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

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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-immunodeficent 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 ^{111}Indium-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.

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