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

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ABBREVIATIONS

A_{405}	Absorbance at 405 nm
A_{450}	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)
IL-	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
LAIV	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
PBPs	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

$TCID_{50}$	50% tissue culture infective dose
TCR	T Cell receptor
Tfh	Follicular $CD4^+$ T helper
Th17	$CD4^+$ T helper 17
Th1	$CD4^+$ T helper 1
THY	Todd-Hewitt broth
TLR	Toll-like receptor
TNF	Tumour Necrosis Factor
TRM	Tissue resident memory
WC	Whole-cell
WCV	Whole-cell vaccine
WT	Wild type
α-GalCer	Alpha-galactosylceramide
μg	microgram/s
μL	microlitre/s
γδ T	Gamma-delta T
γδ T17	Gamma-delta T cells secreting $IL\text{-}17^+$
γ-FLU	Gamma-irradiated influenza vaccine
γ-PN	Gamma-irradiated *Streptococcus pneumoniae* vaccine
γ-SFV	Gamma-irradiated Semliki Forest vaccine

DECLARATION

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Rachelle Babb

Date

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PATENTS, PUBLICATIONS AND CONFERENCE PRESENTATIONS ARISING FROM THIS THESIS

PATENTS		
Patent application No	**Title**	**Inventors**
PCT/AU2016/050231	Streptococcal Vaccine	Rachelle Babb, Mohammed Alsharifi, Austen Yannis Chen Shannon Christa David, Timothy Raymond Hirst, Abiodun David Ogunniyi, James Cleland Paton

PUBLICATIONS		
Thesis chapter	**Title**	**Publication status**
3	Intranasal vaccination with gamma-irradiated Streptococcus pneumoniae whole-cell vaccine provides serotype-independent protection mediated by B cells and innate IL-17 responses *Appendix	Published
5	Enhanced protective CD4+ T cell responses to a serotype independent pneumococcal vaccine when combined with an inactivated influenza vaccine	In submission

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.