THE DEVELOPMENT AND EVALUATION
OF DNA VACCINES AGAINST WHOOPING
COUGH USING A MURINE RESPIRATORY
MODEL OF INFECTION

A dissertation submitted by

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Abstract

*Bordetella pertussis* is the aetiological agent of whooping cough, a respiratory disease of humans that causes severe and potentially fatal manifestations in children. Whooping cough is endemic world-wide accounting for around 50 million cases and 300,000 deaths every year, with epidemics occurring every two to five years (Kerr & Matthews 2000). Although the current whole-cell and acellular vaccines are effective in conferring protection from clinical pertussis, they have been associated with serious systemic and local side reactions in up to a maximum of 18 percent of paediatric vaccinees after repeated boosters (Gold et al. 2003).

In this study, a suite of single antigen DNA vaccines, combination DNA vaccines and dual modality vaccines, were developed and evaluated for their potential to induce humoral and cell-mediated immune responses, and protective efficacy against *B. pertussis*, using the mouse respiratory challenge model. This study was based on the reported claims that DNA vaccines are capable of generating potent humoral and cell-mediated responses, and protection against numerous viral, parasitic and bacterial pathogens in small animal models. Four protective antigens, three of which are included in the currently-marketed acellular pertussis vaccine [aP], were evaluated as single antigen DNA vaccines, namely filamentous hemagglutinin (FHAB), pertactin (PRN), a genetically toxoided S1 subunit of pertussis toxin (PTS1.13L.129G) and genetically toxoided adenylate cyclase-hemolysin (CYAAL58), delivered either by the intramuscular (IM) route or by the oral route via attenuated *Salmonella typhimurium* strain SL3261. The immunogenicity and protective efficacy of these DNA vaccines was compared to that imparted by the DTaP (Infantrix™), a placebo-immunised group, and a vector-immunised group of mice. Two DNA vaccines encoding truncated FHA antigens, representing the entire immunodominant region (pcDNA3.1D/haB1) and dominant B cell epitopes (pcDNA3.1D/haB2), induced a predominantly Th1 response with high levels of IFN-γ and IL-2 produced by stimulated splenocytes *in vitro*. An antigen-specific IgG response was detected in the serum of mice but this was negated by an equivalent or larger IgG response to FHA in the serum of vector-immunised mice. Two AC-Hly DNA vaccines, encoding a genetically inactivated CyaA protoxin either alone (pcDNA3.1D/cyaAL58) or in combination with the accessory protein cyaC
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(pDNA3.1D/cyaAL58 + pDNA3.1D/cyaC), both induced a potent Th1 cytokine response. However, the serum IgG response was not significant due to the presence of cross-reactive antibodies in the sera of vector-immunised and sham-immunised mice (placebo).

A DNA vaccine encoding a genetically inactivated S1 subunit of pertussis toxin (pDNA3.1D/pts1.13L.129G), induced a purely Th1 response with high levels of IFN-\(\gamma\). No antigen-specific or cross-reactive IgG was detected in the sera of mice immunised with pDNA3.1D/pts1.13L.129G or vector only respectively. In contrast to the other antigens tested, the pertactin DNA vaccine induced a Th2-type response as indicated by a significant serum IgG response, the majority of which was IgG1, and lower levels of IFN-\(\gamma\) and IL-2. Mice immunised with each of the single antigen DNA vaccines showed a significantly improved rates of clearance compared to mice that received the vector only or placebo. Overall, their protective efficacy was inferior to that of the DTaP. It has been well established that effective immunisation against such a complex pathogen as B. pertussis requires multiple antigenic priming, with multi-component acellular vaccines often providing an improved level of protection compared to mono-component and two-component acellular vaccines. In order to induce a broad-spectrum immune response and thereby assess the true protective potential of DNA vaccination, a five-gene combination DNA vaccine was tested by direct IM injection of naked DNA (without any added adjuvant). The IM five-gene combination DNA vaccine generated strong Th1 responses to FHA, the inactivated S1 subunit of PT and inactivated CYAA, and a moderate serum IgG response to PRN. Importantly, the response to each antigen was equivalent or better than the respective single antigen DNA vaccines, which indicated that there was no antigenic competition. In fact, co-administration of the five genes resulted in an enhanced response to each antigen. The portal of entry for B. pertussis, like many other human pathogens, is via the mucosa and in the case of pertussis this is limited to the respiratory tract with no systemic dissemination in otherwise healthy individuals. Whilst the whole cell and acellular vaccines induce a potent serological response that can protect against B. pertussis infection, it is likely that the additional priming of a secretory response at the site of colonisation would inhibit colonisation, improve clearance of the pathogen from the lungs, and limit transmission. Hence, a five-gene combination DNA vaccine was delivered via the oral route using attenuated Salmonella typhimurium as the delivery vector, with the aim of stimulating a
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mucosal response in the respiratory tract via the common mucosal pathway. The oral combination DNA vaccine generated a strong systemic response but a poor mucosal antibody response to recombinant or native B. pertussis antigens. Mice immunised with the oral combination DNA vaccine cleared an experimental infection at a significantly faster rate than mice immunised with the oral vector (S. typhimurium harbouring the pcDNA3.1D vector), but the clearance data showed that it was not nearly as effective as the IM combination DNA vaccine or DTaP.

In an attempt to induce a dichotomous humoral and cell-mediated immune response, two DNA vaccine prime-acellular vaccine boost regimens were evaluated: a parenteral-parenteral strategy referred to as the parenteral dual modality vaccine and an oral-parenteral strategy referred to as the oral dual modality vaccine. Priming involved administration of a five-gene combination DNA vaccine via either IM injection or oral gavage, and the boosters consisted of a laboratory constituted three-component acellular vaccine given via SC injection. Dual modality vaccination successfully induced a dichotomous Th1/Th2 response that conferred a degree of protection that was equivalent to that obtained with the commercial DTaP. Interestingly, a mucosal IgG response was also detected in the lung washes of mice immunised with the laboratory constituted acellular vaccine (as part of the dual modality vaccines) or Infanrix™ DTaP that was considered to be due to transudation of antibodies from serum. This study has been the first to demonstrate that immunisation with a five-gene combination DNA vaccine can elicit a protective immune response that approaches the level of protection conferred by DTaP, as judged by the number of days required for clearance of the pathogen from the lungs of vaccinated mice. In addition, both the parental and the oral dual modality vaccines were found to provide an equivalent or better protection against challenge with virulent B pertussis than that imparted by the commercial DTaP. This was despite the fact that the oral DNA combination vaccine was essentially non-protective. The induction of humoral and CMI responses, particularly by the parenteral dual modality vaccine, are highly encouraging and warrant further investigations on the safety and characterisation of anamnestic immune responses induced by the inclusion of different types of adjuvants in the vaccine formulations.
**Declaration**

I declare that all experimental work, results and analyses reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original, has not been previously submitted for any other award and was conducted during my enrolment as a Doctor of Philosophy candidate at the University of Southern Queensland. Animal experiments were approved by the University of Southern Queensland Animal Ethics Committee (Approval no. 03STU194).

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List of Abbreviations

AC  adenylate cyclase
AC-Hly  adenylate cyclase-hemolysin
ACT  adenylate cyclase toxin
ADP  adenosine diphosphate
ADRAc  Australian Adverse Drug Reaction Assessment Committee
AGRf  Australian Genome Research Facility
ANGIS  Australian National Genome Information Service
AP  alkaline phosphatase
APC  antigen presenting cell
ATP  adenosine triphosphate
BAL  bronchoalveolar lavage
BG  Bordet Gengou media
BSA  bovine serum albumin
bp  base pair
cAMP  cyclic adenosine monophosphate
CD  cluster of differentiation
cDMEM  complete Dulbecco’s Modified Eagle Media
CFU  colony forming units
CHO  Chinese Hamster Ovary cell line
CI  clearance index
CMI  cell-mediated immunity
CMV  cytomegalovirus
CNS  central nervous system
ConA  concanavalin A
CpG  cytosine-phosphate-guanosine
DC  dendritic cell
DMEM  Dulbecco’s Modified Eagle Media
DMSO  dimethyl sulfoxide
DNA  deoxyribonucleic acid
DNase  deoxyribonuclease
DNT  dermonecrotic toxin
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<table>
<thead>
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<tbody>
<tr>
<td>dNTP</td>
<td>deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>DTaP</td>
<td>Diptheria-Tetanus-acellular Pertussis vaccine</td>
</tr>
<tr>
<td>DTP</td>
<td>Diptheria-Tetanus-whole cell Pertussis vaccine</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EDAC</td>
<td>1-ethyl-3(3-dimethylaminopropyl) carbodiimide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>EGTA</td>
<td>ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid</td>
</tr>
<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FAE</td>
<td>follicle-associated epithelium</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FcR</td>
<td>receptor for the Fc portion of immunoglobulin</td>
</tr>
<tr>
<td>FFST</td>
<td>formalin-fixed <em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>FHA</td>
<td>filamentous hemagglutinin</td>
</tr>
<tr>
<td>FIM</td>
<td>fimbriae</td>
</tr>
<tr>
<td>GALT</td>
<td>gut-associated lymphoid tissue</td>
</tr>
<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
</tr>
<tr>
<td>GKO</td>
<td>gene knock-out</td>
</tr>
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<td>GSK</td>
<td>GlaxoSmithKline</td>
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<td>hydrochloric acid</td>
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<td>His</td>
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<td>heat-killed <em>Bordetella pertussis</em></td>
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</tr>
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<td>IFN-γ</td>
<td>interferon-gamma</td>
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</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase pair</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo dalton</td>
</tr>
<tr>
<td>KO</td>
<td>knock-out</td>
</tr>
</tbody>
</table>
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>Luria-Betani media</td>
</tr>
<tr>
<td>LBA</td>
<td>Luria-Betani media with ampicillin</td>
</tr>
<tr>
<td>LBAC</td>
<td>Luria-Betani media with ampicillin &amp; chloramphenicol</td>
</tr>
<tr>
<td>LBAG</td>
<td>Luria-Betani media with ampicillin &amp; glucose</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>lethal dose which causes 50% death of a group of test animals</td>
</tr>
<tr>
<td>LF2000</td>
<td>lipofectamine 2000 reagent</td>
</tr>
<tr>
<td>LN</td>
<td>lymph nodes</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>LRT</td>
<td>lower respiratory tract</td>
</tr>
<tr>
<td>LRTI</td>
<td>lower respiratory tract infection</td>
</tr>
<tr>
<td>MALT</td>
<td>mucosal-associated lymphoid tissue</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>MgSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>magnesium sulphate</td>
</tr>
<tr>
<td>MgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>magnesium chloride</td>
</tr>
<tr>
<td>MSS</td>
<td>modified Stainer-Scholte media</td>
</tr>
<tr>
<td>MV</td>
<td>modified Verwey media</td>
</tr>
<tr>
<td>NALT</td>
<td>nasal-associated lymphoid tissue</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer cells</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>O/N</td>
<td>overnight</td>
</tr>
<tr>
<td>OPD</td>
<td>o-phenylene diamine</td>
</tr>
<tr>
<td>ORF</td>
<td>open reading frame</td>
</tr>
<tr>
<td>P69</td>
<td>69 kDa outer membrane protein of <em>B. pertussis</em></td>
</tr>
<tr>
<td>Pa</td>
<td>acellular pertussis vaccine</td>
</tr>
<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PBST</td>
<td>phosphate-buffered saline with Tween-20</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PMNL</td>
<td>polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PMSF</td>
<td>phenylmethylsulfonyl fluoride</td>
</tr>
<tr>
<td>PP</td>
<td>Peyer’s patches</td>
</tr>
<tr>
<td>PRN</td>
<td>pertactin</td>
</tr>
</tbody>
</table>

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List of Abbreviations

PT       pertussis toxin
PVDF     polyvinylidene fluoride
Pw       whole-cell pertussis vaccine
RNA      ribonucleic acid
RNase    ribonuclease
rpm      revolutions per minute
RT       room temperature
S1       S1 subunit of pertussis toxin
SC       subcutaneous
SDS      sodium dodecyl sulfate
sec      second
SLID     sub-lethal infectious dose
SS       Stainer-Scholte media
TAE      tris acetate EDTA
TB       terrific broth
Tc       CD8+ cytotoxic T lymphocyte
TcR      T-cell receptor
TCT      tracheal cytotoxin
TGF-β    tumour growth factor-beta
TNF      tumour necrosis factor
Tween-20 polyoxyethylenesorbitan monolaurate
URT      upper respiratory tract
URTI     upper respiratory tract infection
USQ      University of Southern Queensland
vag      virulence-associated gene
vir      virulence
vrg      virulence-repressed gene
VLP      virus-like protein
WCV      whole-cell pertussis vaccine
WHO      World Health Organisation
wt       wild-type
X-Gal    5-bromo-4-chloro-3-indolyl-b-D-galactoside