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

Dr Natasha Rogers

MBBS (Hons), FRACP

Transplantation Immunology Laboratory
Basil Hetzel Institute for Medical Research,
The Queen Elizabeth Hospital, and
Hanson Institute

Department of Medicine,
Faculty of Health Sciences,
University of Adelaide

Submitted in fulfilment of the
Degree of Doctor of Philosophy,
University of Adelaide,
December 2010

TABLE OF CONTENTS

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 immunmodulatory 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 NFkappaB 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

ACKNOWLEDGEMENTS

Firstly, I sincerely thank my supervisor, A/Prof Toby Coates for his mentorship. I have greatly respected and admired Toby's enthusiasm and scientific thinking, and it has been a privilege to work with him.

I would also like to thank Prof Graeme Russ (RAH, Adelaide), Prof Chris Parish (ANU, Canberra), Dr Shane Grey (Garvan Institute, Sydney) and Prof Ranjeny Thomas (Diamantine Institute, Brisbane) for their valuable intellectual input. I also sincerely thank Prof Clive Prestidge and Dr Timothy Barnes (Ian Wark Institute, UniSA) who allowed me to use the necessary equipment to make liposome preparations, without which I could not have performed so many experiments.

I would like to acknowledge the support of the National Health and Medical Research Council for the provision of the scholarship (and extension) that has enabled me to undertake my PhD.

I am indebted to the most important person in our laboratory, Svjetlana Kireta, who taught and helped me with everything. She was never too busy, and always ready to assist, teach, and listen. I am also grateful to all the staff and students of the TIL during my tenure, especially Julie Johnston, Matthew Stephenson, Clyde Milner, Chris Drogemuller, Darling Rojas, Claire Jessup, Michael Collins, Daisy Mohanasundaram, Amy Hughes, Boris Fedoric, and Austin Milton, for practical assistance, teaching, helpful discussions, insights and thoughtful feedback. Thank you for providing a friendly, accommodating and generous environment in which to develop skills and learn. The staff at the IMVS Animal Facility require mention, particularly Kelly Wicks for her assistance with all the mouse injections.

I would like to thank Chris Drogemuller (RAH/Hanson Institute) for guidance with marmoset DC-SIGN cloning and sequence analysis, John Brealy (TQEH) for electron microscopy assistance, Katherine Pilkington (Detmold Facility, Hanson Institute) for flow sorting expertise and friendship, staff at the South Australian Red Cross Blood Service and the donors for providing blood samples. A special mention must go to Prof John Horowitz whose objective insight, judgement and humour was a great salve in moments of frustration.

I have been blessed with magnificent parents who have been utterly reliable back-up baby sitters on innumerable occasions, and provided scientific advice, moral support and encouragement at every step. Thank you all for everything; this would not have been achieved without your continuous help.

To my husband David, your support of me has made all this possible.

.

And finally, beautiful child, Orli, born before this madness began. You have provided me with laughter, frustration, sleepness nights and joy. I hope one day you might be interested enough to look at this thesis, disregard the fact it is not bound in pink, and understand what I was doing on those nights away from you.

ABBREVIATIONS

[^{3}H] 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 recpetor

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 - cychrome

DAPI - 4’,6-diamindino-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 – diethylenepyrocarbonate

dH_2O – 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 – glucorticoid-induced tumour necrosis factor receptor

GM-CSF – granulocyte-macrophage colony stimulating factor

GVHD – graft versus host disease

H_3PO_4 – 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

IFNγ – 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- Malooney 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-κB – nuclear factor kappa B

NH_4Cl – ammonium chloride

NHP – non-human primate

NK – natural killer

NOS – nitric oxide synthase

NTA_3-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

TGFβ - 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

TNFα - 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