Studies of Immune Biology of The Common Marmoset: A Novel Non-Human Primate Transplant Model.

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THESIS ABSTRACT

Donor-specific immune tolerance is a highly desirable goal in clinical transplantation. Dendritic cells (DC) are potent immune system regulators, and promoting both anti-donor immunity and immune tolerance. DC are therefore an important target for potential tolerance-inducing therapies, which must be validated in non-human primate models before clinical trials. The common marmoset is a small, New World primate which our group is developing as a novel transplant model. The scope of this thesis involves the development of methodology and characterisation of critical aspects of marmoset immune biology pertinent to transplantation and DC immunotherapy.

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 describes a new technique for genotyping marmoset major histocompatibility complex (MHC) Class II DRB genes, to facilitate choosing mismatched donor and recipient animals for transplant studies. Genotype-based matching was predictive of *in-vitro* immune reactivity, and therefore validated as a method for selecting immunologically disparate animal pairs. Two new alleles were also identified. This work has given rise to two publications, and the methodology subsequently extended to marmoset MHC Class I and other Class II genes by others in our group.

Chapter 4 describes the first-ever studies of propagation of marmoset DC *in-vitro* from peripheral blood DC precursors (monocytes and stem cells) mobilised by the growth factor G-CSF. These methods enabled large-scale DC production from small volumes of peripheral blood. Marmoset DC were characterised extensively by morphology, phenotype and function, with many similarities to human and NHP DC. As with all animal models, specific differences

were also identified. In particular, marmoset monocyte-derived DC were maturation-resistant, whereas stem-cell derived DC were semi-mature. This work establishes that marmoset DC exist within the paradigm of human and NHP DC systems, and is therefore a feasible model for DC-based tolerance studies.

Chapter 5 describes for the first time the *in-vivo* propagation of marmoset DC following treatment with the growth factor FLT3-Ligand. A three-colour flow cytometry strategy for identifying and sorting marmoset putative peripheral blood myeloid DC was validated. The rare myeloid DC population was expanded massively by FLT3-Ligand, and could be isolated in numbers sufficient for therapeutic use. These DC had typical myeloid DC morphology and were capable of immune stimulation *in-vitro*. In addition, new markers for plasmacytoid DC were evaluated. This work forms the basis for ongoing studies of *in-vivo* marmoset DC.

Chapter 6 describes the culmination of the studies outlined in earlier chapters, with the first-ever studies of DC immunotherapy in marmoset monkeys. Three donor and recipient pairs were chosen on the basis of MHC genotype mismatch. Donor animals were treated with G-CSF and immature monocyte-derived DC propagated *in-vitro*. Recipient animals were treated with intravenous infusion of unmodified immature donor DC, and immune responses monitored. Two animals exhibited reduction in anti-donor (and third party) immune responses, whereas one animal was initially sensitized to donor cells. These preliminary studies establish the feasibility of DC-based immunotherapy in this model, and demonstrate that DC-induced immune modification can occur and be successfully monitored.

Thus, the work presented in this thesis creates a platform from which future studies of DC-based immune tolerance strategies can be developed in this novel 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 Shilpanjali Prasad 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. 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. Permission to reproduce has been obtained from the publishers as shown below.

1. Prasad S, Humphreys I, Kireta S, Gilchrist RB, Bardy P, Russ GR, Coates PT. MHC Class II DRB genotyping is highly predictive of *in-vitro* alloreactivity in the common marmoset. J Immunol Methods. 2006 Jul 31;314(1-2):153-63. (Publisher: ELSEVIER; License Agreement Date 27.10.08; Number 2056931094502)

2. Prasad S, Humphreys I, Kireta S, et al. The common marmoset as a novel preclinical transplant model: identification of new MHC class II DRB alleles and prediction of *in-vitro* alloreactivity. Tissue Antigens 2007; 69 Suppl 1: 72-75. (Publisher: WILEY-BLACKWELL; permission not required for reproduction of own article in self-edited publication).

PRESENTATIONS AND AWARDS

2008 Australasian Society for Immunology, Annual Scientific Meeting, Canberra (Poster)

2008 XXII International Congress of The Transplantation Society, Sydney (ePoster)
Recipient of TSANZ Young Investigators Award

2008 Postgraduate Research Expo, Faculty of Health Sciences, University of Adelaide, Adelaide (Poster)

2007 TQEH Research Day, Adelaide (Oral Presentation)
Winner, Best Higher Degree Presentation (Medical)

2007 Australia and New Zealand Society of Nephrology, Annual Scientific Meeting, Gold Coast (Oral Presentation)
Finalist, Young Investigators Award

2007 World Congress of Nephrology, Rio de Janeiro (Poster)

2007 Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra (Mini-Oral Presentation)

2007 Australasian Society for Immunology, Annual Scientific Meeting, Sydney (Oral Presentation)

2006 Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra (Oral Presentation)
Recipient of TSANZ Young Investigators Award

2006 TQEH Research Day, Adelaide (Oral Presentation)

2005 14th International HLA and Immunogenetics Workshop (IHIWS), Melbourne (Oral Presentation)

2005 Australasian Society for Immunology, Annual Scientific Meeting, Melbourne (Poster)

2004 Australasian Society for Immunology, Annual Scientific Meeting, Adelaide (Oral Presentation)

2004 Australasian and South East Asian Tissue Typing Association (ASEATTA) Annual Meeting, Sydney (Poster)

2004 TQEH Research Day, Adelaide (Poster)

2004 Australia and New Zealand Society of Nephrology, Annual Scientific Meeting, Adelaide (Poster)

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I would like to thank Ian Humphries (Australian Red Cross Blood Service Tissue Typing Laboratory, Adelaide) for his invaluable assistance with sequencing and analysis performed in Chapter 3, Chris Drogemuller (TQEH) for guidance with RelB sequence analysis, John Brealy for electron microscopy assistance, Dr Ian Lewis (Department of Haematology, Royal Adelaide Hospital) for facilitating access to the Allogeneic Stem Cell Transplant Program, and the donors for providing blood samples.

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To my beautiful precious children, Daniel (born between Chapters 3 and 4) and Sonali (born between Chapters 6 and 7), you have without doubt been the most important thing I have produced during these years. I hope one day you might read this thesis, and see what I was up to when you were babies. Failing that, I hope at least you don't attempt to use it as a dinner plate, embellish it with your art work, or test its ability to float.

And finally, to my husband David. Your unwavering support of me and wonderful devotion to our family has made all this possible, and to you I dedicate this thesis.

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ADDENDUM

Please note that references 232,236, 237, 239, 240 and 241 have been withdrawn by the authors. This came to my attention following the submission of this thesis. This is a body of work from the University of Alabama (Professor Judy Thomas) relating to the STEALTH protocol for tolerance induction in rhesus monkeys. The data relating to graft and animal survival in these studies have been found to be inaccurate. Full details may be obtained at http://ori.dhhs.gov/misconduct/cases/Thomas_Judith.shtml and http://ori.dhhs.gov/misconduct/cases/Contreras_Juan_Luis.shtml.

I have retained text in Chapter 1 relating to these studies of non-human primate transplant tolerance induction, because at the time of publication and in the years following they were important studies that related to other studies in this area and seemingly advanced the field. Therefore the existence of these studies remains relevant, despite the fact the data is now believed to be misleading. However, I have clearly identified discussion relating to withdrawn publications.

ABBREVIATIONS

AA – alternatively activated DC

APC – antigen presenting cell

BDCA – blood dendritic cell antigen

BM- bone marrow

Caja – Callithrix jacchus

CD – cluster of differentiation

CD40L – CD40 ligand

cDC – conventional DC

CpG – cytosine-guanine oligonucleotide

CTLA-4 – cytotoxic T lymphocyte associated antigen-4

DC – dendritic cell

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

DNA – deoxyribonucleic acid

DSG - Deoxyspergualin

EDTA – ethylenediamine tetra acetic acid

ELIspot – enzyme linked immuno-spot assay

FCS – foetal calf serum

FLT3 – fms-like tyrosine kinase 3

FLT3-L – FLT3-ligand

FOXP3 – forkhead box protein 3

G-CSF –granulocyte colony stimulating factor

GM-CSF – granulocyte-macrophage colony stimulating factor

HLA – human leukocyte antigen

HP – haematopoietic precursor

HPDC – DC cultured from haematopoietic precursor cells

iDC – immature DC

IDO – indoleamine 2,3-dioxygenase

IFN – interferon

Ig - immunoglobulin

IL- interleukin

Lin - lineage

LPS - lipopolysaccharide

MDC – myeloid DC

MHC – major histocompatibility complex

MLR – mixed lymphocyte (leukocyte) reaction

MoDC – monocyte-derived DC

NF – nuclear factor

NHP – non-human primate

NK – natural killer

PB – peripheral blood

PBS – phosphate buffered saline

PBMC – peripheral blood mononuclear cell

PCR – polymerase chain reaction

PDC – plasmacytoid DC

RNA – ribonucleic acid

RPMI – Roswell Park Memorial Institute medium

SCF – stem cell factor

Treg – regulatory T-cell

TLR – toll-like receptor

TNF – tumour necrosis factor

TPO – thrombopoietin

TQEH – The Queen Elizabeth Hospital

WCC – White cell count