Superantigens as vaccine delivery vehicles for the generation of cellular immune responses

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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2006

Abstract

The constant battle between pathogen and host has led to substantial diversity and adaptability of the host immune system. Pathogens too, have evolved unique mechanisms to evade their hosts. The production of superantigens is one of these mechanisms.

Superantigens are potent T cell mitogens that have the unique ability to bind simultaneously to major histocompatibility complex (MHC) class II molecules and T cell receptors (TCRs). The resulting uncontrolled activation of up to 20% of all T cells and the subsequent cytokine release, can lead to fever, shock and death. Superantigens are not processed intracellularly like conventional antigens but instead bind as intact proteins to MHC class II molecules expressed on the surface of professional antigen presenting cells.

On the hypothesis that the unique properties of superantigens may serve useful for vaccine delivery, several bacterial superantigens were selectively mutated at their TCR-binding site with the ultimate goal of creating a safe, non-toxic carrier protein that could target antigen presenting cells by binding to MHC class II.

Antigen presenting cells that expressed MHC class II were indeed targeted by the TCR-binding-deficient superantigens. Cellular internalisation of the superantigen into vesicles was observed as early as 30 min. These superantigens were also shown to traffic to, and be captured by, the lymph nodes of immunised mice. Using TCR-binding-deficient superantigens as vaccine carrier proteins, enhanced antigenicity and immunogenicity of the conjugated MHC class I-restricted peptide antigen, GP33, was observed in a mouse model. *In vitro* studies revealed up to 200-fold enhancement of antigenicity when GP33 was conjugated to superantigen. Enhanced immunogenicity was also observed *in vivo*, with

conjugates providing protection against Lymphocytic choriomeningitis virus infection after only a single immunisation. These results indicate that modified superantigens are able to safely deliver peptides for cross-presentation, and may serve as a novel mechanism for vaccine delivery.

In memory of Donald Loh

Your love, support, and guidance will be with me always

Acknowledgements

I would like to thank Professor John Fraser for giving me the incredible opportunity to work on this project. His inspirational attitude towards science will always be remembered.

Thank you to my co-supervisors Birgit Schrage and Diana Schuhbauer for their guidance, advice and friendship.

To past, present and honorary members of the Lab: Indira Basu, Fiona Clow, Matthew Chung, Vanessa Handley, Nicola Jackson, Ries Langely, Guy Moffatt, Thomas Proft, Amanda Taylor, Natasha Willoughby, Phillip Webb and Lily Yang, thank you for your contributions and for being a second family to me.

Thank you to all my friends, especially to the "dinner out girls": Carissa Fonseca, Nimali Withana and Lily Yang, for which without their love and support would not have made this chapter in my life complete.

To Donald, Rosie and Selina, for being the best family anyone could hope for. Mum, thank you for sacrificing so much for me and for putting up with me for all these years.

To Regan Hughes, not only for the tough job of proof-reading this thesis, but also for believing in me and helping me through the tough times. Thank you for your endless encouragement and unconditional love.

This work was generously supported by scholarships and research grants from the University of Auckland, the Health Research Council of New Zealand and Auckland UniServices.

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Abbreviations

°C	Degrees Celsius
2-ME	2-mercaptoethanol
APC	Antigen presenting cell
APS	Ammonium persulfate
ATP	Adenosine triphosphate
β_2m	β_2-microglobulin
BiP	Immunoglobulin heavy chain binding protein
BMDC	Bone marrow-derived dendritic cell
CD	Cluster of differentiation
CDR	Complementary determining region
CFA	Complete Freund's adjuvant
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CLIP	Class II-associated invariant chain peptide
CPM	Counts per minute
C-terminal	Carboxy-terminal
CTL	Cytotoxic T lymphocyte
CTLA	Cytotoxic T lymphocyte-associated antigen
CV	Column volumes
CyaA	Adenylate cyclase toxin
Cα	Constant region of the T cell receptor α-chain
Cβ	Constant region of the T cell receptor β-chain
DAPI	4',6-diamidino-2-phenylindole
DC	Dendritic cell
ddH_2O	Deionised water
DMA	N,N-dimethylacetamide
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
dTTP	Deoxythymidine triphosphate
E:T	Effector:target
EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
EF	edema factor
EMEM	Eagle's minimum essential medium
ER	Endoplasmic reticulum
Fab	Antigen binding fragment
FACS	Fluorescent activated cell sorter
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
FR	Framework region
g	Gram or gravity
GM-CSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GSH	Glutathione
GST	Glutathione-S-transferase
h	Hour or hours
HA	Hem agglutinin

HBsAg	Hepatitis B surface antigen
HCMV	Human cytomegalovirus
Hib	*Haemophilus influenza* type B
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
HPV	Human papilloma virus
HSP	Heat-shock protein
HSV	Herpes simplex virus
HV	Hypervariable region
ICAM	Intracellular adhesion molecule
IDA	Iminodiacetic Acid
IFA	Incomplete Freund's adjuvant
IFN	Interferon
Ii	Invariant chain
IL	Interleukin
IPTG	Isopropylthiogalactose
IVIG	Intravenous immunoglobulin
kDa	Kilo Daltons
KSHV	Kaposi's sarcoma-associated herpes virus
L	Litre
LB	Luria-Bertani broth
LCMV	Lymphocytic choriomeningitis virus
LF	Lethal factor
LFA	Leukocyte function-associated antigen
LMP	Low molecular weight protein
LPS	Lipopolysaccharide
M	Molar
mA	Milliamperes
mBMDC	Murine bone marrow-derived dendritic cell
MCS	Multiple cloning site
MECL	Multicatalytic endopeptidase complex like
mg	Milligram
MHC	Major histocompatibility complex
MHV	Mouse hepatitis virus
MIIC	MHC class II compartment
µl	Microlitre
µM	Micromolar
min	Minutes
ml	Millilitre
mM	Millimolar
MOPS	3-(N-Morpholino) propanesulfonic acid
MQ H_2O	MilliQ water
MW	Molecular weight
ng	Nanogram
NHS	N-hydroxysuccinimide
N-terminal	Amino-terminal
OMP	Outer membrane protein
PA	Protective antigen
PAMP	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cell

pBS	pBlueScript
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
pfu	Plaque-forming units
pg	Picogram
pMHC	peptide-MHC complex
PMSF	Phenyl methyl sulphonyl fluoride
RBC	Red blood cell
RNA	Ribonucleic acid
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute medium
RT	Room temperature
SCID	Severe combined immune deficiency
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	Staphylococcal enterotoxin
SMBS	Sulfo-*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester
SPE	Streptococcal pyrogenic exotoxin
Spp.	Denotes more than one species
SSA	Streptococcal superantigen
TAP	Transporter associated with antigen processing
TB	Terrific broth
TCR	T cell receptor
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFB	Transformation buffer
tg	Transgenic
TGN	Transgolgi network
Th	T helper cell
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TP	Tryptone-phosphate medium
TPCK	L-1-tosylamido-2-phenylethyl chloromethyl ketone
Tris	2-amino-2-(hydroxymethyl)propane-1,3-diol
TSS	Toxic shock syndrome
TSST	Toxic shock syndrome toxin
VCAM	Vascular cell adhesion molecule
VLP	Virus-like particles
Vα	Variable region of the T cell receptor α-chain
Vβ	Variable region of the T cell receptor β-chain
WT	Wild-type