Development of a Novel
Co-vaccination Approach for
Pneumococcal and Influenza Infections

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A thesis submitted for the fulfilment of the
Degree of Doctor of Philosophy

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ABBREVIATIONS

A₄₀₅  | Absorbance at 405 nm
A₄₅₀  | Absorbance at 450 nm
ABC   | ATP-binding cassette
ANT3  | Adenine nucleotide translocator 3
APC   | Antigen presenting cells
A/PC  | A/Port Chalmers/1/73 [H3N2]
A/PR8 | A/Puerto Rico/8/34 [H1N1] influenza strain
CbpA  | Choline-binding protein A
CD    | Cluster of differentiation
CFU   | Colony forming unit
ChoP  | Phosphorylcholine
CPG ODN | Cytosine phosphate guanosine oligodeoxynucleotides
CPS   | Capsular polysaccharides
CRP   | C-reactive protein
CTL   | Cytotoxic T lymphocytes
CT    | Cholera toxin
DC    | Dendritic cell
DI    | Dry ice
DMEM  | Dulbecco’ Modified Eagle’s Medium
DTaP  | Diphtheria-tetanus-acellular pertussis vaccine
Eno   | Enolase
FACS  | Fluorescent activated cell sorting
FCS   | Foetal Calf Serum
FcR   | Fc Receptor
FFI   | Focus forming inhibition
Foxp3 | Forkhead box P3
HA    | Hemagglutinin
Hep B | Hepatitis B virus
Hib   | Haemophilus influenza type b
HPV   | Human papilloma virus
HRP   | Horse Radish Peroxidase
IFN-I | Type I Interferon (α/β)
IF-   | Interleukin
IFN   | Interferon
IFN-γ | Interferon gamma
Ig    | Immunoglobulin
IN    | Intranasally
IP    | Intraperitoneally
IPD   | Invasive pneumococcal disease
IPV   | Inactivated poliovirus
IRF   | Interferon regulatory factors
IV    | Intravenously
KO    | Knock out
kGy   | kiloGray
LAIIV | Live attenuated influenza vaccines
LT    | Labile toxin
LytA  | Autolysin
M1/2 Matrix protein 1/2
MARCO Macrophage receptor with collagenous structure
M cells Microfold cells
MC Mannosylated Chitosan
MFI Mean fluorescence intensity
mg milligram/s
MHC Major histocompatibility complex
mL millilitre/s
MMR Measles, Mumps and Rubella vaccine
MPL Monophosphoryl lipid A
NA Neuraminidase
NALT Nasopharynx-associated lymphoid tissue
NEP Nuclear Export Protein
NF- Nuclear Factor
NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells
NK Natural Killer cell
NKT Natural Killer T cell
NLR Nod-like receptor/s
NP Nucleoprotein
NPP Nucleoprotein peptide
NS1 Non-structural protein 1
OD Optical density
PA Acidic polymerase
PAFr Platelet-activating factor receptor
PAMPs Pathogen associated molecular patterns
PavA Pneumococcal adhesion and virulence A
PB1/2 Basic polymerase protein 1/2
PBS Phosphate buffered saline
PBPss Penicillin Binding proteins
PCR Polymerase chain reaction
PCVs Pneumococcal conjugate vaccines
PdT Pneumolysin mutant
PhtD Pneumococcal histidine triad D
PhtE Pneumococcal histidine triad E
Ply Pneumolysin
PKR Protein Kinase R
PsaA Pneumococcal surface antigen A
PspA Pneumococcal surface protein A
PspC Pneumococcal surface protein C
RNA Ribonucleic acid
RNP Ribonucleoprotein
PRR Pattern recognition receptor
RT Room temperature
RIG Retinoic acid–inducible gene like receptors
*S. pneumoniae* Streptococcus pneumoniae
*S. aureus* Staphylococcus aureus
SD Standard deviation
SFV Semliki Forest Virus
ssRNA Single-stranded ribonucleic acid
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<tr>
<td>TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% tissue culture infective dose</td>
</tr>
<tr>
<td>TCR</td>
<td>T Cell receptor</td>
</tr>
<tr>
<td>Tfh</td>
<td>Follicular CD&lt;sup&gt;4&lt;/sup&gt; T helper</td>
</tr>
<tr>
<td>Th17</td>
<td>CD&lt;sup&gt;4&lt;/sup&gt; T helper 17</td>
</tr>
<tr>
<td>Th1</td>
<td>CD&lt;sup&gt;4&lt;/sup&gt; T helper 1</td>
</tr>
<tr>
<td>THY</td>
<td>Todd-Hewitt broth</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
</tr>
<tr>
<td>TRM</td>
<td>Tissue resident memory</td>
</tr>
<tr>
<td>WC</td>
<td>Whole-cell</td>
</tr>
<tr>
<td>WCV</td>
<td>Whole-cell vaccine</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type</td>
</tr>
<tr>
<td>α-GalCer</td>
<td>Alpha-galactosylceramide</td>
</tr>
<tr>
<td>μg</td>
<td>microgram/s</td>
</tr>
<tr>
<td>μL</td>
<td>microlitre/s</td>
</tr>
<tr>
<td>γδ T</td>
<td>Gamma-delta T</td>
</tr>
<tr>
<td>γδ T&lt;sub&gt;17&lt;/sub&gt;</td>
<td>Gamma-delta T cells secreting IL-17&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>γ-FLU</td>
<td>Gamma-irradiated influenza vaccine</td>
</tr>
<tr>
<td>γ-PN</td>
<td>Gamma-irradiated <em>Streptococcus pneumoniae</em> vaccine</td>
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<tr>
<td>γ-SFV</td>
<td>Gamma-irradiated Semliki Forest vaccine</td>
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DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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________________________________________
Rachelle Babb

Date
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# PATENTS, PUBLICATIONS AND CONFERENCE PRESENTATIONS ARISING FROM THIS THESIS

## PATENTS

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<td>Streptococcal Vaccine</td>
<td>Rachelle Babb, Mohammed Alsharifi, Austen Yannis Chen, Shannon Christa David, Timothy Raymond Hirst, Abiodun David Ogunniyi, James Cleland Paton</td>
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## PUBLICATIONS

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<td>Intranasal vaccination with gamma-irradiated Streptococcus pneumoniae whole-cell vaccine provides serotype-independent protection mediated by B cells and innate IL-17 responses</td>
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<td>Enhanced protective CD4+ T cell responses to a serotype independent pneumococcal vaccine when combined with an inactivated influenza vaccine</td>
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## CONFERENCE PRESENTATIONS

- The 12th European Meeting on the Molecular Biology of the Pneumococcus (Oxford University, Oxford, UK, 2015).  
  **Poster**

- School of Biological Sciences Research Symposium (University of Adelaide, Adelaide, Australia, 2013-2014).  
  **Presentation**
ABSTRACT

*Streptococcus pneumoniae* and influenza are the world’s foremost bacterial and viral respiratory pathogens. In addition to their individual clinical significance, co-infection with these pathogens enhances disease progression and is associated with substantially increased mortality rates. Vaccination is the best preventative method to control disease caused by individual pathogens as well as co-infection. Gamma-irradiation is considered a safe sterilization method, used routinely to sterilize medical devices, pharmaceuticals and most commonly food products. It can also be utilised as an inactivation technique to generate whole cell bacterial and viral vaccines with minimal impact on pathogen structure and antigenic determinants. This study presents the first evidence illustrating the use of this inactivation technique for development of a mucosal *S. pneumoniae* whole cell vaccine (γ-PN). Gamma-irradiation was utilised to inactivate an unencapsulated *S. pneumoniae* strain Rx1 with an unmarked deletion of the autolysin gene and with the pneumolysin gene replaced with an allele encoding a non-toxic pneumolysoid. Intranasal administration of mice with γ-PN without an adjuvant was shown to elicit serotype-independent protection against pneumococcal challenge in models of sepsis and pneumonia. In particular, vaccine efficacy was shown to be reliant on B cells and IL-17 responses. Importantly, immunisation promoted IL-17 production by γδ T cells, as opposed to conventional Th17 cells commonly reported with other pneumococcal whole cell vaccines. Moreover, this study also illustrated that the immunogenicity and protective efficacy of the γ-PN vaccine can be enhanced in the presence of the mucosal adjuvant, cholera toxin.

In addition, this study describes a novel combination vaccine approach comprising inactivated whole bacterial cells and whole virions to *S. pneumoniae* and influenza respectively. In this study mice were co-immunised intranasally with the un-adjuvanted γ-PN vaccine and a gamma-irradiated influenza vaccine (γ-FLU). Interestingly, co-immunisation was shown to enhance γ-PN vaccine efficacy and immunogenicity against virulent pneumococcal challenge, which was dependent on CD4⁺ T cell responses. In contrast to vaccination with γ-PN alone, co-immunisation enhanced pneumococcal-specific effector Th17 and Th1 memory cells, promoted development of CD4⁺ tissue-resident memory cells, and enhanced pneumococcus-specific antibody responses. In addition, this combination approach was shown to elicit significant protection against lethal influenza challenge, as well
as against co-infection with both influenza and *S. pneumoniae*. These data support the notion that γ-FLU exhibits adjuvant-like properties to enhance immunogenicity of a co-administered vaccine without compromising pathogen-specific immune responses. Future work will be focused on clinical development of individual and combination vaccines.