



**Gene Therapy Studies of Adenoviral IL-10
Transduced Dendritic Cells in Allotransplantation**

A Thesis submitted to the University of Adelaide
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by

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Thesis Abstract

The search for novel means of inducing permanent allograft acceptance without recourse to ongoing immunosuppressive therapy is a major goal for Transplantation Immunologists. Recent advances in immunology have identified Dendritic Cells (DC) as initiators and modulators of the alloimmune response to transplanted organs. As such they are potentially novel targets for therapeutic intervention to promote allograft acceptance. Under the influence of regulatory cytokines DC can behave in either a tolerogenic or immunogenic manner. Using a gene therapy strategy to target donor DC with immunosuppressive cytokines is a novel means of inhibiting the alloimmune response. The principal aim of this thesis was to study the capacity of DC transduced with the immunosuppressive gene construct adenoviral interleukin-10 (AdV IL-10) to inhibit alloimmune responses in both small and large animal transplantation models.

The first results chapter of this thesis describes the generation of human DC from monocyte precursors using the recombinant human cytokines IL-4 and GM-CSF. These cells were then transduced with AdV IL-10 and their *in vitro* allostimulatory properties studied. AdV IL-10 transduced DC showed down regulation of the co-stimulatory molecules CD80 / CD86 and impaired secretion of the pro-inflammatory cytokine IL-12. AdV IL-10 transduced DC were potent inhibitors of the alloimmune response in the MLR.

In chapter 4 a chimeric human-immunodeficient mouse skin transplantation model was used to test the capacity of AdV IL-10 transduced DC to modify human skin

graft rejection. DC transduced with AdV IL-10 inhibited skin graft rejection in comparison to DC transduced with the control gene construct adenoviral MX-17 (AdV MX-17) or fibroblasts transduced with AdV IL-10 indicating specificity of the AdV IL-10 DC effect.

Chapter 5 describes the characterization and transduction of pseudoafferent ovine DC with adenoviral gene constructs. Ovine DC were collected via cannulation of a pseudoafferent lymphatic vessel. Using the conditions derived from human DC experiments, ovine DC were transduced with AdV IL-10 and showed similar *in vitro* allo-inhibitory properties to human DC.

Chapter 6 describes migration studies of allogeneic ovine DC within an ovine system. For local migration to draining lymph nodes, AdV IL-10 DC were labeled with the fluorochrome PKH-26 and injected into allogeneic recipients. Dendritic cells migrated to draining lymph nodes and co-localized with endogenous CD83 positive cells. The transcript for AdV IL-10 could be detected by polymerase chain reaction analysis from the draining lymph node. Untransduced ovine DC were also labeled with ¹¹¹Indium-oxine and the systemic distribution followed by gamma camera for up to 7 days post intravenous or intra-dermal injection.

Chapter 7 describes renal transplantation experiments in the ovine heterotopic renal allograft model. Kidney donor DC transduced with either AdV IL-10 or AdV MX-17 were administered to recipient sheep either pre-transplant (day-7 and day - 1) or daily post transplant for 7 days as sole immunosuppressive therapy. Neither regimen was associated with prolonged allograft survival beyond 7 days.

These studies have shown promising *in vitro* evidence for gene therapy to modify DC function, which in small animal models can modify skin graft rejection. In large animals, despite promising *in vitro* and *in vivo* data genetically modified DC alone were not capable of prolongation of allograft survival, suggesting that these cells may require adjuvant immunosuppressive therapy to be used in future protocols.

Table of Contents

Title Page	1
Thesis Declaration	2
Acknowledgements	3
Thesis Abstract	6
Publications and Presentations	9
Table of Contents	12
Chapter 1 Literature Review	19
1.1 <i>Introduction</i>	19
1.2 <i>Dendritic Cell biology</i>	20
1.3 <i>Myeloid Dendritic Cells</i>	20
1.4 <i>Lymphoid Dendritic Cells</i>	23
1.5 <i>Dendritic Cells and Self Tolerance</i>	25
1.6 <i>Dendritic Cells and Transplantation</i>	27
1.7 <i>Dendritic Cells and Chimerism</i>	30
1.8 <i>Dendritic Cells and Tolerance Induction</i>	31
1.9 <i>Dendritic Cell Subsets and Transplantation</i>	33
1.10 <i>Interleukin-10 and Transplantation</i>	35
1.11 <i>Gene Therapy</i>	36
1.12 <i>Genetic Engineering of Dendritic Cells for Transplantation</i>	37
1.13 <i>Summary</i>	42
Thesis Hypothesis	43

Chapter 2	Materials and Methods	44
2.1	<i>Mononuclear Cell Protocols</i>	44
2.1.1	Isolation Of Peripheral Blood Mononuclear Cells	44
2.1.2	Two way Mixed Leukocyte Culture	45
2.2.2	One-way Mixed Leukocyte Culture	45
2.2.3	Concanavalin A (Con A) Stimulation Of PBMNC to generate blast cells	45
2.3	<i>RNA Extraction</i>	46
2.4	<i>Reverse Transcription (RT)</i>	47
2.5	<i>Polymerase Chain Reaction (PCR)</i>	47
2.6	<i>Gel Electrophoresis</i>	49
2.7	<i>Flow Cytometric Analysis (FACS)</i>	50
2.7.1	One colour analysis	50
2.7.2	Two colour analysis	50
2.8	<i>Electron Microscopy</i>	51
2.9	<i>Buffer Solutions</i>	51
2.9.1	General buffers	51
2.9.2	RNA buffers	51
2.9.3	PCR buffers	52
2.9.4	Gel electrophoresis buffers	52
2.10	<i>Reagents</i>	52

Chapter 3 Characterization and Transduction of Human Dendritic Cells with Adenoviral Gene Constructs

3.1	<i>Introduction</i>	56
3.2	<i>Materials and Methods</i>	59
3.2.1	Growth of Monocyte-Derived Dendritic Cells	59
3.2.2	Flow Cytometric Analysis	60
3.2.3	Transduction of Human Dendritic Cells with Adenoviral Gene Constructs	61
3.2.4	Cytokine Analysis of IL-10 Transduced DC in MLC	62
3.2.5	Metabolic labeling and immunoprecipitation of IL-10 by ³⁵ S Methionine	62
3.3	<i>Results</i>	63
3.3.1	Growth of human monocyte-derived dendritic cells	63
3.3.2	IL-4/GM-CSF treated monocytes develop into immature dendritic cells	63
3.3.3	Addition of TNF- α enhances maturation of immature dendritic cells to mature dendritic cells	63
3.3.4	Transduction of human monocyte-derived DC with adenoviral gene constructs	64
3.3.5	Transduction of human DC with AdV IL-10 inhibits the alloimmune response	64
3.3.6	Transduction of human DC with AdV IL-10 downregulates CD80-CD86	64
3.3.7	Transduction of monocyte-derived DC with AdV IL-10 downregulates DC cell surface expression of co-stimulatory molecules CD80-CD86	66

3.3.8	Transduction of human DC with AdV IL-10 inhibits interleukin 12 production in the MLR	68
3.4	<i>Discussion</i>	69
Chapter 4 An Experimental <i>In Vivo</i> Model System to Study the Capacity of Genetically Modified DC to inhibit Human Skin Graft Rejection in chimeric Humanized NOD-<i>scid</i> Mice		
4.1	<i>Introduction</i>	76
4.2	<i>Materials and Methods</i>	78
4.2.1	NOD- <i>scid</i> mice	78
4.2.2	Skin Grafting	78
4.2.3	Human Leukocyte Isolation and Reconstitution of the NOD- <i>scid</i> mouse and processing of skin grafts	79
4.2.4	Histological Analysis	80
4.2.5	Rejection Scoring	80
4.3	<i>Results</i>	81
4.3.1	NOD- <i>scid</i> mice engrafted with human skin will accept skin grafts indefinitely in the absence of allogeneic cells (>100 day survival)	81
4.3.2	Injection of allogeneic mononuclear cells produces rejection of human skin grafts	81
4.3.3	Injection of autologous mononuclear cells does not produce rejection of human skin grafts	82
4.3.4	Co-Injection of Dendritic Cells autologous to the skin graft produces accelerated human skin graft rejection	83
4.3.5	Injection of Dendritic Cells transduced with AdV IL-10 protects human skin grafts from rejection	83

4.3.6	Dendritic cells transduced with Adenoviral - β Galactosidase given via intraperitoneal injection migrate to the skin graft	83
4.3.7	Skin Donor Derived Fibroblasts Transduced with AdV IL-10 do not inhibit skin graft rejection in NOD-scid mice	84
4.3.8	Rejection Associated Cytokine Analysis of Human Skin Grafts	85
4.4	<i>Discussion</i>	86
Chapter 5 Characterization and Transduction of Ovine Pseudoafferent Dendritic Cells with Adenoviral Gene Constructs		
5.1	<i>Introduction</i>	91
5.2	<i>Materials and Methods</i>	93
5.2.1	Cannulation of Pseudoafferent Ovine Lymphatic Vessels	94
5.2.2	Separation of Ovine DC from Ovine Lymph using Metrizamide® Gradient Separation	94
5.2.3	Electron Microscopy of Ovine Dendritic Cells	95
5.2.4	Transduction of Ovine Dendritic Cells with Adenoviral Gene Constructs AdV GFP	96
5.2.5	Isolation of CD8 ⁺ Dendritic cells by Positive Selection using Labeled Immuno-magnetic Beads	96
5.3	<i>Results</i>	97
5.3.1	Flow Cytometric Characterization of Ovine DC	97
5.3.2	Electron Microscopic Characterization of Ovine DC	97
5.3.3	Pseudoafferent Ovine DC are Capable of Uptaking FITC-dextran	98
5.3.4	Pseudoafferent DC are Potent Stimulators of the Mixed Leukocyte Reaction	98

5.3.5	Transduction of Ovine Pseudoafferent DC with Adenoviral Gene Constructs	99
5.3.6	Transduction of Ovine DC with AdV IL-10 inhibits the Mixed Leukocyte Reaction	99
5.3.7	Effect of Transduction of DC with AdV IL-10 upon cytokine production in the Mixed Leukocyte Reaction	100
5.3.8	A population of ovine pseudoafferent dendritic cells express CD8	100
5.3.9	Electron Microscopic Analysis of Ovine CD8 ⁺ Dendritic cells	101
5.4	<i>Discussion</i>	102
Chapter 6 Migration Studies of allogeneic ovine DC in the sheep model		
6.1	<i>Introduction</i>	108
6.2.	<i>Materials and Methods</i>	110
6.2.1	Fluorochrome Labeling of Ovine DC and Microscopic Migration Studies	110
6.2.2	Labeling of Ovine Dendritic Cells with ¹¹¹ Indium oxine	111
6.2.3	Studies on the draining lymph node	111
6.2.4	Enrichment of Effector Cells from Draining Lymph Nodes	112
6.2.5	Cytotoxic T cell assay	112
6.2.6	Radioisotopic detection of ¹¹¹ Indium-oxine labeled cells	113
6.3	<i>Results</i>	113
6.3.1	Allogeneic Ovine DC migrate to T cell dependent areas within draining lymph nodes	113
6.3.2	Cytokine Profiles within Draining Lymph Nodes	114

6.3.3	AdV IL-10 Transduced DC inhibit the formation of donor-specific CTL within draining lymph nodes	115
6.3.4	Migration pattern of allogeneic ovine Dendritic Cells injected via intra-dermal injection	116
6.3.5	Migration pattern of allogeneic ovine Dendritic Cells injected via intravenous injection	117
6.0	<i>Discussion</i>	119
Chapter 7	A Large Animal Renal Transplantation Model using Dendritic Cell Therapy	
7.1	<i>Introduction</i>	126
7.2	<i>Materials and Methods</i>	129
7.2.1	Heterotopic Renal Allografting	129
7.2.2	Pre Transplantation Measurements of Alloreactivity	131
7.2.3	DC Administration Protocols	131
7.3	<i>Results</i>	133
7.3.1	Protocol 1: Pre-transplant administration of intra-dermal AdV IL-10 and AdV MX-17 transduced DC	133
7.3.2	Protocol 2: Post-transplant administration of AdV IL-10 and AdV MX-17 transduced DC	134
7.4	<i>Discussion</i>	137
Chapter 8	Concluding Remarks	145
	References	151
	Published papers	187