

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-1 α (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 *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 *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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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
DDH2O	double distilled water
DEPC	diethylpyrocarbonate

DNA	deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium
dNTP	deoxynucleotide triphosphate
DTH	delayed-type hypersensitivity
DTT	dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylene diamine tetra acetic acid
EF	elongation factor 1 alpha
eGFP	enhanced green fluorescent protein
EIAV	equine infectious anaemia virus
ELISA	enzyme linked immunosorbent assay
eYFP	enhanced yellow fluorescent protein
FACS	fluorescence activated cell sorter
FCS	foetal calf serum
g	gram; unit of gravity
G	gauge
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
h	hour
HEPES	N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulphonic acid)
his	histidine
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HRP	horseradish peroxidase
IFN- γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
IU	international unit

iu	infectious unit
Kan	kanamycin
kb	kilobase
kDa	kilodalton
L	litre
LPS	lipopolysaccharide
LTR	long terminal repeat
LV	lentiviral vector
M	molar
mg	milligram
MHC	major histocompatibility complex
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
MW	molecular weight
n	sample size
ng	nanogram
NK	natural killer
nm	nanometre
°C	degree Celsius
OD _x	optical density at wavelength X (nanometres)
ori	origin of replication, part of adenoviral genome
PBS	phosphate buffered saline
PCR	polymerase chain reaction

pfu	plaque forming unit
PGK	phosphoglycerate kinase
polyA	polyadenylation site
qRT-PCR	quantitative reverse transcription polymerase chain reaction
rAAV	recombinant adeno-associated virus vector
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
RRE	Rev response element
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
s	second
SA	streptavidin
SD	standard deviation of the mean
SV40	simian virus type-40
TCR	T cell receptor
TGF- β	transforming growth factor beta
T _m	melting temperature
TU	transducing unit
TNF- α	tumour necrosis factor alpha
UV	ultraviolet light
v/v	volume per volume
VEGF	vascular endothelial growth factor
w/v	weight per volume