Novel particulate vaccine candidates recombinantly produced by pathogenic and nonpathogenic bacterial hosts

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Abstract

Polyhydroxyalkanoates (PHAs) are biopolyesters synthesized as small spherical cytoplasmic inclusion bodies by a range of bacteria. Recently, PHA beads have been investigated for use as a vaccine delivery platform by using engineered heterologous production hosts that allowed the efficient display of vaccine candidate antigens on the beads surface and were found to greatly improve immunogenicity of the displayed antigens. However, like other subunit vaccines, these antigen-displaying (vaccine) PHA beads only provide a limited repertoire of antigens.

In this thesis we investigate the idea of directly utilizing the disease causative pathogen or model organism to produce vaccine PHA beads with a large antigenic repertoire. These beads are hypothesized to have the potential to induce greater protective immunity compared to production of the same PHA bead in a heterologous production host.

This concept was exemplified with *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* as model human pathogens. For *P. aeruginosa* we describe the engineering of this bacterium to promote PHA and Psl (polysaccharide) production. This represents a new mode of functional display for the engineering, production, and validation of a novel OprI/F-AlgE fusion antigen-displayed on PHA beads. For the disease tuberculosis we investigated the use of nonpathogenic *M. smegmatis* as a model organism for *M. tuberculosis.* We described the bioengineering, production, and validation of Ag85A-ESAT-6 displayed on PHA beads produced in *M. smegmatis.*

Here we showed that both organisms were harnessed to produce custom-made PHA beads for use as particulate subunit vaccines that carried copurifying pathogen-derived proteins as a large antigenic repertoire and the ability of these vaccine PHA beads to generate a protective immune response.

This novel bioengineering concept of particulate subunit vaccine production could be applied to a range of pathogens naturally producing PHA inclusions for developing efficacious subunit vaccines for infectious diseases.

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"Success, 100% persistence and a bit of luck"

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Preface

Below lists the publication status of all chapters in this thesis.

Chapter 1.

General introduction.

This chapter review was written as an introductory chapter for this thesis by Jason Lee.

Chapter 2.

Bioengineering *Pseudomonas aeruginosa* to assemble its own particulate vaccine capable of inducing cellular immunity.

Published: Lee, J. W., Parlane, N. A., Wedlock, D. N., & Rehm, B. H. A. (2017). Bioengineering a bacterial pathogen to assemble its own particulate vaccine capable of inducing cellular immunity. *Scientific Reports, 7*.

This article was written by Jason Lee and reviewed by all other authors. The concept was conceived by Jason Lee and Bernd H. A. Rehm. Experimental design was performed by Jason Lee with the advice of Bernd H.A. Rehm. All experiments were performed by Jason Lee with the exception of some immunological experiments which were planned and performed with the guidance of Natalie Parlane.

Chapter 3.

Engineering mycobacteria for the production of self-assembling biopolyesters displaying mycobacterial antigens for use as tuberculosis vaccine.

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This article was written by Jason Lee and reviewed by all other authors. The concept was conceived by Jason Lee, Axel Heiser, and Bernd H. A. Rehm. All experiments and planning related to molecular biology (Cloning, expression, isolation, and analysis) was performed by Jason Lee with the advice of Axel Heiser and Bernd H.A. Rehm. All experiments related to immunology were planned and performed by Axel Heiser and Natalie Parlane.

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Abbreviations

3-HB	methyl 3-hydroxybutanoate
3-HD	methyl 3-hydroxydecanoate
3-HDD	methyl 3-hydroxydodecanoate
3-HH	methyl 3-hydroxyhexanoate
3-HHD	methyl 3-hydroxyhexadecanote
3-HN	methyl 3-hydroxynonanoate
3-HO	methyl 3-hydroxyoctanoate
3-HTD	methyl 3-hydroxytetradecanoate
3-HUD	methyl 3-hydroxyundecanoate
A:E	Fusion antigen of Ag85A and ESAT-6 epitopes
A:E-MBB	Ag85A-ESAT-6 displaying mycobacterial biobeads
Ag	Fusion antigen of OprI/F-AlgE
Ag-PhaC1$_{Pa}$	N terminal fusion of OprI/F-AlgE to the PHA synthase
Ag85A	Antigen 85A
Alum	Aluminum hydroxide
Ap	Ampicillin
APC	Antigen Presenting Cell
BCG	Bacillus Calmette–Guérin
BDW	Bead Dry Weight
Cb	Carbenicillin
CD	Cluster of Differentiation
CD40L	Cluster of Differentiation 40 ligand
CDW	Cell Dry Weight
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Regulator
CLR	C-type Ligand Receptor
CLSM	Confocal Laser Scanning Fluorescence Microscopy
ConA	Concanavalin A
CTL	Cytotoxic T Lymphocytes
DAMP	Damage Associated Molecular Patterns
DC	Dendritic cell
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPS	Exopolysaccharide

ESAT-6	6 kDa early secretory antigenic target
FM	Fluorescence microscopy
GAP	Granule Associated Protein
GC/MS	Gas Chromatography/Mass Spectrometry
GFP	Green Fluorescent Protein
Gm	Gentamicin
HCP	Host Cell Protein
HCV	Hepatitis C Virus
His_{10}-Ag	10x His-tagged fusion antigen
HIV	Human Immunodeficiency Virus
HRP	Horseradish peroxidase
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
ISCOM	Immune stimulating complex
kDa	Kilodalton
LB	Luria Broth
LPS	Lipopolysaccharide
MAC	Membrane Attack Complex
MALDI-TOF MS	Matrix-Assisted Laser Desorption-Ionization Time-Of-Flight Mass Spectroscopy
MASPs	MBL-Associated Serine Proteases
MBB	Mycobacterial biobeads
MBL	Mannose Binding Lectin
MDR	Multidrug-resistance
MHC	Major Histocompatibility Complex
MOG	Myelin Oligodendrocyte Glycoprotein
MSM	Mineral Salt Medium
MVC	Mycbacterial vector control
ND	Not detected
NF-κB	Nuclear Factor-κB
NK	Natural Killer
NLR	(NOD)-Like Receptor
NLRA	NOD-Like Receptor Acidic transactivating domain
NLRB	NOD-Like Receptor Baculovirus inhibitor of apoptosis protein repeat
NLRC	NOD-Like Receptor Caspase activation and recruitment domains
NLRP	NOD-Like Receptor Pyrin domain
OD	Optical Density

OMP	Outer membrane protein
OMV	Outer Membrane Vesicle
OpdA	Organophosphorus pesticide hydrolase
OprF	Outer membrane protein F
OprI	Outer membrane lipoprotein I
OprI/F-AlgE	Fusion antigen of OprI, OprF, and AlgE (loops 5 & 6) epitopes
PAMP	Pathogen Associated Molecular Patterns
PAO1 ΔCΔ8ΔF	*P. aeruginosa* PAO1 triple knockout mutant
PBS	Phosphate Buffer Saline
PBST	Phosphate buffer saline + tween 20
PHA	Polyhydroxyalkanoate
$PhaC1_{Pa}$-Ag	C terminal fusion of OprI/F-AlgE to the PHA synthase
$PhaC_{Pa}$	PHA synthase from *P. aeruginosa*
$PhaC_{Re}$	PHA synthase from *Ralstonia eutropha*
PHA_{LCL}	Long chain length polyhydroxyalkanoate
PHA_{MCL}	Medium chain length polyhydroxyalkanoate
PHA_{SCL}	Short chain length polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHBHHx	Copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate
PHBV	Copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate
PHO	Poly 3-hydroxyoctanoate
PLGA	Poly(lactic-co-glycolic acid)
PMLA	Poly(β,L-malic acid)
RLH	(RIG-I)-Like Helicases
RNA	Ribonucleic acid
scFv	Single-chain antibody variable fragment
TB	Tuberculosis
TCR	T Cell Receptor
TEM	Transmission Electron Microscopy
Tfh	T Follicular helper cell
Th	T helper
TIR	Toll/Interleukin-1 Receptor
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor
TRIF	TIR-domain-containing adapter-inducing interferon-beta
VLP	Virus Like Particle
WHO	World Health Organization
XDR	Extensively drug-resistant