

TUBERCULOSIS: PROSPECTS FOR AN ORAL VACCINE USING NOVEL ANTIGENS AND ADJUVANTS

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I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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Abstract

In spite of vaccine and treatment strategies, *Mycobacterium tuberculosis* kills more than 3 people per minute. The emergence of drug-resistant strains makes treating the disease complicated and expensive for government health departments, and unpleasant and laborious for patients. The current vaccine, parenterally administered BCG, is only 50% effective. Oral vaccination has the advantage of targeting the mucosal immune system, which acts at the direct site of initial exposure to the infecting airborne pathogen. In addition, oral vaccines are cheaper and safer to administer than parenteral vaccines. This dissertation provides a conceptual framework for the prevention of the disease by means of oral vaccination and outlines methods that were developed for the production of concentrated purified somatic and extracellular antigens. Immune responses to somatic antigens were also examined in conjunction with established and novel adjuvants. The role of *Propionibacterium jensenii* 702 as a suitable mucosal adjuvant was supported by the results obtained.

Abbreviations

”	inch
%	percent
α	alpha
Ag	antigen
AIDS	Acquired Immunodeficiency Syndrome
A-SA	apical-subapical
ATCC	American Type Culture Collection
β	beta
BCG	Bacille Calmette-Guérin
BSA	bovine serum albumin
°C	degrees Celsius
CD	cluster of differentiation
CDC	Centers for Diseases Control and Prevention
CFP	culture filtrate protein
cfu	colony-forming unit
cm	centimetres
CMI	cell-mediated immunity
Con A	Concanavalin A
cpm	counts per minute
CSF	cerebrospinal fluid
CT	cholera toxin
δ	delta
Da	Daltons
DDA	Dimethyl dioctadecyl ammonium bromide
dd-mm-yy	date, in the format day-month-year, each with two digits
dH ₂ O	distilled water
DN	double negative
DNA	deoxyribonucleic acid
DOTS	Directly Observed Therapy – Short-course

DTH	delayed-type hypersensitivity
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
ESAT	early secretory antigen target
et al.	and others
FCA	Freund's Complete Adjuvant
FDCs	fixed dose combinations
g	gram
γ	gamma
HCl	hydrochloric acid
HIV	human immunodeficiency virus
IFA	Incomplete Freund's Adjuvant
IFN	interferon
Ig	immunoglobulin
IL	interleukin
<i>Ipr1</i>	Intracellular pathogen resistance 1
ISCOMS	immunostimulant complexes
IV	intravenous
kDa	kilo Daltons
L	litre
LabVISE	Laboratory Virology and Serology Reporting Scheme
LJ	Lowenstein-Jensen (medium)
LT	heat-labile enterotoxin
M	molar
<i>M.</i>	<i>Mycobacterium</i>
mA	milli Amperes
MDR	multi-drug-resistant
mg	milligram
MHC	major histocompatibility complex
min	minutes
ml	millilitres

mm	millimetres
MPB	<i>Mycobacterium bovis</i> protein
MPL	Monophosphoryl lipid A
MPT	<i>Mycobacterium tuberculosis</i> protein
MSM	Modified Sauton's Medium
MWCO	Molecular Weight Cut-off
N	no
N/A	not applicable
NaOH	sodium hydroxide
nm	nanometres
NNDSS	National Notifiable Disease Surveillance System
NSW	New South Wales, Australia
OD	optical density
<i>P.</i>	<i>Propionibacterium</i>
PAGE	poly-acrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pg	picograms
PI	Protease Inhibitor solution
PJ702	<i>Propionibacterium jensenii</i> 702
PLG	polylactide-coglycolides
PPD	purified protein derivative
rpm	revolutions per minute
RNA	ribonucleic acid
SAF	Syntex Adjuvant Formulation
SDS	sodium dodecylsulfate
sec	seconds
STCF	short-term culture filtrate
TB	tuberculosis
Tc	cytotoxic T-lymphocyte
Th	helper T-lymphocyte

TLR	Toll-like receptor
TNF	Tumour necrosis factor
TST	Tuberculin skin test
μCi	micro Curie
μg	micrograms
μL	microliters
μm	micrometres
US	United States
UV	ultraviolet
V	Volts
VIC	Victoria, Australia
WA	Fraction A from whole <i>Mycobacterium tuberculosis</i> Sonicate
WCAB	Wilkins-Chalgren Anaerobe Broth
WE	Fraction E from whole <i>Mycobacterium tuberculosis</i> Sonicate
WHO	World Health Organization
WTB	Whole <i>Mycobacterium tuberculosis</i> Sonicate
x g	times gravity
Y	yes