Immunomodulatory Effects of Adenoviral CTLA4-EGFP Transfected Dendritic Cells in Allotransplantation.

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Summary

T cell activation occurs by the recognition of antigens presented by antigen presenting cells in the presence of sufficient CD28 costimulation. CTLA4 fusion proteins and interleukin 10 (IL10) are able to abrogate CD28 costimulation and therefore T cell activation. This thesis investigates the combined activity of these agents on allo-immunity and characterises the development of a gene therapy strategy utilising dendritic cells (DC) as a cellular vehicle to deliver CTLA4 fusion proteins.

Chapter 3 describes the effect of combined treatment of the DC-MLR with suboptimal doses of CTLA4-Ig and IL10. Inhibition of the MLR was augmented using the combination of the agents and interestingly a key role for Natural Killer (NK) cells in sensitising both DC and T cells to the inhibitory function of CTLA4-Ig and IL10 at low concentrations was highlighted.

Chapter 4 details the isolation and characterisation of ovine DC obtained by cannulation of the afferent lymphatics of the prefemoral lymph node of sheep. Importantly the isolated DC were potent allo-stimulators of the DC-MLR *in vitro* and were able to rapidly migrate to secondary lymphoid tissues upon *in vivo* administration. Moreover the intradermal injection of allogeneic DC and lymphocytes provoked a strong cutaneous reaction, confirming *in vivo* function and supporting the use of these cells for genetic manipulation to induce alloreactive T cell hyporesponsiveness.

Chapter 5 describes the generation of an adenoviral construct encoding a fusion of the extracellular domain of ovine CTLA4 and the gene for Enhanced Green Fluorescent Protein (EGFP). The adenoviral vector was able to infect both fibroblasts

and dendritic cells allowing production of CTLA4-EGFP proteins and detection of CTLA4-EGFP expression in transfected cells by virtue of the inherent fluorescence of EGFP. CTLA4-EGFP was able to bind to the CD80/86 ligands expressed on DC resulting in alloreactive T cell hyporesponsiveness. Moreover both ovine and human DC transfected with the adenoviral CTLA4-EGFP construct were able to inhibit the DC-MLR providing *in vitro* proof of concept and supporting the assessment of adenoviral CTLA4-EGFP transduced DC in an *in vivo* model of alloreactivity.

In **Chapter 6** an immunocompromised NOD-*scid* model of vascularised ovine skin transplantation was used to test the ability of adenoviral CTLA4-EGFP transduced DC to modify ovine skin allograft rejection after challenge with allogeneic ovine lymphocytes. Adenoviral CTLA4-EGFP transduced DC were able to migrate to the skin allograft and in comparison to DC transfected with the adenoviral vector blank control, inhibited rejection of the skin allograft. Moreover the inhibition of rejection was not associated with detectable levels of CTLA4-EGFP in circulation, indicating that adenoviral CTLA4-EGFP transduced DC are able to inhibit alloreactivity without the requirement of systemic immunosuppression. These data indicate that CTLA4-EGFP transduced DC are able to induce alloreactive T cell hyporesponsiveness *in vitro* and *in vivo* and supports further investigation in a preclinical ovine model of renal transplantation.