LENTIVIRUS-MEDIATED GENE EXPRESSION
IN CORNEAL ENDOTHELium

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To the memory of my father, R.W.R. Parker QC, who always encouraged me to have a love of learning.
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Summary

Modulation of corneal transplant rejection using gene therapy shows promise in experimental models but the most appropriate vector for gene transfer is yet to be determined. The overarching aim of the thesis was to evaluate the potential of a lentiviral vector for use in human corneal transplantation. Specific aims were: (i) to assess the ability of an HIV-1-based lentiviral vector to mediate expression of the enhanced yellow fluorescent protein (eYFP), and a model secreted protein interleukin-10 (IL10), in ovine and human corneal endothelium; and (ii) to examine the influence of lentivirus-mediated IL10 expression on the survival of ovine corneal allografts.

Four lentiviral vectors expressing eYFP under the control of different promoters, were tested: the simian virus type-40 (SV40) early promoter, the phosphoglycerate kinase (PGK) promoter, the elongation factor-1α (EF) promoter, and the cytomegalovirus (CMV) promoter. Two lentiviral vectors expressing IL10 were tested: one containing the SV40 promoter and another containing a steroid-inducible promoter (GRE5). Lentivirus-mediated expression in transduced ovine and human corneal endothelium was assessed by fluorescence microscopy, real-time quantitative RT-PCR and ELISA, following alterations of transduction period duration (2–24 hr) and vector dose, as well as in the presence or absence of polybrene or dexamethasone (GRE5 vector). It was also compared to expression mediated by adenoviral vectors. Orthotopic transplantation of ex vivo transduced donor corneas was performed in outbred sheep. Allografts were reviewed daily for vascularisation and signs of immunological rejection.

Lentivirus-mediated eYFP expression was delayed in ovine corneal endothelium compared to human. However, in both species the final transduction
rate was >80% and expression was stable for at least 14 d \textit{in vitro}. Lentivirus-mediated expression in ovine and human corneal endothelium was higher with the viral promoters in comparison to the mammalian promoters. A 24 h transduction of ovine corneal endothelium with the lentiviral vector encoding IL10 resulted in expression levels which were increasing after 15 d of organ culture but logarithmically lower than those achieved by adenovirus. Shortening the lentiviral transduction period to 2 h led to a reduction in expression, but the addition of polybrene (40 µg / ml) to the transduction mixture restored expression to levels comparable to those attained after a 24 h transduction period. Lentivirus-mediated IL10 expression was higher and more rapid in human corneal endothelium compared to ovine corneas. Dexamethasone-responsive transgene expression was observed in both ovine and human corneal endothelium using the lentiviral vector containing the GRE5 promoter. Lentivirus-mediated expression in ovine corneal endothelium was stable for 28 d \textit{in vivo}. A modest prolongation of ovine corneal allograft survival (median of 7 d) was achieved by transduction of donor corneas for 2–3 h with the lentivirus expressing IL10. Attempts to increase the expression of IL10 by the addition of polybrene (40 µg / ml) to the transduction mixture, resulted in a toxic effect on corneal allografts which abrogated the beneficial effect of IL10.

The lentiviral vector shows potential for the stable expression of therapeutic transgenes in human corneal transplantation. However, the mechanisms underlying the species-specific differences in HIV-1-mediated transgene expression will need to be elucidated and overcome if the ovine preclinical model is to provide justification for a clinical trial.
Declaration

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Douglas G.A. Parker
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Publication arising from this thesis

Abbreviations and symbols

< less than

> greater than

µg microgram

µl microlitre

µm micrometre

AAV adeno-associated virus

ACAID anterior chamber-associated immune deviation

Ad adenoviral vector

Amp ampicillin

APC antigen-presenting cell

BIV bovine immunodeficiency virus

bp base pair

BSS balanced salt solution (balanced for intraocular use)

CD cluster defined antigen

cDNA complementary DNA

cm centimetre

CMV cytomegalovirus

CPE cytopathic effect

cPPT central polypurine tract

CsCl caesium chloride

CTL cytotoxic T lymphocyte

CTLA-4 cytotoxic T lymphocyte-associated protein-4 (CD152)

Da dalton

DDH20 double distilled water

DEPC diethylpyrocarbonate
<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Medium</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>DTH</td>
<td>delayed-type hypersensitivity</td>
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<td>DTT</td>
<td>dithiothreitol</td>
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<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
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<tr>
<td>EDTA</td>
<td>ethylene diamine tetra acetic acid</td>
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<tr>
<td>EF</td>
<td>elongation factor 1 alpha</td>
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<tr>
<td>eGFP</td>
<td>enhanced green fluorescent protein</td>
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<tr>
<td>EIAV</td>
<td>equine infectious anaemia virus</td>
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<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
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<td>eYFP</td>
<td>enhanced yellow fluorescent protein</td>
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<tr>
<td>FACS</td>
<td>fluorescence activated cell sorter</td>
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<td>FCS</td>
<td>foetal calf serum</td>
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<td>gauge</td>
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<td>GAPDH</td>
<td>glyceraldehyde 3-phosphate dehydrogenase</td>
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<td>HEPES</td>
<td>N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulphonic acid)</td>
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<td>his</td>
<td>histidine</td>
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<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HRP</td>
<td>horseradish peroxidase</td>
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<td>IFN-γ</td>
<td>interferon gamma</td>
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<td>Ig</td>
<td>immunoglobulin</td>
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<td>IL</td>
<td>interleukin</td>
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<td>IU</td>
<td>international unit</td>
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<td>iu</td>
<td>infectious unit</td>
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<td>Kan</td>
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<td>kilobase</td>
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<td>kilodalton</td>
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<td>LPS</td>
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<td>long terminal repeat</td>
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<td>major histocompatibility complex</td>
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<td>millilitre</td>
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<td>mM</td>
<td>millimolar</td>
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<tr>
<td>MOI</td>
<td>multiplicity of infection</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>MW</td>
<td>molecular weight</td>
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<tr>
<td>n</td>
<td>sample size</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
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<tr>
<td>NK</td>
<td>natural killer</td>
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<td>nm</td>
<td>nanometre</td>
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<tr>
<td>°C</td>
<td>degree Celsius</td>
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<td>OD&lt;sub&gt;X&lt;/sub&gt;</td>
<td>optical density at wavelength X (nanometres)</td>
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<tr>
<td>ori</td>
<td>origin of replication, part of adenoviral genome</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>pfu</td>
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<td>PGK</td>
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<td>qRT-PCR</td>
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<td>rAAV</td>
<td>recombinant adeno-associated virus vector</td>
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<td>rpm</td>
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<td>RRE</td>
<td>Rev response element</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
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<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
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<tr>
<td>s</td>
<td>second</td>
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<tr>
<td>SA</td>
<td>streptavidin</td>
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<tr>
<td>SD</td>
<td>standard deviation of the mean</td>
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<td>SV40</td>
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<tr>
<td>TCR</td>
<td>T cell receptor</td>
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<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
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<tr>
<td>T_m</td>
<td>melting temperature</td>
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<tr>
<td>TU</td>
<td>transducing unit</td>
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<tr>
<td>TNF-α</td>
<td>tumour necrosis factor alpha</td>
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<tr>
<td>UV</td>
<td>ultraviolet light</td>
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<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
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