Developing a non-human primate model of dendritic cell based immunotherapy in transplantation: Studies in the common marmoset monkey (Callithrix jacchus)

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THESIS ABSTRACT

Kidney transplantation represents the best treatment for end-stage kidney disease, and in comparison to dialysis treatment has been shown to improve survival, quality of life, and reduce health-care costs over time. However, in order to prevent transplant failure from allograft rejection, immunosuppressive drug therapy is required. Immunosuppression is associated with significant systemic toxicities, and continues to impair optimal patient and graft outcomes. The avoidance or minimisation of immunosuppression via the promotion of tolerance of the allograft, or the use of targeted therapeutic strategies, in clinical transplantation is therefore an important goal that could have many benefits for patients. Dendritic cells (DC) are potent antigen-presenting cells that play a pivotal role in the initiation and maintenance of immune responses, and therapies utilising or targeting DC offer the potential to manipulate immune responses towards tolerance. This thesis seeks to develop the potential of DC based immunotherapies in a small and clinically relevant non-human primate (NHP) transplant model, the common marmoset monkey, and thereby facilitate translation of these therapies towards human clinical trials.

Chapter 1 establishes the context for this thesis by outlining the background and providing a comprehensive review of relevant literature.

Chapter 2 describes the materials and methods utilised in this thesis. Additional details of methods are contained in relevant chapters.

Chapter 3 presents a comprehensive study of renal pathology in a colony of laboratory marmosets, including histology, immunofluorescence and electron microscopy, and correlates this for the first time with serum and urine biochemistry. This work demonstrates that the spontaneously observed glomerular pathology in marmosets represents a benign occurrence that would not impact on the assessment of renal function or histology in a marmoset kidney transplant model.

Chapter 4 examines the trafficking behaviour *in vivo* of intravenously and subcutaneously administered allogeneic marmoset DC propagated *in vitro* from genetically disparate marmoset donors. The findings indicate that allogeneic marmoset

DC do not necessarily exhibit normal trafficking behaviour *in vivo*, as they are not found in secondary lymphoid tissues at 48 hours, in contrast to similarly administered autologous DC. This finding lends weight to other recent studies of donor DC cellular therapy that indicate that the tolerogenic effects of this therapy are not mediated through cell to cell interactions with recipient T-cells, but rather through providing a source of donor antigen for acquisition and processing by recipient DC.

Chapter 5 describes studies to develop a monoclonal antibody to marmoset DC-specific ICAM 3-grabbing non-integrin (DC-SIGN), which is a DC-specific marker. Ultimately, a marmoset cross-reactive commercially available anti-human DC-SIGN antibody (DCN46) was identified, and found to be suitable to utilise in the development of DC-SIGN targeted cell-specific therapy. Using this antibody, marmoset DC-SIGN positive cells were identified in the Lineage$^-$ CD11c$^+$ Class II$^+$ fraction of marmoset spleen; in contrast *in vitro* propagated marmoset monocyte-derived DC have been confirmed to lack DC-SIGN expression.

Chapter 6 describes the successful development of a novel nanocarrier targeted to DC: PLGA nanoparticles that target DC using the human and marmoset DC-SIGN cross-reactive antibody identified in Chapter 5. A series of preliminary studies have demonstrated that DC-SIGN targeted PLGA nanoparticles are taken up by Class II$^+$ CD11c$^+$ marmoset spleen cells, and that loading of the nanoparticles with the immunomodulatory drug curcumin shows evidence of *in vitro* immunosuppressive capacity, as shown in mixed leucocyte reaction; however the specificity for DC of immunosuppressive targeted PLGA nanoparticles remains to be demonstrated.

Chapter 7 summarises the overall findings from this thesis, and proposes a series of necessary studies to exploit the identified potentials from this work further.

Overall, the work in this thesis significantly advances the marmoset NHP model as a means to translate the potential of DC based immunotherapies towards clinical transplantation. The feasibility of DC-targeted therapy using nanoparticles has been established, and represents an opportunity to specifically target DC with immunosuppressive drugs *in vivo*, and thereby manipulate the immune response towards tolerance, while reducing the burden of non-targeted immunosuppression.

DECLARATION

I certify that 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 Michael Gerard Collins 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

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Michael Gerard Collins

Date

AWARDS

2013	Transplantation Society of Australia and New Zealand **Young Investigator Award**
2012	The Queen Elizabeth Hospital – Research Day **Winner, Best Poster Presentation**
2011	The Queen Elizabeth Hospital – Research Day **Finalist, Best Presentation – Basic Science Higher Degree**
2009	National Health and Medical Research Council **Medical Postgraduate Scholarship**
2008	The University of Adelaide **Australian Postgraduate Award**
2008	Royal Australasian College of Physicians **Jacquot Research Entry Scholarship**

PUBLICATIONS

Published papers

Jesudason S, **Collins MG**, Rogers NM, Kireta S, and Coates PT. Non-human primate dendritic cells. *J Leukoc Biol* 2012; 91: 217-28.

Manuscripts in preparation

Collins MG, Rogers NM, Kireta S, Brealey J and Coates PT. Spontaneous glomerular mesangial lesions in common marmoset monkeys (Callithrix jacchus): a benign non-progressive glomerulopathy. *[Manuscript in preparation]*

Collins MG, Jesudason S, Kireta S, and Coates PT. Infusion of allogeneic donor derived dendritic cells in marmoset monkeys to promote tolerance: studies of recipient immune responses and trafficking of administered cells in vivo. *[Manuscript in preparation*

Jesudason S, Kireta S, **Collins MG**, Rogers NM, and Coates PT. Blood and tissue dendritic cell subsets in common marmoset monkeys. *[Manuscript in preparation]*

Published abstracts

Collins MG, Rogers NM, Jesudason S, Kireta S, Brealey J and Coates PT. Spontaneous immune complex deposition and proteinuria in the common marmoset monkey (*Callithrix jacchus*) – a model of benign non-progressive glomerulopathy. *Nephrology* 2012; 17 Suppl 2: 43-44.

Collins MG, Rogers NM, Kireta S, Jesudason S, and Coates PT. Developing a monoclonal antibody to target DC-SIGN in non-human primates: a novel tolerogenic cell-specific therapy. *Nephrology* 2011; 16 Suppl 1: 54.

PRESENTATIONS

Invited presentations

"Targeting dendritic cells via DC-SIGN to deliver cell-specific therapy in transplantation: studies in a non-human primate model"

- Basil Hetzel Institute for Medical Research, Adelaide, SA – May 2013
- The annual *DC Down Under* Symposium 2012: Applications in Transplantation and Immunotherapy. Sydney, NSW – August 2012
- Department of Nephrology, Prince of Wales Hospital, Sydney, NSW – May 2012

"Dendritic cell research in transplantation: preclinical cellular transplantation therapy in non-human primates"

- Department of Virology and Immunology, Southwest National Primate Research Center. San Antonio, Texas, USA– November 2009

Conference Presentations

Oral Presentations

Collins MG, Kitto LJ, Jesudason S, Thierry B, Coates PT. Dendritic cell targeted therapy: polymeric nanoparticles targeting human and non-human primate DC-SIGN to inhibit dendritic cell function.

- Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra ACT, June 2013

Collins MG, Rogers NM, Kireta S, Jesudason S, and Coates PT. Developing a monoclonal antibody to target DC-SIGN in non-human primates: a novel tolerogenic cell-specific therapy

- The Queen Elizabeth Hospital Research Day, Adelaide SA – October 2011

Mini-oral presentations

Collins MG, Rogers NM, Jesudason S, Kireta S, Brealey J and Coates PT. Spontaneous immune complex deposition and proteinuria in the common marmoset monkey (*Callithrix jacchus*) – a model of benign non-progressive glomerulopathy

- Australian and New Zealand Society of Nephrology, Annual Scientific Meeting, Auckland, NZ – August 2012

Collins MG, Rogers NM, Kireta S, Jesudason S, and Coates PT. Development of a novel antibody to target DC-SIGN in non-human primate models of DC immunotherapy for transplant tolerance

- Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra ACT – June 2012

Poster presentations

Collins MG, Kitto LJ, Jesudason S, Barnes TJ, Thierry B, Prestidge CA, and Coates PT. Targeting dendritic cells using anti-DC-SIGN conjugated immunoliposomes: a novel approach to immunotherapy

- The Queen Elizabeth Hospital Research Day, Adelaide SA – October 2012

Collins MG, Rogers NM, Kireta S, Jesudason S, and Coates PT. Targeting dendritic cells via the dendritic cell-specific C type lectin DC-SIGN in non-human primates: towards a novel tolerogenic cell-specific therapy

- XXIV International Congress of the Transplantation Society, Berlin, Germany – July 2012

Collins MG, Rogers NM, Kireta S, Jesudason S, and Coates PT. Developing a monoclonal antibody to target DC-SIGN in non-human primates: a novel tolerogenic cell-specific therapy

- Australasian Society of Immunology, Annual Scientific Meeting, Adelaide, SA – December 2011
- Australian and New Zealand Society of Nephrology, Annual Scientific Meeting, Adelaide, SA – September 2011

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And finally, to my wife and best friend, Sarah. We weren't yet married when I started this PhD, and I'm sure you've thought at times since that it would never be completed. However, it has been your love, unwavering support and constant encouragement that have made the difference in getting this completed. I dedicate this thesis to you, and to our daughter Eliza, whose birth before I had quite finished helped spur me to get across the finish line.

ABBREVIATIONS

7AAD	7-amino-actinomycin D
AEC	animal ethics committee
ALP	alkaline phosphatase
ANOVA	analysis of variance
APC	allophycocyanin
APC	antigen presenting cell(s)
BDCA	blood dendritic cell antigen
BM	bone marrow
BSA	bovine serum albumin
C3	complement component 3
CCL	chemokine ligand
CCR	chemokine receptor
CD	cluster of differentiation
cDC	conventional dendritic cell(s)
CFSE	carboxyfluorescein diacetate succinmidyl ester
CHO	Chinese Hamster Ovary
CLEC	C-type lectin
CLR	C-type lectin (receptor)
CM	complete medium
COOH	carboxyl group
CRD	carbohydrate recognition domain
CTLA4	cytotoxic T-lymphocyte antigen 4
CTLA4-Ig	CTLA4 immunoglobulin fusion protein
DAPI	4’, 6-diamidino-2-phenylindole dihydrochloride
DC	dendritic cell(s)
DC-SIGN	dendritic cell specific intercellular adhesion molecule (ICAM) 3-grabbing non-integrin
DiI	1,1’-dioctadecyl-3, 3, 3’, 3’-tetramethylindocarbocyanine perchlorate
DMSO	dimethyl sufoxide
DNA	deoxyribonucleic acid
DPPC	1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine
DSA	donor specific antibody
DSPE	1,2-distearoyl-phosphatidyl ethanolamine

EDC	1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
EM	electron microscopy
ESKD	end-stage kidney disease
Fab	fragment antigen-binding region (of an immunoglobulin)
Fc	fragment crystallisable region (of an immunoglobulin)
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
Flt3	fms-related tyrosine kinase 3
Flt3L	Flt3 ligand
FMO	fluorescence minus one
G-CSF	granulocyte-colony stimulating factor
GBM	glomerular basement membrane
GM-CSF	granulocyte macrophage-colony stimulating factor
GVHD	graft versus host disease
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HP	haematopoietic progenitor
HPDC	haematopoietic progenitor-derived dendritic cell(s)
HSA	human serum albumin
ICAM	intercellular adhesion molecule
ICOS	inducible costimulator
ICOS-L	ICOS ligand
IFN	interferon
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
iNOS	inducible nitric oxide synthase
IPTG	isopropyl-β-D-1-thiogalactopyranoside
IQR	interquartile range
ISIS	International Species Information System
KLH	keyhole limpet haemocyanin

LB	liquid broth
LC	Langerhan cell(s)
Lin	lineage
LPS	(bacterial) lipopolysaccharide
M-CSF	macrophage colony-stimulating factor
M-CSFR	M-CSF receptor
mal	maleimide
MHC	major histocompatibility complex
MLR	mixed leukocyte reaction
MoDC	monocyte-derived dendritic cell(s)
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor of activated T-cells kappa B
NHMRC	National Health and Medical Research Council
NHP	non-human primate
NHS	*N*-hydroxysuccinimide
NK	natural killer
NWP	new world primate
NWT	nylon wool T-cells
OCT	optimal cutting temperature compound
OWP	old world primate
PAMPs	pathogen-associated molecular patterns
PAS	periodic acid-Schiff
PBMC	peripheral blood mononuclear cell(s)
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell(s)
PDL	programmed death ligand
PE	phycoerythrin
PEG	polyethylene glycol
PLA	polylactic acid
PLGA	polylactic-co-glycolic acid
pre-DC	dendritic cell precursor(s)
PRRs	pattern recognition receptors
RCF	relative centrifugal force
RES	reticuloendothelial system
rh	recombinant human

RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute medium (aka RPMI-1640)
SCF	stem cell factor
SD	standard deviation
SIV	simian immunodeficiency virus
TGFβ	transforming growth factor beta
Th1	T helper type 1
Th17	T helper type 17
Th2	T helper type 2
TLR	toll-like receptor
TNF	tumour necrosis factor
TPO	thrombopoietin
Tr1	T regulatory type 1 cell(s)
Treg	T regulatory cell(s)
WB	whole blood
Xgal	5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside