting cells. Submitted to *Br J Pharmacol*, minor revision undertaken and resubmitted.

Rogers NM, Collins MG, Coates PTH. Marmoset kidney histology and progression: implications for disease models. Submitted to *Am J Primatol*.

Rogers NM, Jesudason S, Kireta S, Lim WH, Russ GR, Coates PTH. Blood and tissue dendritic cell subsets in common marmoset monkeys. Manuscript in preparation, to be submitted to *Exp Haematol* March 2011.

Prasad S, Rogers NM, Collins MG, Coates PTH. Non-human primate dendritic cells. Manuscript to be submitted to *Immunol Cell Biol* March 2011.

Abstract publications

Rogers NM, Stephenson MD, Coates PT. Liposomal curcumin ameliorates renal ischaemia-reperfusion injury via NFκappaB inhibition and antioxidant pathways. *Immunol Cell Biol* 2010; 88(6): A28

Rogers NM, Kireta S, Coates PT. Curcumin generates maturation-resistant dendritic cells and T regulatory cells *in vitro*. *Immunol Cell Biol* 2010; 88(6): A24

Rogers NM, Coates PT. Curcumin generates maturation-arrested “FAST” dendritic cells that expand regulatory T cells *in vitro* and *in vivo*. *Nephrology* 2010; 15(S4): 40

PRESENTATIONS

Invited presentations

“Modulation of innate and adaptive immunity to facilitate organ transplantation”

- Department of Ophthalmology, Flinders Medical Centre, South Australia, November 2010
- Welcome Centre, Oxford, UK, November 2010
- Beth Israel Deaconess Medical Centre, Boston, USA October 2010
- Basil Hetzel Institute for Medical Research, South Australia, October 2010
- Thomas E. Starzl Institute, University of Pittsburgh, USA, September 2010
- Flinders Medical Centre Seminar Series, South Australia, August 2010
- Vascular Medicine Institute, University of Pittsburgh, USA, May 2010

Conference presentations

Oral presentations

Rogers NM, Stephenson MD, Coates PT. “Liposomal curcumin ameliorates renal ischaemia-reperfusion injury via NFkappaB inhibition and antioxidant pathways”

- Australian Society for Medical Research Annual Scientific Meeting, Adelaide, June 2010
- Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra, June 2010
- XXIII International Congress of the Transplantation Society, Vancouver, August 2010
- Young Investigator Award, Australian and New Zealand Society of Nephrology, Perth, September 2010
- The Queen Elizabeth Hospital Research Day, Adelaide, October 2010

Rogers NM, Kireta S, Coates PT. “Curcumin generates maturation-resistant dendritic cells and T regulatory cells *in vitro* and *in vivo*”

- President’s Prize, Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, June 2010

Rogers NM, Stephenson M, Kireta S, Coates PTH. “Amelioration of ischaemia-reperfusion injury using liposomal curcumin”

- The Queen Elizabeth Hospital Research Day, Adelaide, October 2009

Mini-oral presentations

Rogers NM, Coates PT. Curcumin generates maturation-arrested “FAST” dendritic cells that expand regulatory T cells *in vitro* and *in vivo*”

- Australian and New Zealand Society of Nephrology Annual Scientific Meeting, Perth, September 2010

Poster presentations

Rogers NM, Coates PT. Curcumin generates maturation-arrested “FAST” dendritic cells that expand regulatory T cells *in vitro* and *in vivo*”

- XXIII International Congress of the Transplantation Society, Vancouver, August 2010

Rogers NM, Stephenson M, Parish CR, Thomas R, Coates PTH. “Alteration of innate and adaptive immune responses using liposomal curcumin”

- Australasian Society of Immunology Conference, Gold Coast, December 2009

Rogers NM, Parish CR, Russ GR, Coates PTH. “Specific targeting of dendritic cells using tolerogenic liposomes”

- The Queen Elizabeth Hospital Research Day, Adelaide, October 2008

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ABBREVIATIONS

[³H] thymidine – tritiated thymidine
AA-DC – alternatively activated dendritic cells
Ab/anti- – antibody
ADCC – antibody-dependent cell-mediated cytotoxicity
Ag – antigen
ALP – alkaline phosphatase
APC – antigen presenting cell
APC – allophycocyanin
ATP – adenosine triphosphate
Automacs® – automated magnetic cells separator
bp – base pair
BDCA – blood dendritic cell antigen
BM- bone marrow
BODIPY – boron-dipyrromethene
cDC – conventional dendritic cells
CCL – CC chemokine ligand
CCR – CC chemokine receptor
CD – cluster of differentiation
CD40L – CD40 ligand
CD62L – CD62 ligand
cDC – conventional DC
cDNA - complementary deoxyribonucleic acid
CHO cell – chinese hamster ovary cell
CM – complete medium
CNI – calcineurin inhibitor
CpG – cytosine-guanine oligonucleotide
CPM – counts per minute
CsA - cyclosporine
CTL – cytotoxic lymphocyte
CTLA-4 – cytotoxic T lymphocyte associated antigen-4

CTRL - control

CurcDC – curcumin-treated dendritic cells

CYC - cytochrome

DAPI - 4',6-diamidino-2-phenylindole

DC – dendritic cell

DC-LAMP – dendritic-cell-lysosome-associated membrane protein

DC-SIGN – dendritic cell-specific intercellular adhesion molecule [ICAM]-3 grabbing non integrin

DEPC – diethylenetripyrocarbonate

dH₂O – distilled water

DiI - 1,1'-dioctadecyl 3,3',3',3'-tetramethylindocarbocyanine perchlorate

DNA – deoxyribonucleic acid

dNTP – deoxynucleotide triphosphate

DOGS-NTA-Ni - 1,2-dioleoyl-*sn*-glycero-3-[(N-(5-amino-1 carboxypentyl)iminodiacetic acid)succinyl] (nickel salt)

DSG - Deoxyspergualin

EDTA – ethylenediamine tetra acetic acid

ELISA – enzyme-linked immunosorbent assay

EM –electron microscopy

EPG – egg phosphatidylcholine

FcR – Fc receptor

FCS – foetal calf serum

FITC - Fluorescein isothiocyanate

FKBP – FK binding protein

Flt – FMS-like tyrosine kinase

Flt3L – fms-like tyrosine kinase 3 ligand

FoxP3 – forkhead box protein 3

G-CSF –granulocyte colony stimulating factor

GITR – glucocorticoid-induced tumour necrosis factor receptor

GM-CSF – granulocyte-macrophage colony stimulating factor

GVHD – graft versus host disease

H₃PO₄ – phosphoric acid

H&E – haematoxylin and eosin
HI FCS – heat-inactivated foetal calf serum
HLA – human leukocyte antigen
HP – haematopoietic precursor
HPDC – dendritic cell cultured from haematopoietic precursor cells
HPRT1 - hypoxanthine phosphoribosyltransferase 1
HSP – heat shock protein
Hu MoDC – human monocyte-derived DC
iDC – immature DC
IDO – indoleamine 2,3-dioxygenase
IFN – interferon
IFN γ – interferon gamma
Ig – immunoglobulin
IL – interleukin
IMVS – Institute of Medical and Veterinary Science
IKDC – interferon-producing killer dendritic cells
IP – intraperitoneal
IPTG – isopropyl β -D-1-thiogalactopyranoside
IRI – ischaemia-reperfusion injury
IV – intravenous
LAG 3 – lymphocyte activated gene 3
LB – Luria broth
LC – Langerhans cells
Lin - lineage
LPS – lipopolysaccharide
MAPK – mitogen activated protein kinase
MBL – mannose binding lectin
MCP - monocyte chemoattractant protein
MDC – myeloid DC
MFI – mean fluorescence intensity
MHC – major histocompatibility complex
MIP – macrophage inflammatory protein

MLR – mixed lymphocyte (leukocyte) reaction
MMLV- Maloney murine leukemia virus
MMR – macrophage-mannose receptor
MNC – mononuclear cells
mRNA – messenger ribonucleic acid
mTOR – mammalian target of rapamycin
MW – molecular weight
NaOH – sodium hydroxide
NF – nuclear factor
NFAT – nuclear factor of activated T-cells
NF- κ B – nuclear factor kappa B
NH₄Cl – ammonium chloride
NHP – non-human primate
NK – natural killer
NOS – nitric oxide synthase
NTA₃-DTDA – 3-nitriloacetic acid ditetradecylamine
NWT – nylon wool T-cells
OCT – optimal cutting tissue
OD – optical density
OligodT – oligodeoxythymidylic acid
PB – peripheral blood
PBS – phosphate buffered saline
PBMC – peripheral blood mononuclear cell
PCR – polymerase chain reaction
PD-1 – programmed death-1
pDC – plasmacytoid DC
PD-L1 – programmed death ligand-1
PD-L2 – programmed death ligand-2
PE – phycoerythrin
PE-Cy5 - phycoerythrin-Cy-5
PE-Cy5.5- phycoerythrin-Cy-5.5
PE-Cy7- phycoerythrin-Cy-7

PG - prostaglandin
PI – propidium iodide
PMV – plasma membrane vesicles
Pre-DC – DC precursors
Pre-MDC – myeloid dendritic cell precursors
Pre-PDC – plasmacytoid dendritic cell precursors
PTLD – post-transplant lymphoproliferative disorder
RB – round bottom
rh - human recombinant
RNA – ribonucleic acid
RNAsin - RNase inhibitor
rpm – revolutions per minute
RPMI – Roswell Park Memorial Institute
RT-PCR – real-time polymerase chain reaction
SCF – stem cell factor
SD – standard deviation
SEB – streptococcal enterotoxin B
SEM – standard error of mean
SOD – superoxide dismutase
SOT – solid organ transplant
STAT – signal transducers and activators of transcription
Tac – tacrolimus
TCR – T-cell receptor
Th – T-helper
TGF – transforming growth factor
TGF β - transforming growth factor beta
tolDC – tolerogenic dendritic cell
T_R1 – T regulatory type 1 cells
Treg – regulatory T-cell
TLR – toll-like receptor
TLR4 – toll-like receptor 4
TNF – tumour necrosis factor

TNF α - tumour necrosis factor alpha

TolDC – tolerogenic dendritic cells

TPO – thrombopoietin

TQEH – The Queen Elizabeth Hospital

WB – western blot

WCC – White cell count

Xgal – 5-bromo-4-chloro-3-indoyl-beta-D-galactopyranoside