Development and use of polyhydroxybutyrate biopolyester as particulate vaccine beads.

A thesis presented in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Microbiology

at Massey University, Manawatu,

New Zealand.

Natalie Anne Parlane

2012

Abstract

Poly(3-hydroxybutyric acid) (PHB) is the most commonly produced polyhydroxyalkanoate formed naturally inside many genera of bacteria and archaea when nutrients are limited and a carbon-source is available in excess. These water-insoluble biopolyester spherical beads in the size range of 20-800 nm can be recombinantly produced by insertion of the required PHB biosynthesis genes into alternative bacterial hosts and then culturing the organisms under suitable conditions. A gene fusion can also be made to enable production of PHB beads which display the selected proteins abundantly at the surface of the bead.

Vaccines are needed which stimulate cell-mediated immunity and are effective at reducing intracellular infections such as tuberculosis, neosporosis and many viral infections. These diseases are responsible for a huge burden to human and animal health. Particulate vaccines target antigen presenting cells and cellular immune responses to protein antigens are enhanced when particulate vaccines are used.

This thesis describes the development of a novel vaccine delivery system in which PHB beads were engineered to display vaccine antigen on the surface of the beads.

Investigations were made into the process of vaccine bead design, production and validation to enable their use in vaccine trials. PHB synthesis genes from *Cupriavidus necator* were inserted into production strains to enable production of PHB. *Escherichia coli* was initially used as a bacterial production host and then *Lactococcus lactis* was introduced as an alternative, due to its lack of lipopolysaccharide, previous use as a production host for recombinant proteins and history of safe use for a range of human foods and products. To expand the repertoire of PHB vaccine beads, different vaccine antigens were used: hepatitis C core antigen and mycobacterial antigens (antigen-85A and 6 kDA early secretory antigenic target). Antigen specific cellular immune responses were produced in mice vaccinated with PHB vaccine beads and protection against tuberculosis was observed in mice immunized with these vaccines.

Preliminary studies into the mechanism of uptake of PHB beads by dendritic cells (DCs) showed PHB beads were taken up readily by DCs, with maturation of DCs and subsequent secretion of interleukin-12.

Acknowledgements:

I would like to thank my supervisors for excellent guidance and support. Professor Bernd Rehm first inspired my interest in the topic of biopolyesters when he gave a seminar entitled "Microbial cell factories: Production of tailor-made biopolymers and nanostructures" at AgResearch on 23 February 2005. I therefore really appreciated the later opportunity to do PhD research into the use of these versatile polyester beads as vaccine delivery vehicles. Bernd's encouragement, scientific advice and extensive knowledge have been greatly valued. Dr Bryce Buddle and Dr Neil Wedlock have been a great co-supervisors and I respect their vast experience and support. I am grateful for the helpful suggestions and objective counsel when required.

I thank members of the Rehm group. In the initial stages, it was simple help with finding things and basic protocols. Also, weekly student meetings provided another opportunity to gain knowledge and the cake rounds ensured a friendly atmosphere. There were ongoing interactions through the Friday seminars where we presented more formal project updates and new methods. These stimulating interactions have been invaluable in broadening my scientific knowledge and were an ideal opportunity to practice preparation and presentation skills to a friendly but challenging audience. Co-authoring review papers entailed a rapid learning curve on my part but stimulated a good team spirit due to the co-operation and interactions needed. I value your on-going friendship and support.

I am very appreciative to the members of the AgResearch Hopkirk Infectious Diseases lab. Thanks to Gina Pedersen for help with initial cloning of the Ag85A-ESAT-6 gene and particularly for her support with the recombinant protein production. Her commitment and sense of humour were greatly appreciated, especially when yet another recombinant protein was insoluble and suitable methods needed to be found and trialed. Allison McCarthy has shared much with me over the time of my PhD; an office, the ups and downs of moving to a new city and new building and life in general.

AgResearch have been very supportive and I appreciate this greatly. I thank them for their financial contribution and the opportunity for training in software such as End-note and Minitab. My statistical education has been increased thanks to Dr Bryce Buddle and Dr Dongwen Luo. The library support has enabled me to access a broad range of papers which aided my research immensely.

AGMARDT provided me with a PhD scholarship and for this I am very grateful. They also provided the opportunities to present my research to the AGMARDT board annually and then to an international audience in 2010 at the Modern Veterinary Vaccines and Adjuvants conference in Budapest, Hungary.

Finally, I thank my family for allowing me the time to pursue this PhD. Your love, support and encouragement have been invaluable.

Preface:

This thesis is written according to the regulations of the latest version of the Handbook for Doctoral Study, Version 7 – January 2011, published by the Doctoral Research Committee. This thesis complies with the format of a thesis based on publication as described in the handbook.

The list below presents the publication status of all chapters in this thesis. Published papers do not appear in chronological order.

Chapter 1A

Biopolyester particles: preparation and applications. I. A. Rasiah, N. Parlane, K. Grage, R. Palanisamy, A.C. Jahns, J.A. Atwood and B. H. A. Rehm.

Published: Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, edited by Michael C. Flickinger, 2010 John Wiley & Sons, Inc.

This book chapter review was jointly written by all authors. Natalie Parlane made particular contributions to the section describing poly lactic-co-glycolic acid.

Chapter 1B

Bacterial polyhydroxyalkanoate granules: Biogenesis, structure and potential use as micro-/ nano-beads in biomedical applications. Katrin Grage, Anika C. Jahns, Natalie Parlane, Rajasekaran Palanisamy, Indira A. Rasiah, Jane A. Atwood and Bernd H. A. Rehm.

Published: Biomacromolecules, 2009, 10(4), 660–669

This review was jointly written by all authors. Natalie Parlane made a specific focus on the section describing structure of PHA granules.

Chapter 1 C

Introduction to Immunity and Vaccines.

This chapter has been written by Natalie Parlane as an introductory chapter for this thesis only and is not intended for publication

Chapter 2

Bacterial polyester inclusions engineered to display vaccine candidate antigens for use as a novel class of safe and efficient vaccine delivery agents. Natalie A. Parlane, D. Neil Wedlock, Bryce M. Buddle and Bernd H. A. Rehm.

Published: Applied and Environmental Microbiology, 2009, 75(24), 7739-7744

All experiments were carried out by Natalie Parlane except for construction of the pCWE *Spe*I-Ag85A-ESAT-6 and pHAS-Ag85A_ESAT-6 plasmid, which were done by Jessica Koach and Gina Pedersen.

Chapter 3

Vaccines displaying mycobacterial proteins on biopolyester beads stimulate cellular immunity and induce protection against tuberculosis. Natalie A. Parlane, Katrin Grage, Jun Mifune, Randall J. Basaraba, D. Neil Wedlock, Bernd H. A. Rehm, Bryce M. Buddle

Published: Clinical and Vaccine Immunology, 2012, 19(1), 37-44

All immunological experiments were devised and carried out by Natalie Parlane. Construction of Ag85A-ESAT-6 plasmids and cloning into *L. lactis* was done by Katrin Grage and Jun Mifune. Randall Basaraba analysed all histopathology.

Chapter 4

Production of a particulate Hepatitis C vaccine candidate by engineered *Lactococcus lactis.* Natalie A. Parlane, Katrin Grage, Jason W. Lee, Bryce M. Buddle, Michel Denis and Bernd H. A. Rehm

Published: Applied and Environmental Microbiology, 2011, 77(24), 8516-8522

All immunological experiments were devised and completed by Natalie Parlane. Construction of plasmids pET-HCc-phaC and pNZ-HCcCAB and cloning was done by Katrin Grage and Jason Lee.

Chapter 5

Uptake by dendritic cells of polyhydroxybutryate vaccine beads produced in *Lactococcus lactis* and *Escherichia coli.* Natalie A. Parlane, Bryce M. Buddle, D. Neil Wedlock and Bernd H. A. Rehm

Manuscript in preparation: For submission to Immunology and Cell Biology with additional experimental results.

All experiments were devisd and carried out by Natalie Parlane.

Table of Contents

List of Figures

Chapter 1A

Chapter 1B

Chapter 2

Chapter3

Chapter 4

Chapter 5

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Abbreviations

AFB	acid-fast bacilli
Ag85A	antigen 85A
APC	antigen presenting cell
BCG	bacille Calmette-Guérin
BSA	bovine serum albumin
CBA	Cytometric bead array
CFU	colony forming units
CTL	Cytotoxic t cell
DC	dendritic cell
BMDC	bone-marrow derived dendritic cell
MoDC	monocyte derived dendritic cell
DMEM	Dulbeco's Modified Eagle media
ELISA	enzyme linked immunosorbent assay
ESAT-6	early secreted antigenic target- 6kDa
FCS	foetal calf serum
GC-MS	gas chromatography-mass spectroscopy
H&E	haematoxylin and eosin
IL	interleukin
IFN	interferon
LPS	lipopolysaccharide
LB	Luria-Bertani

MALDI-TOF MS	matrix-assisted laser desorption-ionization time-of-flight mass spectroscopy
MFI	median fluorescence intensity
MHC	major histocompatibility complex
NLR	NOD-like receptor
OD	optical density
PAMP	pathogen associated molecular pattern
PBS	phosphate buffered saline
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
PLA	Polylactic acid
PLGA	poly(lactic-co-glycolic acid)
PRR	pattern recognition receptor
s.c	subcutaneous
SDS-PAGE	sodium dodecyl sulphate - polyacrylamide gel electrophoresis
SEM	standard error of the mean
TB	tuberculosis
TCR	T cell receptor
TEM	transmission electron microscopy
Th	T helper
TLR	Toll-like receptor
TNF	tumour necrosis factor

WT	wild-type
-/-	deficient or knock-out
2-ME	2 mercaptoethanol