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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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The University of Auckland
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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.

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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>2-ME</td>
<td>2-mercaptoethanol</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen presenting cell</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulfate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>$\beta_2$m</td>
<td>$\beta_2$-microglobulin</td>
</tr>
<tr>
<td>BiP</td>
<td>Immunoglobin heavy chain binding protein</td>
</tr>
<tr>
<td>BMDC</td>
<td>Bone marrow-derived dendritic cell</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CDR</td>
<td>Complementary determining region</td>
</tr>
<tr>
<td>CFA</td>
<td>Complete Freund's adjuvant</td>
</tr>
<tr>
<td>CFSE</td>
<td>Carboxyfluorescein diacetate succinimidyl ester</td>
</tr>
<tr>
<td>CLIP</td>
<td>Class II-associated invariant chain peptide</td>
</tr>
<tr>
<td>CPM</td>
<td>Counts per minute</td>
</tr>
<tr>
<td>C-terminal</td>
<td>Carboxy-terminal</td>
</tr>
<tr>
<td>CTL</td>
<td>Cytotoxic T lymphocyte</td>
</tr>
<tr>
<td>CTLA</td>
<td>Cytotoxic T lymphocyte-associated antigen</td>
</tr>
<tr>
<td>CV</td>
<td>Column volumes</td>
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<td>CyaA</td>
<td>Adenylate cyclase toxin</td>
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<td>Cα</td>
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<td>Antigen binding fragment</td>
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<td>Granulocyte-macrophage colony stimulating factor</td>
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<tr>
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</tr>
<tr>
<td>GST</td>
<td>Glutathione-S-transferase</td>
</tr>
<tr>
<td>h</td>
<td>Hour or hours</td>
</tr>
<tr>
<td>HA</td>
<td>Hem agglutinin</td>
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XVII
HBsAg  Hepatitis B surface antigen  
HCMV  Human cytomegalovirus  
Hib  *Haemophilus influenzae* 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 H₂O  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  

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