REGIONAL IMMUNOSUPPRESSION FOR CORNEAL TRANSPLANTATION

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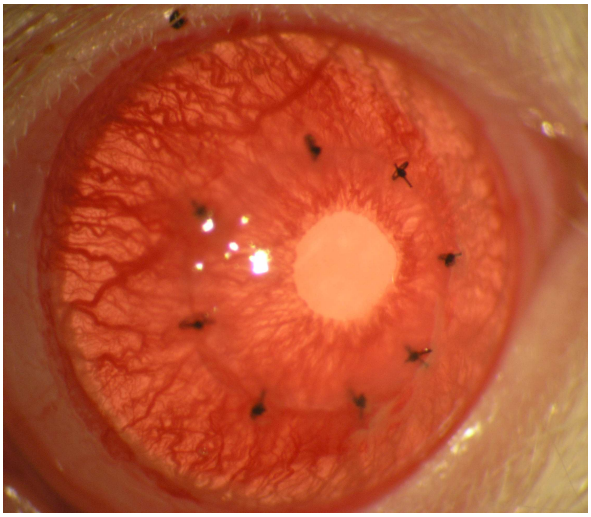

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ABSTRACT

Corneal transplantation is performed to restore vision or to relieve pain in patients with damaged or diseased corneas. However, approximately 40% of corneal allografts fail after 10 years. The most common cause of graft failure is irreversible immunological rejection, primarily mediated by CD4+ T cells, despite the topical application of glucocorticosteroids. The aim of this project was to investigate the anatomic site of antigen presentation during corneal transplantation in the rat, by using a lentiviral vector to express an anti-CD4 antibody fragment at potential sites of antigen presentation, including the donor corneal endothelium, the anterior segment of the eye and the cervical lymph nodes.

Dual-gene lentiviral vectors were constructed by inserting the 2A self-processing sequence between two transgenes. This allowed expression of two transgenes within a single open reading frame. *In vitro* characterisation of the dual-gene vectors was performed in cell culture experiments, which showed that transgenic proteins were expressed at lower levels from dual-gene vectors compared to the expression from single-gene vectors and expression was lowest when the transgene was situated downstream of the 2A self-processing sequence.

To locate the anatomic site of antigen presentation during corneal transplantation in rats, a lentiviral vector carrying an anti-CD4 antibody fragment was delivered to the corneal endothelium either immediately prior to corneal transplantation by *ex vivo* transduction of the donor corneas, or 5 days prior to corneal transplantation by anterior chamber injection into both the recipient and the donor rats. A separate group of recipient rats received intranodal injections of the lentiviral vector carrying

an anti-CD4 antibody fragment into the cervical lymph nodes 2 days prior to corneal transplantation. Another group of rats underwent bilateral lymphadenectomy of the cervical lymph nodes 7 days prior to corneal transplantation. Corneal allografts were scored daily for opacity, inflammation and neovascularisation. Expression of the anti-CD4 antibody fragment from transduced tissues was detected using flow cytometry and polymerase chain reaction. Modest, but significant prolongation of corneal allograft survival was experienced by rats that received *ex vivo* transduction of the donor corneas with a lentiviral vector carrying an anti-CD4 antibody fragment immediately prior to corneal transplantation, but all grafts did eventually reject. Anterior chamber injection of the lentiviral vector carrying the anti-CD4 antibody fragment 5 days prior to corneal transplantation into both recipient and donor eyes did not prolong allograft survival. Intranodal injection of a lentiviral vector carrying an anti-CD4 antibody fragment did not prolong the survival of the corneal allografts, nor did bilateral lymphadenectomy of the cervical lymph nodes 7 days prior to corneal transplantation.

Neither expression of the anti-CD4 antibody fragment in the cervical lymph nodes nor the removal of these nodes was able to prolong corneal allograft survival in rats, suggesting that T cell sensitisation could potentially occur elsewhere in the body. However, expression of the anti-CD4 antibody fragment from the donor corneal endothelium was able to prolong corneal allograft survival, suggesting that some antigen presentation might occur within the anterior segment of the eye. Based on the findings described in this thesis and those of others, I propose that antigen presentation in the rat occurs within anterior segment of the eye and within the secondary lymphoid tissues such as the cervical lymph nodes, and that inhibiting

antigen presentation at one of these sites will delay graft rejection. However, to completely abolish antigen presentation during corneal transplantation in the rat, I hypothesise that antigen presentation within both the anterior segment of the eye and within the secondary lymphoid tissues must be inhibited.

CONFERENCE PRESENTATIONS ARISING FROM THIS THESIS

Brice S.L., Mortimer L.M., Marshall K.A., Brereton H.M., Williams K.A. Lentiviral-mediated gene transfer of anti-CD4 scFv prolongs corneal allograft survival. 2009 May 29-April 1, Australian Gene Therapy Society meeting, Sydney, poster presentation.

Brice S.L., Mortimer L.M., Brereton H.M., Williams K.A. Lentiviral gene transfer to the rat cornea. 2008 August 9-14, The Transplantation Society – XXII International Congress, Sydney, poster presentation.

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.

Sarah L Brice

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Lastly, I would like to dedicate this thesis to my grandparents who have always believed in me. You have been my inspiration and Grandpa I wish you were able to see the final product.

ABBREVIATIONS

>	greater than
<	less than
°C	degrees Celsius
µg	microgram
µl	microlitre
µm	micrometre
A549	human lung adenocarcinoma epithelial cell line
AAV	adeno-associated viral vector
AC	anterior chamber
ACAID	anterior chamber-associated immune deviation
Adv	adenoviral vector
AE	amplification efficiency
Ag	antigen
AIDS	acquired immunodeficiency syndrome
APC	antigen presenting cell
ARBP	acidic ribosomal phosphoprotein
bp	base pair
BSS	balanced salt solution
$CaCl_2$	calcium chloride
CALT	conjunctiva-associated lymphoid tissue
CB-Dx	cascade blue dextran
CCTS	The American Collaborative Corneal Transplant Study
CH	constant domain of immunoglobulin heavy chain
CHO	Chinese hamster ovarian cell line

CD	cluster of differentiation
CD40L	CD40 ligand
cDNA	complementary deoxyribonucleic acid
CGD	chronic granulomatous disease
CL	constant domain of immunoglobulin light chain
CLN	cervical lymph node
cm	centimetre
CMV	cytomegalovirus immediate early promoter
CPE	cytopathic effects
cPPT	central polypurine tract
CT	cycle threshold
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T lymphocyte-associated protein-4 (CD152)
Da	Dalton
DC	dendritic cell
DDH_2O	double distilled water
DEPC	diethylpyrocarbonate
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTP	dinucleotide triphosphate
ds	double stranded
DTH	delayed type hypersensitivity
DTT	dithiothreitol
eGFP	enhanced green fluorescent protein

eYFP	enhanced yellow fluorescent protein
ECACC	European Collection of Cell Cultures
E. Coli	*Escherichia coli*
EK5	human endostatin::kringle-5 fusion protein
ELISA	enzyme-linked immunosorbent assay
ETDA	ethylene diamine tetra acetic acid
EU	endotoxin unit
F2A	FMDV 2A self-processing sequence with a furin cleavage site immediately upstream of 2A, and a 2B proline residue at its C-terminus
F344	Fisher 344 inbred rat strain
Fab	monomeric antigen binding fragment
FACS	fluorescence-activated cell sorting
FasL	Fas-ligand (CD95L)
Fc	crystallisable fragment
FCS	fetal calf serum
FDA	Food and Drug Administration
fHSS	factor H secretory sequence
FITC	fluorescein isothiocyanate
FMDV	foot and mouth disease virus
g	gram
g	unit of gravity
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
HeBS	HEPES-buffered saline

HEK-293A	human embryonic kidney cell line with E1- region of adenovirus 5
HEK-293T	human embryonic kidney cell line that constitutively expresses the SV40 large T cell antigen
HEPES	N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid
HIS6 tag	6 histidine tag
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HRP	horseradish peroxidise
HPRT	hypoxanthine guanine phosphoribosyl-transferase
Hz	Hertz
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
IRES	internal ribosome entry sites
IU/ml	international units/ml
kb	kilobase
kDa	kilodalton
L	litre
LB	luria bertani
LC	Langerhans cells
LCA2	Leber's congenital amaurosis type 2
LIP	liposome-incorporated
log	logarithm
$\log_e$	natural logarithm
LTR	long terminal repeat

LV	lentiviral vector
LYVE-1	lymphatic vessel endothelial hyaluronan receptor 1
M	Molar
mAb	monoclonal antibody
MLN	mesenteric lymph node
MFI	mean fluorescence intensity
mg	milligram
MHC	major histocompatibility complex
ml	millilitre
MLR	mixed lymphocyte reaction
MLV	Molony murine leukaemia viral vector
mm	millimetre
MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
MW	molecular weight
NIH	National Institutes of Health
ng	nanogram
NHMRC	National Health and Medical Research Council
NK	natural killer cell
NTC	no template control
OD	optical density
ORF	open reading frame
OVA	ovalbumin peptide
pA	polyadenylation signal
PBL	peripheral blood lymphocytes

PBS	phosphate buffered saline
PC2	physical containment level 2
PCR	polymerase chain reaction
PE	phycoerythrin
pfu	plaque forming unit
pg	pictogram
PGK	phosphoglycerate kinase
pmol	picomole
polyA	polyadenylation site
PPT	polypurine tract
qPCR	quantitative real-time polymerase chain reaction
qRT-PCR	quantitative reverse transcription real-time polymerase chain reaction
RBC	red blood cells
RCR	replication competent recombinant
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
RRE	rev response element
RREXt	extended rev response element
RT	reverse transcription
SAP	shrimp alkaline phosphatase
SAPE	streptavidin R-phycoerythrin
SCID-X1	x-linked severe combined immunodeficiency disorder
scFv	single chain fragment variable
SD	standard deviation
sFlt-1	soluble vascular endothelial growth factor receptor 1

SIN	self inactivating
SOC	Super Optimal Broth with 20 mM glucose. ‘C’ stands for catobolite repression, reflective of the added glucose.
SOE-PCR	splice overlap extension polymerase chain reaction
ss	single stranded
SV40	simian-like virus type-40 early promoter
Tc	cytotoxic response
$TCID_{50}$	tissue culture infectious dose method
TCR	T cell receptor
TGF-β	transforming growth factor beta
Th	T helper response
T_m	melting temperature
TNF	tumour necrosis factor
TU	transducing units
UV light	ultraviolet light
v/v	volume per volume
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VH	variable domain of immunoglobulin heavy chain
VL	variable domain of immunoglobulin light chain
VSV	vesicular stomatitis virus
VSV-G	vesicular stomatitis virus glycoprotein G
whv	woodchuck hepatitis virus post-transcriptional element
w/v	weight per volume
WF	Wistar Furth inbred rat strain

WT	wild type