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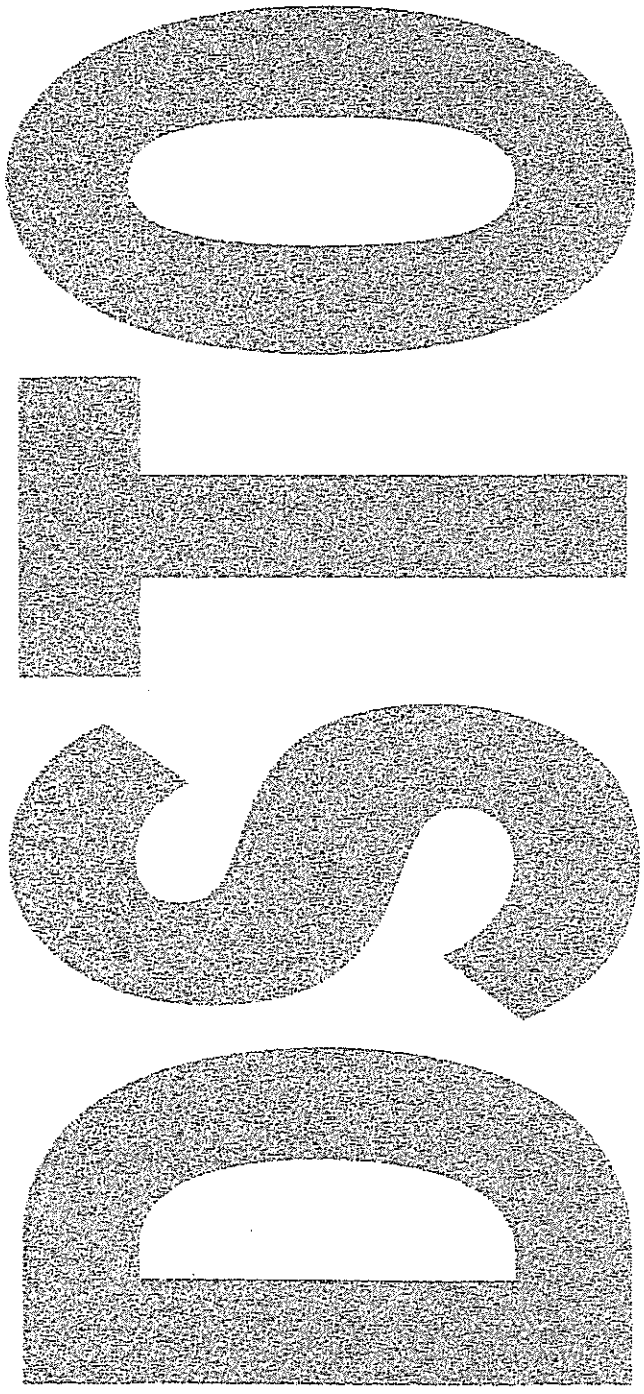

Development of DNA Vaccines Against *Burkholderia pseudomallei*

Jane McAllister, Sharon Lazzaroni, Jodie Barnes, Natkunam Ketheesan and David Proll

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LIMITED RELEASE

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Jane McAllister, Sharon Lazzaroni, Jodie Barnes*, Natkunam Ketheesan* and David Proll*

Human Protection and Performance Division
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* **School of Biomedical Sciences,** James Cook University

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ABSTRACT

Burkholderia pseudomallei is the causative agent of melioidosis, a disease endemic in Southeast Asia and Northern Australia. The bacteria cause infection via subcutaneous or inhaled routes, resulting in either acute lethal sepsis or chronic and eventually fatal disease. Currently no licensed vaccine is available to provide protection against this pathogen. In this study, eleven DNA vaccines encoding the *B. pseudomallei* proteins were constructed and used to immunise mice and subsequent protective capability analysed following live challenge with *B. pseudomallei*.

Mice immunised with DNA vaccines encoding Hag1, Hag2 and heat shock protein demonstrated the greatest level of protection against bacterial infection, however, the levels of protection seen were still quite limited. There was no increase in survival seen following immunisation with the remaining eight vaccines. This study highlights the challenges in designing vaccines against *B. pseudomallei* and suggests that a successful DNA vaccine may need to be used in conjunction with adjuvant such as CpG oligodeoxynucleotide, or combined in a prime-boost regime with recombinant protein in order to increase protective capabilities.

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Development of DNA Vaccines Against *Burkholderia pseudomallei*

Executive Summary

ADF personnel are at risk of being exposed to biological warfare (BW) agents deliberately released by an enemy, and to agents naturally occurring in environments where they are deployed. One such agent is *Burkholderia pseudomallei,* an intracellular bacterium that is the causative agent of melioidosis, a disease endemic in Southeast Asia and Northern Australia. The bacteria cause infection via subcutaneous or inhaled routes, resulting in either acute lethal sepsis or chronic and eventually fatal disease. Currently there is no licenced vaccine to protect against infection with this bacteria.

Recently, scientific advances have seen the use of recombinant DNA techniques to produce DNA vaccines. DNA vaccines act by providing the host with a set of instructions for the synthesis of an encoded bacterial or viral protein. The host cell utilises this information to synthesise the protein, which interacts with the host immune system to generate the immune response and provide protection against disease.

This report describes the construction and assessment of eleven DNA vaccines against *B. pseudomallei.* The vaccines are encoded with known virulence factors against *B. pseudomallei* and proteins shown to be immunogenic in other bacteria. Following construction, the eleven vaccines were assessed in a mammalian cell culture system to see if the encoded protein could be synthesised *in vitro.* Once it had been demonstrated that the constructs contained all the necessary components to produce the protein in mammalian cells, they were then used to vaccinate mice to determine levels of protection when mice were given a lethal dose of *B. pseudomallei.*

Mice immunised with DNA vaccines encoding Hag1, Hag2 and heat shock protein demonstrated the greatest level of protection against bacterial infection, however, the levels of protection seen were still quite limited. There was no increase in survival seen following immunisation with the remaining eight vaccines. This study highlights the challenges in designing vaccines against *B. pseudomallei.* They may need to be used in conjunction with adjuvant, or in combination with recombinant protein boosting in order to provide protective immunity.

Authors

Jane McAllister
Human Protection and Performance Division

Jane McAllister, joined HPPD in 2003. Prior to DSTO, Jane worked at the University of Canberra, identifying potential antigens for vaccines against respiratory pathogens, and assessing their effect on the immune response. Her work in DSTO focuses on the construction and immunological analysis of DNA vaccines against biological agents.

Sharon Lazzaroni
School of Biomedical Sciences, James Cook University

Sharon M Lazzaroni is a Researcher within the Infectious Diseases and Immunopathogenesis Research Group at James Cook University. She completed her BMedLabSci degree at the same institution in 2004 and currently enrolled in an MSc programme. Her primary interest is in determining host-pathogen interactions in melioidosis.

Jodie Barnes
School of Biomedical Sciences, James Cook University

Jodie L Barnes is a Postdoctoral Fellow within the Infectious Diseases and Immunopathogenesis Research Group at James Cook University. She graduated with her BSc Honours at James Cook University in 1998 and went on to complete her PhD at the same institution in 2004. Her major interest is in determining the adaptive immune responses in Burkholderia pseudomallei infection.

Natkunam Ketheesan
School of Biomedical Sciences, James Cook University

Natkunam Ketheesan is an Associate Professor working within the Infectious Diseases and Immunopathogenesis Research Group at James Cook University. He graduated with his MD at the Vinnitsa Medical Institute in 1985, and completed his PhD at the University of Leeds in 1995. Post-doctoral work followed at the Universities of Leeds, Western Australia and Queensland. Currently his major interests are in determining the immune responses in melioidosis, rheumatic fever, poststreptococcal glomerulonephritis and Q fever.

David Proll
Human Protection and Performance Division

David Proll graduated from Monash University in 1993 with a B.Sc(Hons) and went on to complete his PhD in the department of Microbiology. His PhD studies focused on the replication of positive strand RNA viruses. After graduating from university he worked at the Eijkman Institute of Molecular Biology in Jakarta, Indonesia. Here, he investigated the application and development of DNA based vaccines against the parasite that causes Malaria. Upon returning to Australia in 2000, he was recruited by DSTO to initiate a research program investigating DNA vaccines for defence applications.

Contents

1. Introduction

DNA vaccines, a new generation vaccine technology, have the potential to prevent various infectious diseases and have several advantages over conventional vaccine strategies. They pose significantly less risk of side effects than live attenuated vaccines, are cheap to manufacture, have no reliance upon cold chain [1], and can evoke strong cytotoxic lymphocyte (CTL) responses, which are often lacking in strategies such as recombinant protein vaccination [2].

Burkholderia pseudomallei is the causative agent of melioidosis, a potentially fatal disease endemic in sub-tropical areas of South East Asia and Northern Australia, including areas such as Darwin and Townsville, where military exercises are conducted. It is also considered a potential biowarfare agent, due to high pathogenicity, ease of aerosolisation, and the prolonged therapy required to treat subsequent disease. It is therefore of interest to the military to develop an effective vaccine against this pathogen. Due to the intracellular nature of the bacteria, an effective vaccine would have to evoke not only an antibody response but also a strong cellular based response. A DNA vaccination strategy would provide a means of activating both the humoral and cellular arms of the immune system [3-5].

The following report describes the construction and assessment of the immunogenicity and protective capabilities of eleven DNA vaccines targeting *B. pseudomallei*. Six putative *B. pseudomallei* proteins were selected for evaluation as candidate antigens for DNA vaccine development against melioidosis based upon the original *B. pseudomallei* K96423 genome annotation. These were selected on the basis of homology to virulence factors in other pathogens and reports of known immunogenicity properties in other bacteria. These included *B. pseudomallei* flagellin (FliC), the chaperonin/ heat shock protein (HSP) encoded by *GroEL*, homologues to *E. corrodens* Hag2 haemagglutinin, *E. coli* O157:H7 outer membrane protein A (OMPA), *C. acidovorans* outer membrane porin protein (OPC), and a novel protein designated AimA.

In addition, Dstl, Porton Down had previously investigated recombinant protein vaccination with another five potential *B. pseudomallei* antigens, and the encoding sequences of these proteins were provided to us to enable construction of the corresponding DNA vaccines used in this study. These gene sequences encoded homologues to adhesins such as pneumoccoccal surface protein A (PsA) and haemagglutinin (Hag1), enzymes such as urease and cardiolipin synthase (CS), and a putative transmembrane protein (TMP).

Following construction of the vaccines, subsequent analysis of their efficacy to protect against infection in a mouse model was undertaken, with survival and bacterial clearance from the spleen assessed in immunised mice following challenge with live *B. pseudomallei*.

In addition, a secondary trial was also performed, immunising mice with multiple DNA vaccine constructs in an effort to synergistically enhance any protection seen following single antigen DNA vaccination.

2. Materials and Methods

2.1 Bacterial strains and plasmids

Genomic DNA was extracted from *Burkholderia pseudomallei* 08 strains obtained from Griffith University. The pGem T-easy vector was purchased from Promega and the pcrIITOPO and pcDNA3.1 V5-His vectors were purchased from Invitrogen. The general laboratory strains of *E. coli* used in this study included TOPO Top10F' (Invitrogen) and DH5α (Stratagene). Bacterial cultures were grown overnight in LB broth (Sigma) at 37 °C with vigorous shaking. Where appropriate, cultures were supplemented with 50 μg /ml ampicillin.

2.2 Polymerase Chain Reaction (PCR)

The PCR mixture contained 10 ng genomic DNA template, 40 pmol of both the sense and antisense oligonucleotide primers for each amplicon (synthesised by GeneWorks) as indicated in Table 1, 2x Failsafe™ Buffer F, 1.25U Failsafe™ enzyme in a total volume of 25 μl. The thermal cycling parameters, performed using a Peltier Thermal Cycler-200 (MJ Research), were as follows: 94 °C for 5 minutes, 50 °C for 30 seconds, 72 °C for 2 minutes followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 15 seconds, 72 °C for 90 seconds. A final cycle of 94 °C for 30 seconds, 55 °C for 15 seconds and an extension of 72 °C for 5 minutes concluded the thermal cycling. PCR generated DNA was visualised by UV light after electrophoresis through a 1% w/v agarose gel in TAE (40 mM Tris-Acetate, 1mM EDTA) containing 0.5 μg/ml ethidium bromide.

Table 1: Oligonucleotides used for amplification of B. pseudomallei gene targets

Antigen	Sense 5'- 3'	Antisense 5'- 3'
AimA	atctagaggctcgatcgtcgacaag	ggtctagagatcgaggtacccgtgccg
Heat Shock Protein	atctagagctaaagacgtcgtattcgg	ggtctagacggagcgtcttccttcg
FliC	acggctctagaggaatcaacagcaacattaactcg	agaggtctagattgcaggagcttcagcacttg
OMPA	acggctctagagcgtttccgcaaggtattccg	agaggtctagacttttgcttgatgcggatttcc
Hag2	acggctctagaagcccagcctggcgcttag	agaggtctagagtcgagcagatgtgatcggg
OPC	acggctctagagttatgctcgcaacatggctg	agaggtctagagaaagcatgttgaatccccgc
PsA	atctagagcctcttgttcgtcgcgctcg	agaggtctagaggcgccgagcgcgtcgagctg
Hag1	acggctctagaaggaatgaggtcgtgaacagg	agaggtctagattccacctgctggaagagtc
Urease	acggctctagaagggcgtacgcggaaatgtacgg	agaggtctagagaacaggaagtagcgttgcgcc
Cardiolipin Synthase	acggctctagaaccctcgactggctccatctcgg	gaggtctagagaacagccgtgcgacgtgcatcgcc
Transmembrane Protein	acggctctagacagcaccgatatctgacccagg	agaggtctagagccgctcgcgctcgcgttgac

2.3 Recombinant DNA techniques

DNA ligations, restriction endonuclease digestion and agarose gel electrophoresis were performed according to standard techniques [6]. Enzymes were purchased from Promega. DNA was purified from agarose gels using the Perfectprep Gel Cleanup Kit (Eppendorf) as per manufacturer's recommendations. Following ligation of DNA fragments, plasmid DNA was introduced into *E. coli* cells, made competent by calcium chloride, using the heat shock method [6].

2.4 Plasmid DNA preparation

Small-scale plasmid DNA preparations were extracted from 1.5 ml of a bacterial culture that had been grown overnight in LB broth (Sigma) supplemented with 50 µg /ml ampicillin. Plasmid DNA was extracted from the bacterial cells using the Promega Wizard plasmid purification kit as per manufacturer's recommendations.

Large scale plasmid DNA preparations were performed on 500 ml bacterial cultures using the Qiagen Plasmid Maxi Kit as per manufacturer's recommendations.

2.5 Transfection of 293 cells

Six well plates were seeded with $1x10^6$ 293 cells per well in 2 ml of growth medium (5% Bovine Calf Serum (ThermoTrace) / SF-DMEM (Gibco – Life Technologies) / 50 µg /ml penicillin-streptomycin) and incubated overnight at 37 °C, 5% CO_2. Two micrograms of plasmid DNA in 250 µl SF-DMEM (Gibco) per well to be transfected, was added to 10µl of Lipofectamine 2000 (Invitrogen) in 250µl of SF-DMEM that had been pre-incubated for 5 mins at room temperature. The mixture was then incubated for 20 minutes at room temperature. The cells were washed with 1 ml SF-DMEM, and 2 ml of SF-DMEM added to cover the cell monolayer. The plasmid DNA- Lipofectamine 2000 mixture was added to the appropriate well containing the cell monolayer in 2 ml of SF-DMEM and the plate incubated for 6 hrs at 37 °C, in an atmosphere containing 5% CO_2. The 2ml of SF-DMEM was removed and replaced with 2ml of the growth medium. The cells were then incubated for a further 24 hrs at 37 °C, 5% CO_2.

After the addition of 1ml of PBS, the cells were scraped from the plate using a spatula and transferred into a microfuge tube. The cells were washed two times by the addition of 500 ul of PBS followed by centrifugation at 200 g for 5 minutes to pellet the cells. The final cell pellet was resuspended in 100 ul of lysis buffer and incubated on ice for 15 minutes. The solution was then passed through a 22-gauge needle 10 times to shear the DNA.

2.6 Polyacrylamide gel electrophoresis

The harvested cells were prepared for PAGE by resuspending them in an equal volume of 2x Laemmli sample buffer (Sigma) (12.2 mM Tris-HCl pH 6.8, 20% Glycerol v/v, 4% SDS w/v, 10% β-Mercaptoenthanol v/v, 0.004% Bromophenol Blue w/v) and the sample heated to 100 °C for 5 mins, before loading the cell extracts onto a polyacrylamide gel. The prepared

samples were separated by electrophoresis through a 12.5% homogeneous SDS polyacrylamide PhastGel (Pharmacia Biotech) as per manufacturer's instructions. Low range, pre-stained, molecular weight standards (BioRad) were also loaded onto the polyacrylamide gel to enable molecular weight comparisons. Following electrophoresis, gels were either stained with Coomassie Blue (Sigma) or electrophoretically transferred to a nitrocellulose membrane (BioRad) for western blotting using the PhastSystem (Pharmacia Biotech) as per manufacturer's instructions.

2.7 Western blot analysis

Western blot analysis was performed using standard techniques. In brief, following blocking of the membrane in 10 ml of blocking buffer (5% milk powder in PBS -Tween 20 [Sigma]) for 1hr at room temperature the membrane was washed twice in 20 ml of PBS-Tween 20. The membrane was then incubated in 5 ml of blocking buffer containing Anti-V5 antibody (Invitrogen) at a dilution of 1:5000, for 2 hrs at room temperature, with shaking. For the western blots developed by ECL, the membrane was washed twice in 20 ml of PBS-Tween 20 and incubated in 15 ml of blocking buffer containing goat anti-mouse IgG horse radish peroxidase conjugate (Sigma) at a dilution of 1:30000 for 1hr at room temperature. Following washing of the membrane twice in 20 ml of PBS-Tween 20, proteins recognised by the Anti-V5 antibody were visualised by developing the blot onto x-ray film using ECL and Western Blotting Detection kit as per manufacturer's instructions (GE Healthcare). For the western blot developed using BCIP/NBT, a goat anti-mouse IgG alkaline-phosphatase conjugate (Sigma) was used as the secondary antibody for 1hr at RT, at a dilution of 1:2000 in blocking buffer. The blot was then washed twice as above, and developed using BCIP/NBT (Sigma) as per manufacturer's instructions.

2.8 Preparation of DNA microcarriers for gene gun immunisation

The preparation of the microcarriers was performed as described in the Biorad Helios Gene Gun System instruction manual using a microcarrier loading quantity (MLQ) of 0.5 mg/shot of gold and DNA loading ratio (DLR) of 1 μg/shot of plasmid for single plasmids, or a DLR of 3 μg/shot (1 μg each antigen) for multiple plasmids. In brief, 100 μl of 0.05 M spermidine was added to the required amount of gold and vortexed. The designated concentration of plasmid DNA was then added, followed by the dropwise addition of 100 μl 1M $CaCl_2$ while vortexing. The mixture was allowed to precipitate at room temperature for 10 min, then spun to pellet the gold, and the supernatant discarded. The pellet was then washed three times with 100% ethanol, before resuspension in ethanol containing the appropriate concentration of polyvinylpyrolidone (PVP). The gold/plasmid suspension was then coated onto tubing, and 0.5 inch cartridges prepared. In order to ensure quality control of cartridges, random samples of 2 cartridges per batch were selected for assessment of DNA load. The cartridges were resuspended in 200 μl Tris-EDTA and sonicated to strip the gold/DNA off the tubing. The gold was pelleted by centrifugation and the supernatant run on a 1% SDS-PAGE gel to visualise the DNA. Additionally, 2 cartridges per batch were resuspended in 500 μl Tris-EDTA and sonicated, and the gold pelleted as above, then the supernatant was read at OD260 to determine the concentration of DNA on the cartridges.

2.9 Immunisation of mice

2.9.1 Preliminary single antigen animal trial

Groups of 13 Balb/c mice were intradermally (ID) immunised on day 0, day 14 and day 28 with one of the following or the pcDNA3.1start vector [7] that did not contain any *B. pseudomallei* genes. Ten mice from each group were identified for monitoring survival, 3 for calculation of bacterial loads and ID immunisation was achieved using the Helios gene gun (Biorad), which delivered 2 μg DNA at 300 psi into the shaved abdomen of each mouse. On day 42 animals in the survival/bacterial load studies were challenged with live *B. pseudomallei* NCTC 13179 as detailed below.

2.9.2 Multiple antigen animal trial

Groups of 15 Balb/c mice were intradermally immunised on day 0, day 14 and day 28 with either 2 μg of DNA vaccine encoding HSP, Hag2, Haemagglutinin, or urease alone, or a combination DNA vaccine containing 2 μg of each plasmid coated onto the one gene gun bullet. One control group received the pcDNA3.1start vector and the other control group remained unvaccinated. Ten mice from each group were monitored for survival following challenge with live *B. pseudomallei*. The remaining five mice in each group were used to calculate bacterial loads as described below.

2.10 Challenge with *Burkholderia pseudomallei*

B. pseudomallei NCTC 13179, was plated onto blood agar 48 hours prior to challenge and incubated at 37 °C. A dose of 9.4×10^5 cfu (approximately $2 \times LD_{50}$) as described by [8], was used to challenge the vaccinated mice. A suspension of 1×10^8 cells was made up in sterile PBS with an optical density reading at 650 nm of 0.198. The dose was then diluted to achieve the required concentrations in 200 μl and the suspension was then placed into syringes. The mice were infected with the bacteria via intraperitoneal inoculation and monitored for 10–14 days for survival and any moribund mice were euthanised using CO_2 asphyxiation.

To determine the precise inoculum dose for the mice, ten-fold serial dilutions were made in PBS and 20 μl of each dilution were plated in triplicate onto Ashdown's agar and incubated at 37 °C for 24–48 hours. The colonies were counted and the dose was calculated.

2.11 Bacterial loads

Bacterial loads were carried out on 3–5 mice from each immunisation group at three days after challenge with *B. pseudomallei* NCTC 13179. Mice were euthanised via CO_2 asphyxiation. Spleens were removed aseptically and placed into a stomacher bag with PBS. The bags were then placed into a stomacher until the spleens were homogenised. Ten-fold dilutions were made from the homogenate and 20 μl aliquots plated onto Ashdown's agar in triplicate and incubated for 24–48 hours. The colonies were counted and the concentration of bacteria in the whole spleen calculated.

2.12 Western Blot using Melioidosis patient sera

Western blot analysis was performed using standard techniques. In brief, following blocking of the membrane in 10 ml of blocking buffer (3% milk powder in PBS -Tween 20 [Sigma]) overnight at 4 °C, the membrane was washed twice in PBS-Tween20. The membrane was then incubated in 5 ml of blocking buffer containing sera from melioidosis patients or negative control sera at a dilution of 1:500, for 1 hr at room temperature, with shaking. The membrane was washed twice in 20 ml of 1% NaCl in PBS-Tween 20 to help reduce non-specific background, followed by two washes in 20 ml of PBS-Tween 20. The blots were then incubated in 15 ml of blocking buffer containing goat anti-human IgG horse radish peroxidise conjugate (Sigma) at a dilution of 1:50000 for 1hr at room temperature. Following washing of the membrane twice in 20 ml of 1% NaCl in PBS-Tween 20 followed by two washes in 20 ml of PBS-Tween 20, proteins recognised by patient sera were visualised by developing the blot onto x-ray film using ECL and Western Blotting Detection kit as per manufacturer's instructions (GE Healthcare).

3. Results

3.1 Construction of DNA vaccines

The genetic sequences of eleven putative *B. pseudomallei* antigens were obtained either as the result of BLAST searches against homologous sequences from other organisms, or as encoding sequences of recombinant protein vaccines produced by Dstl, Porton Down. The identified full sequences were amplified by PCR using genomic DNA from the 08 *B. pseudomallei* strain as template, with the exception of the PsA sequence, which failed to amplify under several different PCR conditions, and was subsequently truncated at the 3' (N-terminus) end to enable successful amplification of the product. The acquired PCR products were then ligated into the intermediate vector pcrIITOPO™ (Invitrogen). The sequence was then excised and sub-cloned into the mammalian expression vector pcDNA3.1start [7] to create the DNA vaccine constructs listed in Table 2.

Table 2: Gene products encoded by the DNA vaccine constructs

DNA Vaccine Construct	Gene Product
pBps130	Novel protein (AimA)
pBps160	Heat shock protein (HSP)
pBps170	Flagellin (FliC)
pBps1100	OMPA
pBps1120	Hag2
pBps1130	OPC
pBps1140	PsA
pBps1150	Hag1
pBps1160	Urease
pBps1170	Cardiolipin synthase (CS)
pBps1180	Transmembrane protein (TMP)

In order to assess whether the encoded *B. pseudomallei* sequences could be successfully translated into protein antigen within mammalian cells, the DNA vaccines were transfected via a lipoprotein carrier into human HEK 293 kidney cells *in vitro*, and the subsequent protein expression analysed by separating the lysed cell extracts by PAGE and probing with an anti-V5 antibody that recognised the V5 epitope engineered to the carboxy terminal of the protein. The molecular weight estimated for the detected proteins correlated with the expected molecular weight of the *B. pseudomallei* antigens encoded by their corresponding DNA vaccines, with the exception of the PsA homolog, where the molecular weight reflected the truncation of the antigen sequence (Figure 2). The detection of all eleven of the *B. pseudomallei* antigens by western blot demonstrated that the engineered vaccines contained all the necessary information to direct protein synthesis of the encoded proteins in a mammalian cell system, and provided some confidence that when introduced into mice, the vaccines should permit the synthesis of the encoded protein, and potentially allow for peptide display on the surface of transfected cells for recognition by immune cells.

3.2 Immunisation and challenge of mice with a single DNA vaccine construct

Following assessment of protein expression *in vitro*, preliminary animal trials were undertaken in order to test the protective capability of the constructed DNA vaccines. Eleven groups of 15 Balb/c mice received three immunisations ID with one of the DNA constructs over a 6 week period, and were subsequently challenged with $2xLD_{50}$ *B. pseudomallei* NCTC 13179 two weeks after the final immunisation. One group of mice in each trial performed were sham immunised with the pcDNA3.1start vector alone as a negative control. Ten mice in each group were monitored for survival over a 10 day period post-challenge (Figure 3). The groups immunised with the Hag1, Hag2 and heat shock protein DNA vaccines showed the highest level of protection against bacterial infection, with percentage survival at 20%, 25% and 20% respectively. One mouse was also protected in the group immunised with DNA vaccine encoding urease. There was no protection seen in mice immunised with the DNA vaccines encoding the cardiolipin synthase, the transmembrane protein, AimA, OPC, FliC, OMPA or PsA.

In addition, 3 mice per immunisation group were sacrificed on the third day post-challenge in order to assess the ability of immunised animals to effectively clear bacteria from the spleen. The spleens were homogenised and bacterial loads calculated through serial dilutions plated in triplicate on Ashdown's agar and incubated over 24–48hrs (Figure 4). Bacterial loads were unable to be determined in mice immunised with DNA vaccines encoding urease, cardiolipin synthase or the transmembrane protein, as all animals succumbed to challenge prior to day 3. Unfortunately in the remaining immunisation groups, there was no reduction in bacterial loads in the spleens of any of the animals, when compared to sham immunised animals, indicating that although some DNA vaccine constructs provided moderate protection and delayed time to death, they could not promote complete clearance of infection.

3.3 Immunisation and challenge of mice with multiple DNA vaccine constructs

Based on the results observed following immunisation with a single DNA vaccine construct, the four vaccines that provided some protection against challenge (Hag1, Hag2, HSP and urease) were used in a secondary animal trial to determine whether immunisation with a combination of all four plasmid constructs could synergistically enhance survival. Four groups of 15 mice received three ID immunisations at two week intervals of a single vaccine construct. A fifth group received all four plasmids 3 times over the same 6 week period. The concentrations of each vaccination dose were adjusted to ensure that mice receiving multiple plasmids were administered the same concentration of each antigen as those mice receiving a single dose.

As with the previous trial, 10 mice from each group were assessed for survival following challenge with $2xLD_{50}$ *B. pseudomallei* NCTC 13179 IP over a 10 day period. It was planned that the remaining five mice were to be sacrificed on day 3 post-challenge to determine bacterial loads in the spleen, however the majority of mice in each group succumbed to infection prior to this date, and therefore bacterial clearance could only be observed in 2 mice from each immunisation group.

Although immunisation with multiple plasmids failed to significantly enhance any protection seen following vaccination with a single plasmid (Figure 5), there was some decrease in the bacterial loads observed in animals immunised with all four plasmids when compared with both sham or unvaccinated controls and also with animals immunised with the HSP or urease DNA vaccine alone (Figure 6), which may indicate that DNA vaccination with multiple antigens is more effective than single plasmid vaccination. However, due to the low number of animals surviving in each group when bacterial loads were assessed, it is hard to draw conclusions about the significance of this result.

3.4 Western Blot using Melioidosis patient sera

Western blot analysis was performed to determine if antibodies in human sera could recognise the antigens encoded by the constructed DNA vaccines. Sera was taken from two convalescent melioidosis patients and one uninfected individual (negative control) and blotted against 293 cell extracts containing expressed protein encoded by each of the DNA vaccines. Untransfected 293 cell extracts were also probed with the sera to act as a negative control. Unfortunately there was no antigen-specific antibody recognition by either of the melioidosis patient serum samples (data not shown).

4. Discussion

DNA vaccines provide a promising strategy for combating disease caused by intracellular pathogens. Often it proves hard to develop effective vaccines against these organisms, as they are adept at evading the host immune response, and frequently cause latent infection, remaining dormant for months or even years, only to re-emerge when the host is immunocompromised. DNA vaccines allow antigen to be synthesised intracellularly and presented on cell surfaces complexed with MHC I, resulting in the activation of cellular immunity including $CD8^+$ cytotoxic lymphocytes, which can then lyse infected cells and release cytokines to make surrounding cells resistant to subsequent infection.

In this study eleven DNA vaccines were constructed against the intracellular pathogen *Burkholderia pseudomallei*, the causative agent of melioidosis. The vaccines were subsequently assessed *in vitro* for ability to intracellularly express the encoded antigen, and also ability to provide protection in Balb/c mice following administration of a lethal dose of *B. pseudomallei* NCTC 13179.

The groups of mice immunised with the Hag1, Hag2 and heat shock protein DNA vaccines showed the highest level of protection against bacterial infection. The increased protective capabilities of the heat shock protein DNA vaccine support the findings of previous studies, which have shown the immunogenicity of HSP, particularly when used as an adjuvant for DNA vaccines [9, 10]. It is well established that HSPs play a significant role in innate and adaptive immune responses to pathogens, through interactions with antigen presenting cells, mediation of dendritic cell maturation, and cytokine induction [11, 12]. As a stand-alone vaccine it may not be a good choice, due to the highly conserved nature of the antigen across a wide selection of organisms, however as part of a multi-component vaccine it may enhance any protective responses provided by other antigens, as has been shown previously [10].

The Hag1 and Hag2 vaccines both encode adhesins, and are considered important virulence factors, as these proteins have been shown to play a role in attachment of pathogens to host cells to facilitate invasion [13, 14]. It is expected that they may be more immunogenic than other antigens, as they are surface proteins and are therefore more likely to be recognised by the immune system. A homolog to Hag2 was induced *in-vivo* in a rat lung model of chronic *P. aeruginosa* infection [15], indicating that it may be highly expressed during infection, and therefore more accessible to antigen presenting cells and lymphocytes.

Interestingly however, the DNA vaccine encoding the homolog of PsA, also an adhesin known to facilitate invasion [16], failed to induce any increase in survival. This contradicts the effects observed following vaccination with recombinant PsA performed by Dstl, where significant delay time to death was seen in immunised animals when compared with sham or unvaccinated controls (unpublished data). This lack of a protective response seen in our study may be the result of the truncation of the protein, which although led to the translated protein being expressed more easily, may have resulted in a change to the secondary structure, thereby masking epitopes that may be important in immune recognition, or even eliminating epitopes altogether if they were located on the truncated domain.

It is also surprising that there was no increase in protection following immunisation with the FliC vaccine, as this would appear to contradict observations from a study performed by Chen *et al*, who found that intramuscular injection of a DNA plasmid construct encoding flagellin was associated with a decrease in live bacterial counts in both the liver and the spleen following bacterial challenge (17). This difference in responses could however be attributed to different vaccine delivery systems, as gene gun induced responses have been shown to differ from those seen following intra-muscular immunisation (18).

Another explanation for the lack of protective response and bacterial clearance observed in our study could be the degree to which antigens are actually expressed by the organism in natural infection, a claim that is supported by the non-reactivity of sera taken from two convalescent melioidosis patients when immuno-blotted against the protein antigens (data not shown).

For this reason, perhaps immunisation with plasmid DNA is not the most effective vaccination strategy to employ against *B. pseudomallei*. It may be beneficial against viral pathogens, where there are a limited number of structural proteins that can be encoded by the vaccine, thus allowing for all antigens to be incorporated into one vaccine, and therefore covering a larger pool of antigens potentially on display to immune cells during natural infection. But for bacteria, in particular *B. pseudomallei* which has a large genome spread over 2 chromosomes, the range of potential antigens is extensive, and by selecting a mere few, the chance that these antigens will be highly effective at providing protection from disease is quite small. The limited knowledge of the mechanisms of *B. pseudomallei* infection also makes selecting effective candidate antigens quite difficult. While certain virulence factors that contribute to adherence and invasion of the organism have been quite well defined through mutagenesis studies, it is evident both from our study, and from others [19], that virulence in *B. pseudomallei* infection does not necessarily automatically translate to immunogenicity. Indeed, it has even been reported that in some cases, not even naturally acquired infection is enough to guarantee against subsequent re-infection with *B. pseudomallei* [20]. Reverse vaccinology, which uses bioinformatics to determine epitopes from an entire sequenced genome is fast becoming the future for antigenic determination, however it seems that one of the more optimal ways of providing protection is still to use attenuated *B. pseudomallei*, a vaccine strategy that is unlikely to ever be licensed, due to the high risk of reversion of the bacteria to a virulent state.

The use of multiple DNA vaccines to synergistically enhance protective responses has been studied in a number of pathogens, with mixed results. A study comparing single plasmid DNA immunisation against *M. tuberculosis* with a four-plasmid multiple DNA immunisation regime found that the multi-plasmid immunisation not only gave a higher protective response than the single plasmid vaccine, but was also equivalent to the protective response seen by the currently licensed live BCG vaccine [21]. Similar results were also seen in studies of combination vaccination with HIV/HCV immunogens, and even with multi-plasmid vaccines against different targets such as *B. anthracis*, Ebola virus, Marburg and Venezuelan equine encephalitis [22]. However, there have also been cases where multi-plasmid vaccination has resulted in reduction of immune responses due to interference between antigens [23, 24]. Whether this is the case in our study remains to be seen, however it may be one explanation as

to why there was no enhancement in the protective response seen when immunising with a combination of antigens over immunisation with a single antigen.

As an alternative vaccine strategy for *B. pseudomallei*, it may then be better to focus on non-antigen specific means of enhancing immunity. Immunostimulatory adjuvants such as oligodeoxynucleotides (ODN) containing CpG motifs have been shown to stimulate toll-like receptor 9 (TLR9) which is important in innate immune responses. Studies have shown that when CpG ODN 1826 was incorporated into a DNA plasmid construct encoding *B. pseudomallei* flagellin and was administered intra-muscularly to Balb/c mice, it enhanced both the Th1-specific immune response and resistance of the mice to infectivity when compared with animals immunised with an unmodified plasmid [25]. Indeed even when administered alone, CpG ODN has repeatedly shown protective capability against infection with a number of intracellular bacteria [26], including *B. pseudomallei* [27] most likely due to initial innate inflammatory responses subsequently facilitating the development of more specific Th1 based immunity, with rapid stimulation of B and T cells, and enhanced production of immunomodulatory cytokines such as IFN-γ, IL-12, IL-6 and TNF-α [26].

Chemical adjuvants have also proved to be quite promising in enhancing DNA vaccine immunogenicity in animal models. Liposomes have been shown to protect DNA from degradation by serum proteins [28], and cationic polymers aid in transfection and augment DNA expression *in vivo* [29]. Microparticles have also been used, by adsorbing DNA immunogens onto or encapsulating inside polymeric microspheres to help protect the DNA from degradation and also to specifically target the plasmid DNA to antigen presenting cells [28].

5. Conclusion

This study has detailed the construction and assessment of eleven DNA vaccines encoding various antigens from *B. pseudomallei*. Although DNA vaccines have been shown in a number of studies to evoke strong CD8+ cytotoxic responses necessary for protection against intracellular pathogens, we have found in our studies that for *B. pseudomallei* they may not be the optimal delivery system to provide protection against disease in their own right. They may be better suited to viral pathogens, where antigen selection is limited to a few genes, as opposed to the numerous potential antigens for *B. pseudomallei*. The recommendations for the use of a *B. pseudomallei* DNA vaccine would perhaps be to combine it either in a prime boost regime with recombinant protein or with use of an adjuvant such as CpG oligonucleotide or chemical adjuvants such as liposomes, nanoparticles or polymers.

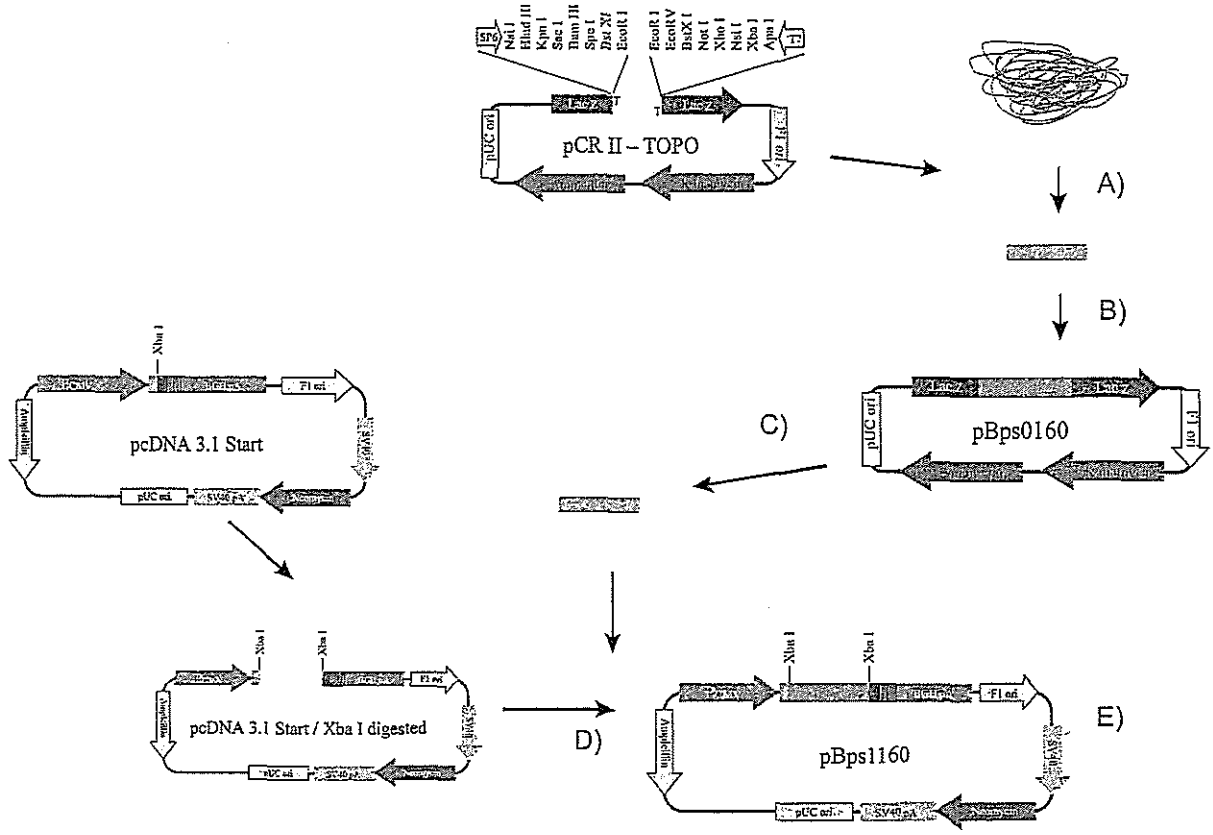

Figure 1: ***Construction of the DNA vaccines.*** *The gene sequence encoding each* B. pseudomallei *antigenic protein was amplified from* B. pseudomallei *genomic DNA using PCR (A). The amplified product was cloned into the plasmid vector pCRII-TOPO. to create an intermediate construct (B). The* B. pseudomallei *gene sequence was then excised from pCRII- TOPO by Xba I restriction endonuclease digestion (C) and subcloned into the modified mammalian cell expression vector pcDNA3.1start (D), to create the DNA vaccine construct (E).*

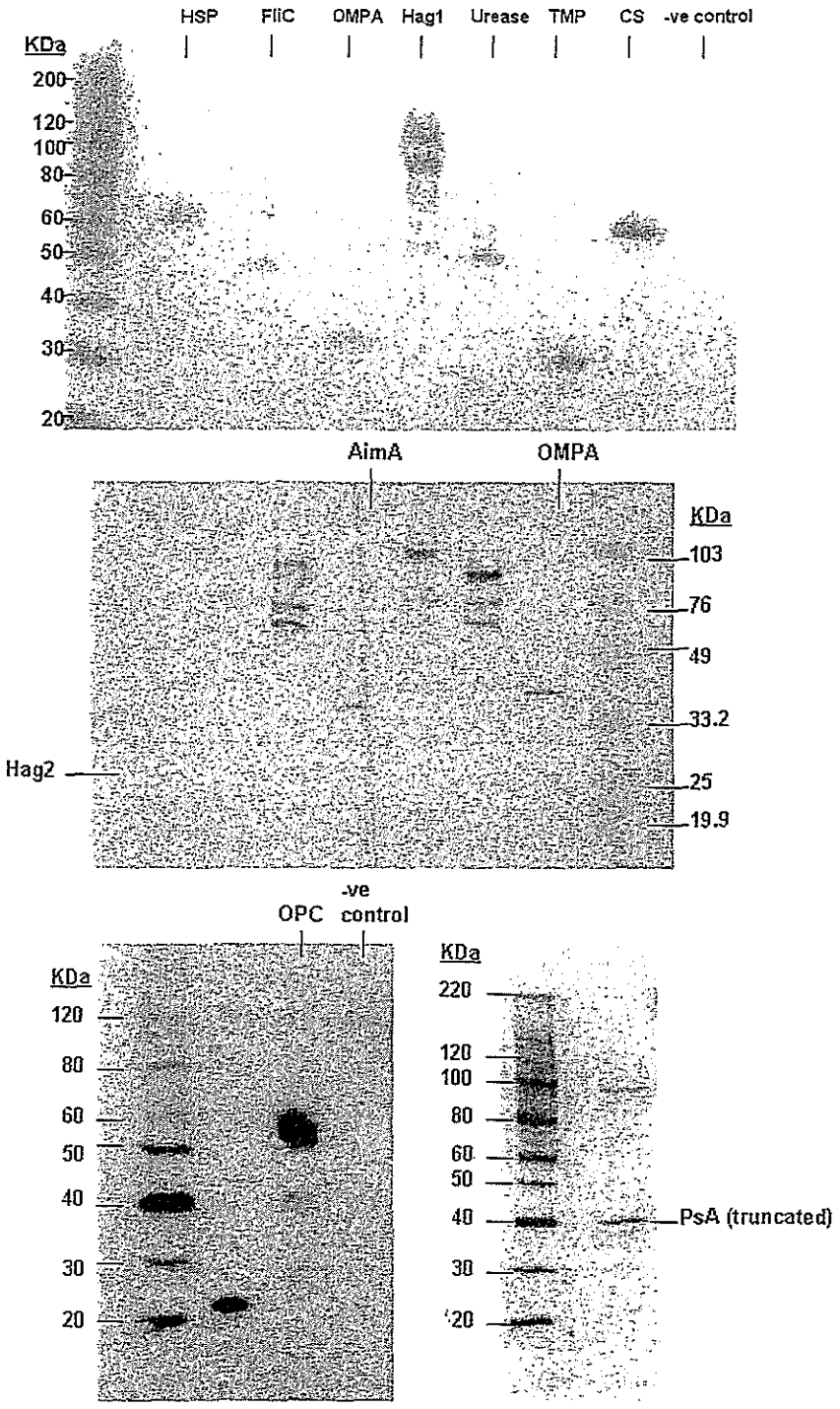

Figure 2: In vitro expression of the B. pseudomallei *proteins encoded by the DNA vaccines, detected by western blot. The DNA plasmid constructs were transfected into mammalian 293S cells using a cationic lipid complex. Extracts from transfected cells and non-transfected negative control cells were resolved by PAGE (12.5%), transferred to a nitrocellulose membrane and probed with anti-V5 epitope antibody. Reactive proteins were visualised through a HRP-conjugated secondary antibody developed by ECL, with the exception of Gel (B), where reactive proteins were visualised through an alkaline-phosphatase conjugated secondary antibody developed by BCIP/NBT.*

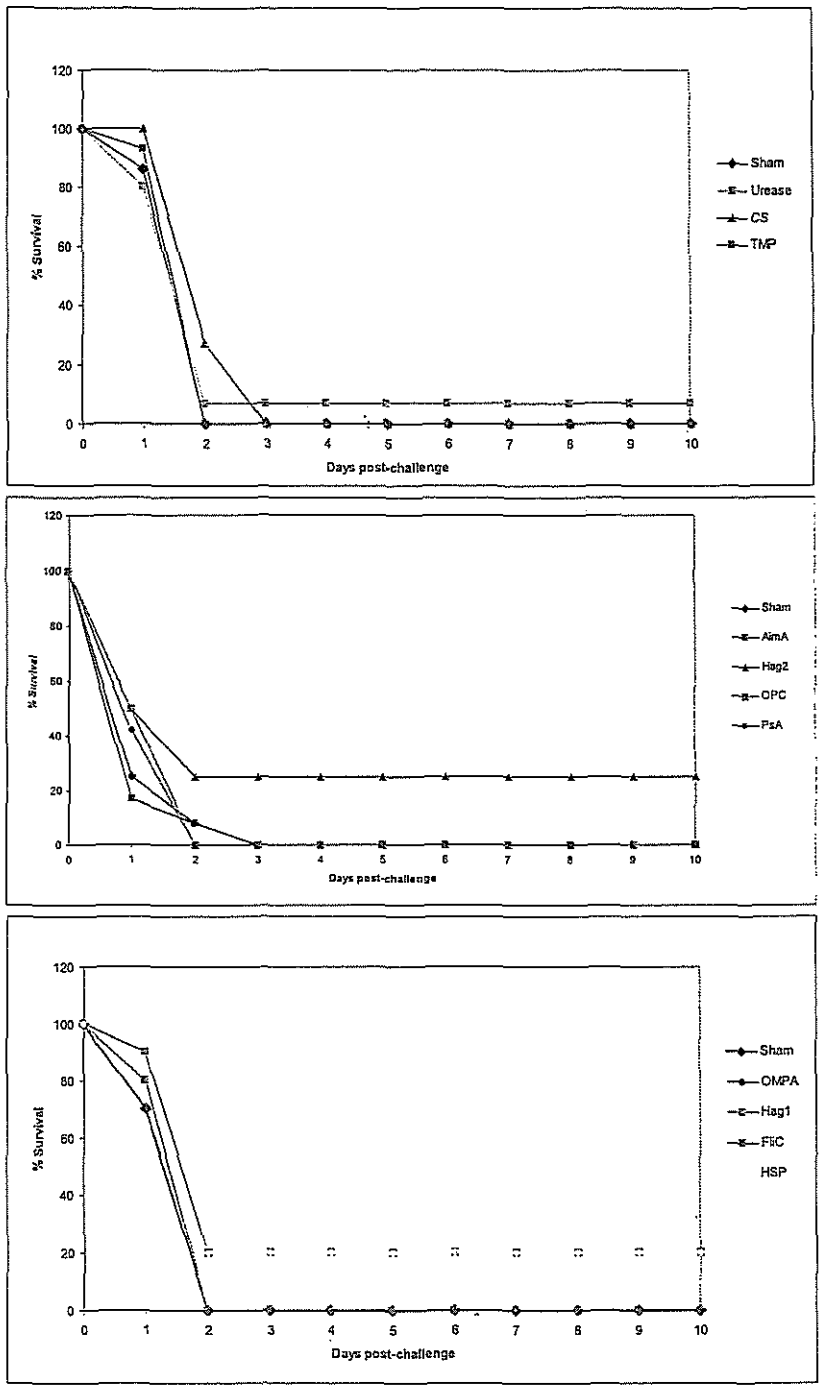

Figure 3: Survival of mice following challenge with live B. pseudomallei NCTC 13179. Ten mice immunised with either pcDNA3.1start alone (sham) or a DNA vaccine encoding one of the following B. pseudomallei *antigens: urease, cardiolipin synthase (CS), transmembrane protein (TMP), OPC, PsA, OMPA, Hag1, Hag2, AimA, FliC, or heat shock protein (HSP). Two weeks after the final immunisation, all animals received 2xLD50 IP of live* B. pseudomallei. *Survival was monitored over a 10 day period post-challenge.*

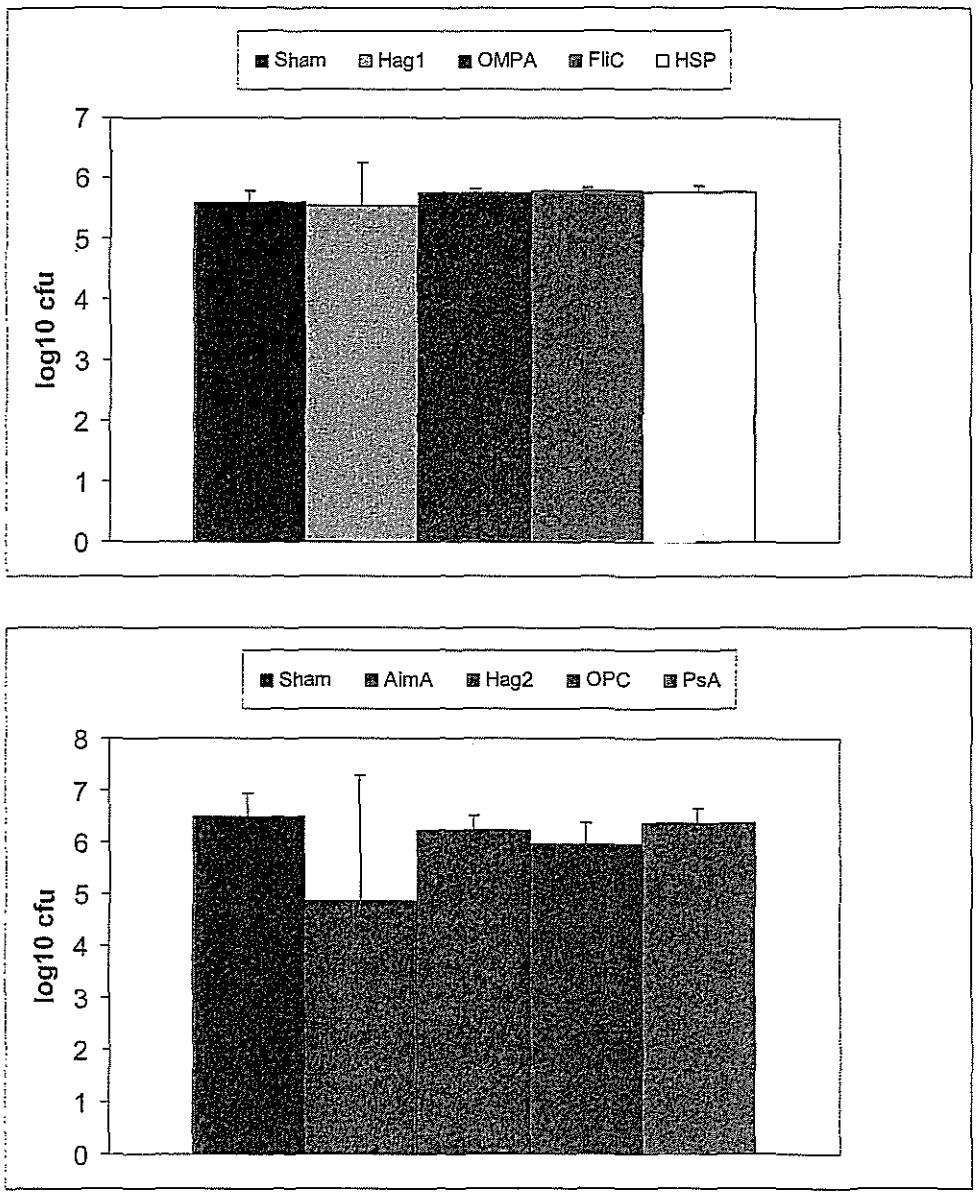

Figure 4: *Bacterial loads in spleens of mice following DNA vaccination and challenge with live* **B. pseudomallei** *NCTC 13179. 3 mice from each immunisation group received 2xLD_{50} of live* B. pseudomallei *NCTC 13179 IP, 2 weeks after the third DNA vaccination. Three days after challenge animals were sacrificed and the spleens homogenised. Ten-fold dilutions were made from the homogenate and plated onto Ashdown's agar in triplicate in 20 µl aliquots and incubated for 24–48 hours. The colonies were counted and the concentration of bacteria in the whole spleen calculated. Values are expressed as the mean±SD*

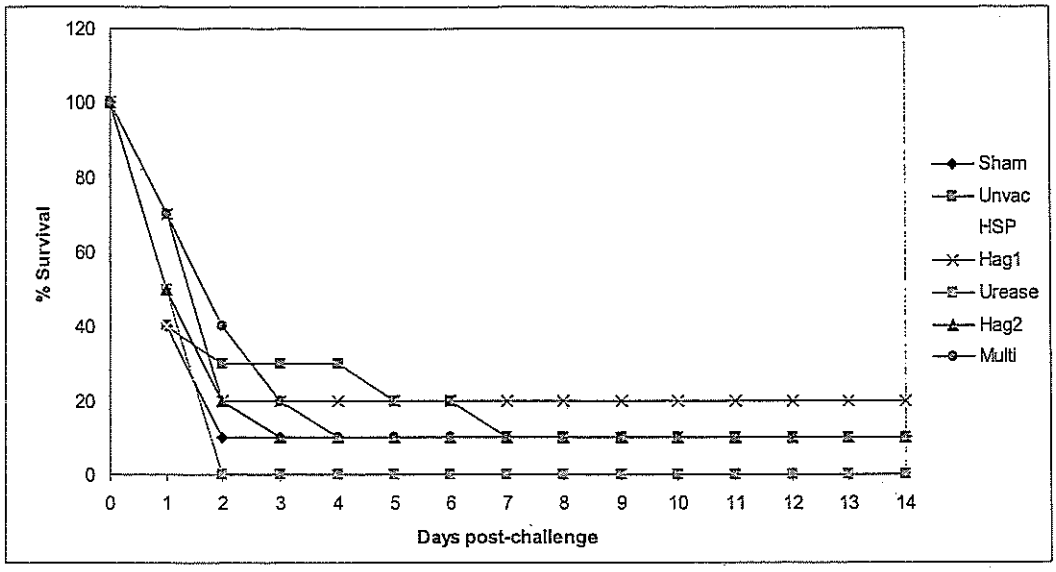

Figure 5: Effects of DNA immunisation with multiple **B. pseudomallei** *antigens on survival followed by live challenge. Groups of ten mice were immunised with DNA vaccines encoding HSP, Hag1, Urease, Hag2, or a combination of all four antigens (Multi). Two control groups received either pcDNA3.1start alone (sham) or remained unvaccinated. Two weeks after the third immunisation, mice received 2xLD_{50} IP of live* B. pseudomallei *NCTC 13179.and survival was monitored over a 14 day period post-challenge.*

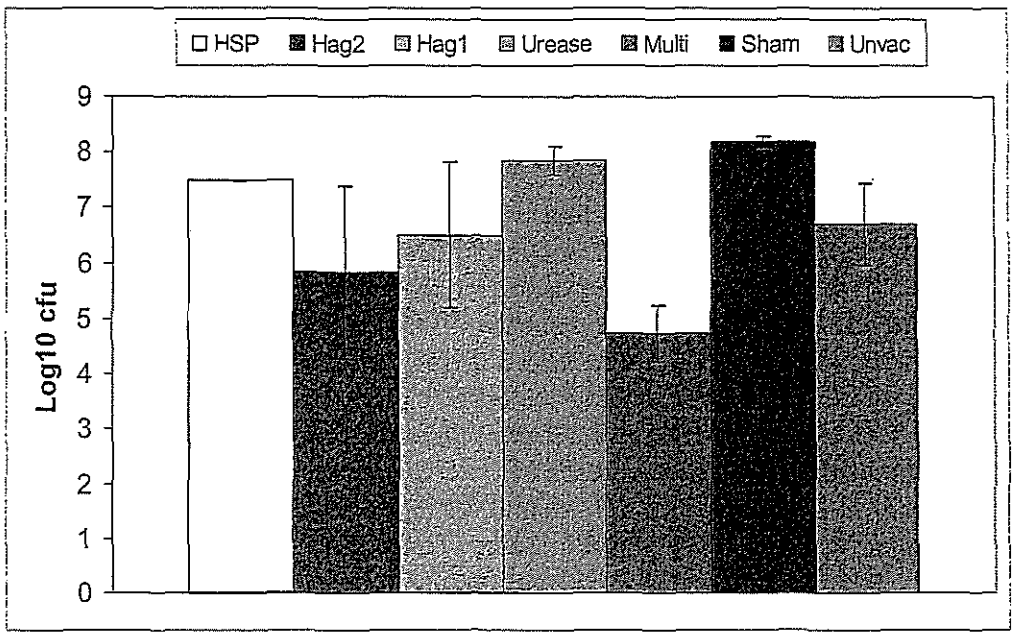

Figure 6: Effects of immunisation with multiple B. pseudomallei *antigens on bacterial loads in spleens of mice following challenge with live* B. pseudomallei *NCTC 13179. 5 mice from each immunisation group received 2xLD50 of live* B. pseudomallei *NCTC 13179 IP, 2 weeks after the third DNA vaccination. Two days after challenge animals were sacrificed and the spleens homogenised. Ten-fold dilutions were made from the homogenate and plated onto Ashdown's agar in triplicate in 20 µl aliquots and incubated for 24–48 hours. The colonies were counted and the concentration of bacteria in the whole spleen calculated. Values are expressed as the mean±SEM*

6. References

1. Giese, M. 1998. DNA-antiviral vaccines: new developments and approaches--a review. *Virus Genes.* 17:219-232.
2. Nagata, T., T. Aoshi, M. Uchijima, M. Suzuki, and Y. Koide. 2004. Cytotoxic T-lymphocyte-, and helper T-lymphocyte-oriented DNA vaccination. *DNA Cell Biol.* 23:93-106.
3. Zhang, D. J., X. Yang, C. Shen, and R. C. Brunham. 1999. Characterization of immune responses following intramuscular DNA immunization with the MOMP gene of Chlamydia trachomatis mouse pneumonitis strain. *Immunology.* 96:314-321.
4. Chikhlikar, P., L. B. de Arruda, M. Maciel, P. Silvera, M. G. Lewis, J. T. August, and E. T. Marques. 2006. DNA Encoding an HIV-1 Gag/Human Lysosome-Associated Membrane Protein-1 Chimera Elicits a Broad Cellular and Humoral Immune Response in Rhesus Macaques. *PLoS ONE.* 1:e135.
5. Laddy, D. J., and D. B. Weiner. 2006. From plasmids to protection: a review of DNA vaccines against infectious diseases. *Int Rev Immunol.* 25:99-123.
6. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: a Laboratory Manual.* Cold Spring Harbor Laboratory Press, USA.
7. Proll, D. F., and P. J. Gauci. 2003. Generation of a gene expression cassette to enable the rapid production of DNA vaccines. *DSTO Technical Report.*
8. Barnes, J. L., and N. Ketheesan. 2005. Route of infection in melioidosis. *Emerg Infect Dis.* 11:638-639.
9. Zhang, X., C. Yu, J. Zhao, L. Fu, S. Yi, S. Liu, T. Yu, and W. Chen. 2007. Vaccination with a DNA vaccine based on human PSCA and HSP70 adjuvant enhances the antigen-specific CD8+ T-cell response and inhibits the PSCA+ tumors growth in mice. *J. Gene Med.* 9:715-726.
10. Segal, B. H., X.-Y. Wang, C. G. Dennis, R. Youn, E. A. Repasky, M. H. Manjili, and J. R. Subjeck. 2006. Heat shock proteins as vaccine adjuvants in infections and cancer. *Drug Discov. Today* 11:534-540.
11. Walker, K. B., J. Keeble, and C. Colaco. 2007. Mycobacterial heat shock proteins as vaccines - A model of facilitated antigen presentation. *Curr. Mol. Med.* 7:339-350.
12. Javid, B., P. A. MacAry, and P. J. Lehner. 2007. Structure and function: heat shock proteins and adaptive immunity. *J. Immunol.* 179:2035-2040.
13. Quin, L. R., Q. C. Moore, 3rd, and L. S. McDaniel. 2007. Pneumolysin, PspA, and PspC contribute to pneumococcal evasion of early innate immune responses during bacteremia in mice. *Infect Immun.* 75:2067-2070. Epub 2007 Jan 2012.
14. Inatsuka, C. S., S. M. Julio, and P. A. Cotter. 2005. Bordetella filamentous hemagglutinin plays a critical role in immunomodulation, suggesting a mechanism for host specificity. *Proc Natl Acad Sci U S A.* 102:18578-18583. Epub 12005 Dec 18579.
15. Handfield, M., D. E. Lehoux, F. Sanschagrin, M. J. Mahan, D. E. Woods, and R. C. Levesque. 2000. In vivo-induced genes in Pseudomonas aeruginosa. *Infect. Immun.* 68:2359-2362.
16. Hammerschmidt, S. 2006. Adherence molecules of pathogenic pneumococci. *Curr. Opin. Microbiol.* 9:12-20.

17. Chen, Y. S., Y. S. Hsiao, H. H. Lin, C. M. Yen, S. C. Chen, and Y. L. Chen. 2006. Immunogenicity and anti-Burkholderia pseudomallei activity in Balb/c mice immunized with plasmid DNA encoding flagellin. *Vaccine.* 24:750-758. Epub 2005 Sep 2006.
18. Wang, S., C. Zhang, L. Zhang, J. Li, Z. Huang, and S. Lu. 2008. The relative immunogenicity of DNA vaccines delivered by the intramuscular needle injection, electroporation and gene gun methods. *Vaccine* 26:2100-2110.
19. Druar, C., F. Yu, J. L. Barnes, R. T. Okinaka, N. Chantratita, S. Beg, C. W. Stratilo, A. J. Olive, G. Soltes, M. L. Russell, D. Limmathurotsakul, R. E. Norton, S. X. Ni, W. D. Picking, P. J. Jackson, D. I. H. Stewart, V. Tsvetnitsky, W. L. Picking, J. W. Cherwonogrodzky, N. Ketheesan, S. J. Peacock, and E. J. Wiersma. 2008. Evaluating Burkholderia pseudomallei Bip proteins as vaccines and Bip antibodies as detection agents. *FEMS Immunol. Med. Microbiol.* 52:78-87.
20. Maharjan, B., N. Chantratita, M. Vesaratchavest, A. Cheng, V. Wuthiekanun, W. Chierakul, W. Chaowagul, N. P. J. Day, and S. J. Peacock. 2005. Recurrent melioidosis in patients in Northeast Thailand is frequently due to reinfection rather than relapse. *J. Clin. Microbiol.* 43:6032-6034.
21. Morris, S., C. Kelley, A. Howard, Z. Li, and F. Collins. 2000. The immunogenicity of single and combination DNA vaccines against tuberculosis. *Vaccine* 18:2155-2163.
22. Riemenschneider, J., A. Garrison, J. Geisbert, P. Jahrling, M. Hevey, D. Negley, A. Schmaljohn, J. Lee, M. K. Hart, L. Vanderzanden, D. Custer, M. Bray, A. Ruff, B. Ivins, A. Bassett, C. Rossi, and C. Schmaljohn. 2003. Comparison of individual and combination DNA vaccines for B. anthracis, Ebola virus, Marburg virus and Venezuelan equine encephalitis virus. *Vaccine* 21:4071-4080.
23. Kjerrstrom, A., J. Hinkula, G. Engstrom, V. Ovod, K. Krohn, R. Benthin, and B. Wahren. 2001. Interactions of single and combined human immunodeficiency virus type 1 (HIV-1) DNA vaccines. *Virology* 284:46-61.
24. Muthumani, K., S. Kudchodkar, D. Zhang, M. L. Bagarazzi, J. J. Kim, J. D. Boyer, V. Ayyavoo, G. N. Pavlakis, and D. B. Weiner. 2002. Issues for improving multiplasmid DNA vaccines for HIV-1. *Vaccine* 20:1999-2003.
25. Chen, Y. S., Y. S. Hsiao, H. H. Lin, Y. Liu, and Y. L. Chen. 2006. CpG-modified plasmid DNA encoding flagellin improves immunogenicity and provides protection against Burkholderia pseudomallei infection in BALB/c mice. *Infect Immun.* 74:1699-1705.
26. Elkins, K. L., T. R. Rhinehart-Jones, S. Stibitz, J. S. Conover, and D. M. Klinman. 1999. Bacterial DNA containing CpG motifs stimulates lymphocyte-dependent protection of mice against lethal infection with intracellular bacteria. *J Immunol.* 162:2291-2298.
27. Wongratanacheewin, S., W. Kespichayawattana, P. Intachote, S. Pichyangkul, R. W. Sermswan, A. M. Krieg, and S. Sirisinha. 2004. Immunostimulatory CpG oligodeoxynucleotide confers protection in a murine model of infection with Burkholderia pseudomallei. *Infect Immun.* 72:4494-4502.
28. Greenland, J. R., and N. L. Letvin. 2007. Chemical adjuvants for plasmid DNA vaccines. *Vaccine* 25:3731-3741.
29. Breunig, M., U. Lungwitz, R. Liebl, C. Fontanari, J. Klar, A. Kurtz, T. Blunk, and A. Goepferich. 2005. Gene delivery with low molecular weight linear polyethylenimines. *J. Gene Med.* 7:1287-1298.

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Jane McAllister, Sharon Lazzaroni, Jodie Barnes, Natkunam Ketheesan and David Proll

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DSTO Defence Science and Technology Organisation
506 Lorimer St
Fishermans Bend Victoria 3207 Australia

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Burkholderia pseudomallei, DNA vaccine, immune response

19. ABSTRACT

Burkholderia pseudomallei is the causative agent of melioidosis, a disease endemic in Southeast Asia and Northern Australia. The bacteria cause infection via subcutaneous or inhaled routes, resulting in either acute lethal sepsis or chronic and eventually fatal disease. Currently no licensed vaccine is available to provide protection against this pathogen. In this study, eleven DNA vaccines encoding the *B. pseudomallei* proteins were constructed and used to immunise mice and subsequent protective capability analysed following live challenge with *B. pseudomallei*.

Mice immunised with DNA vaccines encoding Hag1, Hag2 and heat shock protein demonstrated the greatest level of protection against bacterial infection, however, the levels of protection seen were still quite limited. There was no increase in survival seen following immunisation with the remaining eight vaccines. This study highlights the challenges in designing vaccines against *B. pseudomallei* and suggests that a successful DNA vaccine may need to be used in conjunction with adjuvant such as CpG oligodeoxynucleotide, or combined in a prime-boost regime with recombinant protein in order to increase protective capabilities.

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