GENERATION OF TOLEROGENIC HUMAN DC THROUGH RAPAMYCIN CONDITIONING AND GENETIC MODIFICATION WITH HLA-G

A thesis submitted in partial fulfillment of the
PhD degree

in

The Department of Medicine
Faculty of Health Sciences
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by

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Notes:

Abbreviations

Abbreviation	*Expanded meaning*
AdV	Adenovirus
AICD	Activation Induced Cell Death
APC	Antigen Presenting Cells
CD	Cluster of Differentiation
CMV	Cytomegalovirus
CPM	Counts Per Minute
CsA	Cyclosporin A
DC	Dendritic cell
DMEM	Dulbecco's Modified Eagle Medium
FCS	Foetal Calf Serum
g	Gravity
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
Gy	Greys
H&E	Hematoxylin and Eosin
HEK	Human Embryonic Kidney
HLA-G	Human Leukocyte Antigen G
IDO	Indoleamine 3,3-Dioxygenase
IFN	Interferon
IL	Interleukin
ILT	Immunoglobulin-like Transcript
mAb	Monoclonal antibody
MFI	Mean Fluorescence Intensity
MHC	Major Histocompatability Complex
MLR	Mixed Lymphocyte reaction
NOD	Non obese diabetic
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PHA	phytohaemagglutinin
RAPA	Rapamycin
RPM	Revolutions Per Minute
RPMI	Roswell Park Memorial Institute
SCID	Severe Combined Immunodeficiency
Th	T helper cell
Treg	T regulatory cell

Declaration

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***List of publications**

- The following research article is published:

"Rapamycin downregulates the inhibitory receptors ILT2, ILT3, ILT4 on human dendritic cells and yet induces T cell hyporesponsiveness independent of FoxP3 induction" Immunol Lett. 2008 Oct 30;120(1-2):49-56.

- The following abstracts have been published:
    - Fedoric, B *et al.*. Immunology and Cell Biology, Vol. 84 Issue 3 Page A1 June 2006
    - Fedoric, B *et al.*. American Journal of Transplantation, Vol. 7 Supplement 2 Page 410 May 2007

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Boris Fedoric Dr Ravi Krishnan

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Thesis Summary

Dendritic cells (DC) are potent antigen presenting cells involved in the initiation of the alloimmune response and organ transplant rejection. This thesis, has investigated pharmacological and genetic approaches to manipulate DC in order to generate tolerogenic DC which elicit poor allostimulatory activity as potential cell therapy agents to treat allograft rejection.

In the first aspect of this study, human monocyte-derived DC were used to study the influence of Rapamycin (RAPA) on DC phenotype and function. This study showed that RAPA when added to monocytes prior to DC differentiation or after DC maturation generated tolerogenic DC as evidenced by the ability of these cells to induce T cell hypo-responsiveness. However, T cell hyporesponsiveness was associated with downregulation of costimulatory molecules only when added prior to differentiation and surprisingly was not influenced by the induction of $CD4^{+}FoxP3^{+}$ T cells. To assess the effects of RAPA on DC function in the transplant setting an *in vivo* chimeric model of ovine vascularised skin allograft transplantation was established in immunocompromised NOD/SCID mice as a host. This model was established as a preliminary model to acquire *in vivo* data prior to testing the effect of pharmacologically modified DC in the preclinical ovine model of renal allograft transplantation, also established in the host laboratory. Firstly, comparison of ovine DC obtained from cannulation of the prefemoral lymphatic vessels in sheep demonstrated that RAPA-modified ovine DC acted as poor stimulators of allogeneic ovine T cells similar to human DC treated with RAPA. Secondly, in NOD/SCID mice engrafted with ovine skin, the infusion of allogeneic ovine T cells together with RAPA-modified ovine DC reduced histological rejection in comparison to control DC.

In the second aspect of this study, the effects of genetic manipulation of DC were investigated. In order to investigate the effects of genetic modification of DC, two isoforms of the human HLA-G molecule, HLA-G1 (membrane bound) and HLA-G5 (soluble isoform) were used to generate adenoviral vectors. Unexpectedly, both HLA-G isoforms expressed by human DC transfectants were unable to induce allogeneic T cell hyporesponsiveness in the mixed lymphocyte reaction (MLR). Surprisingly, in the MLR the allogeneic T cells acquired HLA-G1, but not HLA-G5, indicating that direct cell contact and membrane transfer from DC to T cells occurred (Trogocytosis). In addition to HLA-G1, costimulatory molecules (CD40, CD80, CD86 and MHC Class II) were also co-transferred from DC to allogeneic T cells. Accordingly, in secondary proliferation assays T cells immunoselected after co-culture with allogeneic untransfected DC (T_{UT}) demonstrated potent antigen presenting activity when used as stimulators of autologous T cells (analogous to the indirect pathway of antigen presentation). In contrast to T_{UT}, immunoselected T cells that acquired HLA-G1 (T_{HLA-G1}) upon co-culture with DC-transfectants showed poor stimulatory capacity. Thus the data reported in this thesis supports the proposed novel concept that HLA-G acquired by T cells through genetically modified DC, functions to autoregulate T cells via T-T cell interaction through the HLA-G receptor ILT2 (negative signalling receptor) expressed on T cells.

In conclusion, this thesis has firstly provided supportive evidence that the pharmacological modification of human and ovine DC with RAPA has potential therapeutic effects on allograft rejection. Secondly, the genetic modification of DC to induce expression of HLA-G has specifically allowed the transfer of this molecule to T cells by trogocytosis and the inhibition of alloreactive T cell expansion.