Burkholderia pseudomallei and *Burkholderia mallei* vaccines: are we close to clinical trials?

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ABSTRACT

B. pseudomallei is the cause of melioidosis, a serious an often fatal disease of humans and animals. The closely related bacterium *B. mallei*, which cases glanders, is considered to be a clonal derivative of *B. pseudomallei*. Both *B. pseudomallei* and *B. mallei* were evaluated by the United States and the former USSR as potential bioweapons. Much of the effort to devise biodefence vaccines in the past decade has been directed towards the identification and formulation of sub-unit vaccines which could protect against both melioidosis and glanders. A wide range of proteins and polysaccharides have been identified which protective immunity in mice. In this review we highlight the significant progress that has been made in developing glycoconjugates as sub-unit vaccines. We also consider some of the important the criteria for licensing, including the suitability of the “animal rule” for assessing vaccine efficacy, the protection required from a vaccine and the how correlates of protection will be identified. Vaccines developed for biodefence purposes could also be used in regions of the world where naturally occurring disease is endemic

Keywords

melioidosis

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1. Melioidosis; The global incidence

B. pseudomallei is the cause of melioidosis, a serious and often fatal disease of humans and animals. The closely related bacterium *B. mallei*, which causes glanders, is a clonal derivative of *B. pseudomallei* [1], with a reduced host range. In this review we have included references to *B. mallei* where appropriate. Human melioidosis can range from a localised skin infection to an acute septicaemia or a pneumonia. Some individuals develop chronic disease, whilst others apparently clear the infection only to suffer a relapse later [2, 3]. The diverse forms of disease make disease diagnosis based in clinical signs and symptoms challenging.

B. pseudomallei and *B. mallei* were evaluated by the United States and the former USSR as potential bioweapons [4, 5]. They attracted attention because, at least in animal models, they are highly infectious by the airborne route [6]. This is consistent with cases of disease in healthy US helicopter crews during the Vietnam War, believed to be a consequence of the inhalation of soil-derived dusts containing *B. pseudomallei* [7]. Because of the potential for *B. pseudomallei* and *B. mallei* to cause disease in humans and animals these bacteria are classified as tier 1 overlap select agents by the US Centers for Disease Control and Prevention and the US Animal and Plant Health Inspection Services.

Naturally occurring melioidosis is usually associated with South East Asia or Northern Australia. In northeast Thailand melioidosis is the third most common cause of death from infectious diseases after human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and tuberculosis [8]. A wide range of underlying conditions predispose individuals to disease, but diabetes is the main risk factor [9, 10]. Melioidosis is not currently considered to be a neglected tropical disease, but evidence is accumulating that it is present in many sub-tropical and tropical regions of the world [11, 12]. A recent study (Table 1)

predicts that the global incidence of human melioidosis is 165,000 cases (95% credible interval 68,000–412,000) with 89,000 (95% credible interval 36,000–227,000) deaths [11]. If these predications are validated, then the global death toll from melioidosis is comparable to the global mortality from measles (95,600 per year) and higher than the global death toll from leptospirosis (50,000 per year) or dengue (9,100–12,500 per year) [11].

B. pseudomallei (and *B. mallei*) is resistant to many antibiotics including many β-lactams, aminoglycosides, macrolides and polymixins [3] making the treatment of disease difficult. Even with aggressive antibiotic treatment the fatality rate is 50% in Northeast Thailand and 19% in Australia [8]. Against this background there is an urgent need for improved preventative measures, such as vaccines, to protect against disease. Because many antigens are shared *B. pseudomallei* by *B. mallei*, and there is evidence of cross-protective immunity [13-19], it is feasible that a single vaccine can be devised which protects against both diseases.

2. Vaccine candidates

2.1 Live attenuated vaccines

A range of attenuated *B. pseudomallei* (and *B. mallei*) mutants able to induce protective immunity in mice have been reported [14, 20-26]. However, not all attenuated mutants can induce protective immunity. Some are over-attenuated and are cleared too rapidly or the disrupted gene may play a role in biosynthesis of a protective antigen [27]. Immunisation with *Burkholderia thailandensis*, a naturally attenuated species that is related to *B. pseudomallei* can induce a protective immune response [28].

It is not certain that a live attenuated mutant would be acceptable as a human vaccine. The potential problem of reversion to virulence can be solved by introducing multiple mutations

and some mutants capable of inducing protective immunity [29] have been shown to be safe even in immunocompromised (IFNγ -/-, SCID) mice [30]. However, a concern is that, like wild type strains, attenuated mutants may be able to establish latent infections.

In spite of these concerns live attenuated mutants of *B. pseudomallei* and *B. mallei* are some of the most protective vaccine candidates identified to date [31-33]. As such they are useful as a standard in studies comparing the protection afforded after immunisation with non-living vaccines. In addition, live attenuated mutants have proved to be valuable tools for dissecting the nature of protective immunity, at least in mice.

2.2 Killed whole cell vaccines

Immunisation with killed *B. pseudomallei*, *B. thailandensis* or *B. mallei* cells can induce protective immunity [18, 34, 35]. These vaccines may be attractive because they are cheap to produce and because a range of antigens are presented to the immune system. The recent report of the Steering Group on Melioidosis Vaccine Development (SGMVD) highlighted that killed whole cell vaccines could be acceptable if they met the criteria for efficacy, safety and progressed through clinical trials [33]. The principle disadvantages of killed vaccines are that some protective antigens are not expressed when bacteria are grown *in vitro* and some components in the vaccine, such as the lipid A of lipopolysaccharide (LPS), might cause short-term but undesirable side effects [36].

A refinement of whole cell vaccines, exploits outer membrane vesicles (OMVs) [37, 38]. These are naturally shed from bacteria and contain cell wall lipids, polysaccharides and proteins. OMVs induce significant but incomplete protection against an aerosol challenge in mice [37]. In more recent studies OMVs have been shown to be safe and immunogenic in non-human primates [39]. These findings suggest that OMVs are an alternative to killed whole cell vaccines, and might be exploited as a low cost vaccine.

2.3 Sub-unit vaccines

A sub-unit vaccine against melioidosis and or glanders would contain only protective antigens and consequently would not be reactogenic, would potentially be more effective and easier to produce reproducibly. Much of the effort to devise biodefence vaccines against melioidosis and or glanders in the past decade has been directed towards the formulation and testing of sub-unit vaccines in mice (Table 2). Many of these antigens are conserved between *B. pseudomallei* and *B. mallei* and have been shown to play major roles in virulence. For example, the capsular polysaccharide appears to block C3b deposition [40], whilst the lipopolysaccharide confers resistance to serum killing [41]. Many of the proteins tested are components or effectors of secretion systems which play roles in virulence. BopA is a putative effector of the type III secretion system [42], BimA is the type V autotransporter involved in actin polymerisation and motility in cells [43] whilst the Hcp proteins form the needle of the type VI secretion system [44].

It is difficult to compare the relative efficacy of the different sub-units as protective antigens, because different immunisation regimes, adjuvants, animal models and challenge strains doses and routes have been used [33]. An additional concern is that many of the adjuvants used do themselves have protective effects, making the interpretation of protection data difficult.

2.3.1 Protein sub-unit vaccines

A range of proteins have been identified as partially protective sub-units against experimental melioidosis and glanders. These antigens are derived largely from the cell wall. However, to date the goal of finding a single protein that provides high level protection and sterile immunity has been elusive. One approach to address this problem might involve screening additional sub-units [23, 45-48]. Another approach is to use a combination of proteins. One study has shown that a combination of antigens can provide protection against experimental disease although sterile immunity was still not achieved [49]. An alternative to

using combinations of proteins would involve using combinations of epitopes derived from different proteins and there has been some exciting foundation work in this area [50-57].

2.3.2 Naked DNA vaccines

There are two reports that immunisation with DNA vaccines encoding the *B pseudomallei* flagellar subunit gene, *fli*C provided modest levels of protection in mice [58, 59]. In a whole genome screen, using expression library immunisation, 12 *B. mallei* ORFs which could induce protective immunity were identified and the proteins encoded by 3 of these were shown to induce protective immunity towards experimental glanders (Table 2) [48].

2.3.3 Polysaccharide sub-unit vaccines

Bacterial polysaccharides often make excellent vaccines and surface polysaccharides of *B. pseudomallei* and *B. mallei* have been investigated. LPS is attractive as a vaccine candidate but there are at least three different LPS O-antigens (A, B and B2) in *B. pseudomallei* [60] and the O-antigen may be *O*-acetylated and/or *O*-methylated [61] leading to subtle immunological differences. The O-antigen produced by *B. thailandensis* (strain E264) appears to be identical to the *B. pseudomallei* Type A O-antigen [61]. The *B. mallei* O-antigen is similar to the Type A O-antigen but with some differences in acetylation [61]. The capsular polysaccharide is highly conserved between different strains of *B. pseudomallei* and *B. mallei* and some strains of *B. thailandensis* produce a similar capsular polysaccharide [62].

The immunisation of mice or hamsters with purified capsular polysaccharide or LPS results in the induction of protective, but not sterile, immunity [49, 63, 64]. Protection is dependent on antibodies, and the passive transfer of immune sera [63], or monoclonal antibodies against these polysaccharides [15, 65] can protect niaive animals.

One challenge associated with polysaccharide vaccine production, is the isolation of sufficient quantities, and the isolation of polysaccharide from *B. pseudomallei* (or *B. mallei*) can be hazardous. An alternative could involve growing an attenuated strain as a source of the antigen and in the USA some mutants of *B. pseudomallei* are exempt from select agent regulations [66]. The similarity of the *B. thailandensis* and *B. pseudomallei* Type A O-antigens [61] indicates the potential to use the *B. thailandensis* antigen [67-69]. Another approach might be to produce the polysaccharide in an engineered strain of a non-pathogenic species of bacteria. The expression of the *B. mallei* O-antigen gene cluster in an attenuated strain of *Salmonella enterica* serovar Typhimurium [13] indicates the feasibility of this approach. Finally, there has been progress with the chemical synthesis of polysaccharides. A synthetic repeat unit of the capsular polysaccharide (2-O-acetyl-6-deoxy-β-d-*manno*-heptopyranose) has been shown to be immunogenic and protective [70]. Further refinement of the epitope(s) recognised could allow the refinement of the synthetic immunogen [71].

2.3.4 Glycoconjugate vaccines

Polysaccharides are poor immunogens that do not generate an anamnestic response because of the lack of T cell involvement. To elicit a T cell dependent response, polysaccharides can be conjugated to proteins. Conjugates of capsular polysaccharide or LPS linked to tetanus Hc fragment, BSA, flagellin proteins or Hcp1 have been reported (Table 3). These are immunogenic in rabbits, mice and rhesus macaques [64, 68-70, 72-75] and, compared to polysaccharide alone, induce enhanced antibody responses, with a bias towards IgG production [64, 68, 72, 73]. The conjugates tested to date typically use chemically coupled polysaccharide and protein. However, one recent report highlights the potential for exploiting the natural glycosylation system found in *Campylobacter jejuni* but transferred in *E.coli* to devise biological conjugates of the *B. pseudomallei* O-antigen linked to AcrA acceptor protein [76].

3. Criteria for candidate selection

An efficacious melioidosis/glanders vaccine would ideally provide high level protection against multiple routes of infection, protect against multiple LPS types and provide sterilizing immunity. Additionally, it would be both safe and cost-effective to produce. At present, some of the most promising vaccine candidates undergoing pre-clinical evaluation include LPS- and CPS-based glycoconjugates, protein sub-units, OMVs and live attenuated strains. Important properties associated with these types of vaccines are shown in Table 4. Although good progress has been made, the development of a vaccine that possesses all of the attributes listed, in particular sterilizing immunity, may be difficult to achieve since *B. pseudomallei* and *B. mallei* are able to survive intracellularly [77].

3.1 Sterile immunity

None of the vaccine candidates tested to date provide sterile protection in animal models questioning their usefulness for biodefense or public health purposes. The feasibility of using a vaccine that provides protection but not sterile immunity in a public health setting has been examined. A melioidosis vaccine providing only partial protection (50% protection for 12 months or a 50% reduction in disease for 10 years) could be useful in high-risk populations [32]. A recent report from the SGMVD indicated that a lack of sterilizing immunity should not be a barrier to progressing promising candidates [33]. Furthermore, the SGMVD suggested that a partially protective melioidosis vaccine may be useful in endemic areas since it could reduce disease severity and death rates by extending the therapeutic window and allowing the administration of other treatments [33]. Similarly, a vaccine that shifts disease from an acute to a sub-acute or chronic form in endemic regions may provide a similar benefit by increasing the time an individual has to seek treatment.

3.2 Need for head to head comparisons

Due to differences in vaccination protocols, challenge routes and animal models, it is difficult to compare and contrast the various vaccine candidates identified to date. Consequently, the

SGMVD has recommended head-to-head protection studies in mice be conducted for candidate selection [33]. While all details relating to vaccine production, formulation, route of administration and dosage should be the responsibility of the developers, the SGMVD proposed challenges with 2 or 3 *B. pseudomallei* strains selected from the BARDA panel (strains MSHR668, MSHR305, 1026b, 1106a, K96243 and 406a). The preferred route of inoculation is subcutaneous using a challenge dose that results in the negative control group reaching humane endpoints within 7-14 days, with continued monitoring of the test groups for at least 28 days post-challenge. The SGMVD does not recommend a particular NHP model for further testing, does not state the specific criteria that would characterize a successful vaccine candidate and has identified specific hurdles in the process of advancing melioidosis vaccine candidates into early phase clinical trials [33].

3.3 Criteria for the selection of biodefence vaccines

A Broad Agency Announcement from the U.S. Defense Threat Reduction Agency (DTRA; HDTRA1-14-CHEM-BIO-BAA Amd #3, Topic: CBMV-03) has outlined the criteria for protective efficacy of melioidosis and/or glanders vaccine candidates in preclinical studies. The preferred infection model for initial efficacy testing is C57BL/6 mice challenged via aerosol with *B. pseudomallei* (strains HPUB10134a, MSHR5855, or K96343) and *B. mallei* (23344 FMH). Demonstration of protective efficacy in mice is defined as "… >80% survival over 30 days and >50% survival over 60 days OR extension of therapeutic window by >28 days". For further evaluation of vaccine candidates in NHPs, "protective efficacy may be defined as >80% survival over 45 days and >50% survival over 60 days OR extension of therapeutic window by >28 days". Additional criteria dictate that the time to onset of immunity be 28-90 days lasting for >1 year and can be achieved with no more than 3 doses of vaccine. The purpose of these decision points is to guide the development of vaccines to protect the warfighter from disease. It is anticipated, however, that such a vaccine would also be useful in public health settings.

3.4 Utility of Biodefence Vaccines for public health purposes

Biodefence vaccines may be useful for protecting against naturally occurring disease in endemic regions but not all biodefence vaccines would be equally suitable. Biodefence vaccines would protect healthy people from infection, whilst a vaccine against natural disease would need to protect individuals who may be immunocompromised (e.g. diabetics) [33]. A biodefence vaccine would need to protect primarily against an inhalational challenge, whereas natural infection occurs by a number of routes [33]. An additional consideration is whether antibiotic treatment after vaccination or in parallel with vaccination is equally likely in biodefence and/or public health situations.

Notwithstanding these concerns, the cost effectiveness of exploiting biodefence vaccines for the prevention of melioidosis in Thailand was examined recently [32]. The model considered the efficacy of the vaccine, the duration of protection afforded by the vaccine and the cost of the vaccine and revealed that in a number of scenarios vaccination would be cost effective. For example, a vaccine that cost $2 per dose, provided only 50% protection and which protected only for 12 months would be cost effective for vaccination of the population at greatest risk of disease. A vaccine that cost $25 per dose and which reduced disease by 50% and provided protection for 10 years would be cost effective for use in all diabetics. Overall, this study concluded that in Thailand, a vaccine would likely be cost-effective if used in high-risk populations and highlighted the value of vaccines that provide only partial protection against disease [32].

3.5 The animal rule

US Food and Drug Administration's (FDA) Animal Rule was implemented to allow the licensing of medical countermeasures, such as vaccines, for diseases for which clinical trials involving exposure to the pathogen are unethical or impractical. This situation might apply to many biodefence vaccines. A similar mechanism exists for licensing products in Canada but the European Medicines Agency (EMA) currently does not have a similar licensing

mechanism. The Biothrax anthrax vaccine was the first vaccine to be approved under the FDA animal rule [78] which requires that the benefits of a vaccine are demonstrated in more than one animal species and predict the likely response in humans. Alternatively a single animal species can be used if it is accepted to be a well-characterized animal model for predicting human response to the vaccine.

In the case of a meliodosis, a vaccine licensed for public health purposes would have undergone clinical trials but may have limited value as a biodefence vaccine, because it may not meet the criteria outlined above. Therefore, it is possible that a melioidosis biodefence vaccine may need to be approved under the FDA animal rule. In the case of glanders, there are very few naturally occurring cases of human disease and it seems certain that a biodefence vaccine would have to be approved under the FDA animal rule.

There are no single animal models that are accepted as robust indicators of the efficacy of human vaccines against glanders or melioidosis. Therefore licensing under the FDA animal rule would require at least two animal species to demonstrate efficacy. Mouse models of inhalational disease caused by *B. pseudomallei* and *B. mallei* have been used extensively to evaluate vaccine candidates and it is likely that non-human primate models of disease will also be required. There have been several reports of the development of non-human primate models of disease. Marmosets [79] appear to be more susceptible than rhesus macaque [80, 81] or African green monkeys [80] to *B. pseudomallei* infection. Both rhesus macaque aerosol [82] and marmoset intranasal infection models [83] have been described for *B. mallei*, and rhesus macaques have been used to assess vaccine candidates [69]. There is one report of a s.c. infection model for *B. pseudomallei* and *B. mallei* in marmosets [84].

4. Correlates of Protection

All vaccine discovery and evaluation projects benefit from an understanding of the immune responses underlying protection. The term ‘immunological correlates of protection’ describes

an immunological response, typically measured by laboratory assay, which is statistically associated with vaccine efficacy and based on clinical trial data in humans [85, 86]. Correlates of protection may be mechanistic, where the response measured directly mediates protection, or non-mechanistic serving as an indirect indicator of protection. In situations where clinical trial data does not exist, as is currently the case of melioidosis, relevant immunological biomarkers could be identified and verified later as correlates of protection [87].

Immediately following exposure, *B. pseudomallei* is extracellular, and therefore susceptible to antibody mediated defences. However, it also has an intracellular lifestyle able to grow in macrophages, and so would be a target for (T) cell mediated immune responses. Live attenuated, killed whole cell, OMV, and polysaccharide-conjugate vaccines using *B. pseudomallei* proteins as carriers will likely require an analysis of both antibody and cell mediated biomarkers. In contrast, studies with polysaccharide alone and polysaccharides conjugated to heterologous carriers (e.g. tetanus toxoid or CRM197) would likely focus only on antibody responses.

4.1 Antibody mediated correlates of protection

Antibodies are established correlates of protection for many vaccines in use today [85, 86]. The three primary parameters which determine antibody-mediated efficacy are concentration, class/isotype and affinity. Plasma IgM, due to its rapid production and complement fixing ability and to a larger extent IgG responses, because of their greater affinity, extended memory and opsonic activity are the most likely correlates of antibody mediated protection in any *B. pseudomallei* vaccine. The protective properties of mucosal IgA (and IgG) have been considered in other bacterial infections [88] and in theory provide an opportunity for actually preventing infection via the inhalational route, but their importance in melioidosis has not been considered.

Assays of antibody function integrate all three parameters providing direct and accurate correlates of protection. Typically these involve either i) serum bactericidal assays in the presence of complement, ii) Fc receptor mediated uptake of opsonised bacteria by host neutrophils or monocytes (opsonophagocytic-OP assay), iii) subsequent intracellular killing of the bacteria (opsonophagocytic killing -OPK assays) or iv) bacterial agglutination. Flow cytometry based measurement of phagocytosis and respiratory burst have been described for *B. pseudomallei* [89], and intracellular killing can be measured by standard colony forming unit assays [90]. Few studies have used these assays in the context of immune responses to *B. pseudomallei* vaccine candidates [15, 38, 54, 91, 92].

4.2 Cell mediated correlates of protection

Unfortunately, experience with other vaccines has shown that defining cell mediated correlates of protection can be a difficult process. The most dramatic example being that of BCG, a vaccine given to over 4 billion children since the 1930's, where an immune correlate of protection is still not defined with any certainty [87].

The role of antibody in protection against melioidosis indicates a likely involvement of CD4+ T-cells in protection against meloioidosis and especially follicular T-cells in the development of a humoral response. In support of this, in mice there is evidence that CD4+ T-cells play a role in protective immunity [93]. These cells might also provide IFNγ and it is known that *B. pseudomallei* is susceptible to killing by IFNγ activated macrophages further indicating a role for cell mediated protection. Humans do develop CD4+ T-cell responses to *B. pseudomallei* [94] and IFNγ production by Th1 cells might contribute to the survival of melioidosis patients presenting with acute infection [95]. Glycoconjugate vaccines would exploit the involvement of T-cells by promoting both the magnitude, subclass and duration of antibody responses against the polysaccharide and potentially enhancing protective immunity. For example, immunisation with a polysaccharide conjugates generated significantly higher levels of antigen-specific IgG than polysaccharide alone [64, 72]. In addition, the elevated levels of

IgG2a seen after immunisation with a lipopolysaccharide glycoconjugate suggested a bias towards a Th1 responses, whereas immunisation with lipopolysaccharide alone evoked almost no IgG2a [64].

Although *B. pseudomallei* is adapted to replicate in the cytoplasm of infected cells, and should load protein antigens into the Class I MHC antigen presentation pathway, we are relatively ignorant of the biology and role of $CD8^+$ T cells in response to this organism. $CD8^+$ T cells are a source of IFNγ for macrophage activation, but their cytotoxic potential against host cells infected with *B. pseudomallei* is not known. In a murine model of disease protection does not appear to involve $CD8^+$ T cells [93], but it is not known whether $CD8^+$ T cells play a protective role in humans. Further identification of Class-I MHC restricted *B. pseudomallei*-derived protein epitopes recognised by $CD8^+$ T cells is warranted.

NK cells, considered part of the innate immune response, may also need to be examined in future studies on *B. pseudomallei* vaccine induced immunity. These cells provide the initial source of IFNγ in both mice and humans in response to innate cytokines produced by macrophages and probably dendritic cells following exposure to the bacteria [94, 96]. In support of this possibility, activation of the innate immune system has been shown to protect against inhalational challenges with *B. pseudomallei* or *B. mallei* and to involve the activation of NK cells and the production of IFNγ [97].

Just as functional (OP/OPK) assays integrate the key features of antibody dependent immunity, bacterial growth inhibition assays can assess the killing capacity of cell mediated responses induced following vaccination. Viable bacteria are incubated with whole blood or PBMC from vaccinated donors and bacterial CFU measured subsequently; killing being an integrated readout of phagocytosis, T/NK cell cytokine secretion and macrophage activation within the culture. These assays are providing important information in the search for

vaccines against *M. tuberculosis* and need to be developed in both mice and humans for *B. pseudomallei* [98, 99].

Finally, when future human vaccine trials are being conducted it will be important to harmonise the assays used in order to optimise data comparability. Useful precedents for this exist from tuberculosis biomarker discovery which can be applied to *B. pseudomallei*, addressing issues such as standardization of T cell stimulation conditions, batch analyses of frozen peripheral blood samples and use of common flow cytometry antibody panels and gating and analysis strategies [100].

4.3 Systems biology approaches to vaccine evaluation

Use of systems biology and the 'omics technologies is increasingly important in the development and evaluation of vaccines [101, 102]. The search for correlates of protection is dominated by the use of transcriptomics, and in particular the genome-wide transcriptional profiling of peripheral blood immune responses following vaccination [103]. The transcriptional profiles of many polysaccharide and conjugate vaccines against other pathogenic bacteria have been defined, and will provide bench marks for future melioidosis vaccine studies [104]. To date, the peripheral blood gene signatures of both mice and humans infected with *B. pseudomallei* have been reported [105, 106] but there is currently no information on vaccine responses following administration of candidate *B. pseudomallei* vaccines.

5. Conclusion

There have been a number of important developments since the publication of previous reviews on the development of *B. pseudomallei* and *B. mallei* vaccines. In this review we highlight the DTRA guidelines on vaccine performance, and which might drive any assessment of the candidates which could be selected for development and clinical trials. These criteria might be equally applicable to vaccines for biodefence and public health

purposes. We also consider some of the important the criteria for licensing, including the suitability of the “animal rule” for assessing vaccine efficacy and how correlates of protection will be identified. Finally, we review the significant progress that has been made in developing glycoconjugates as sub-unit vaccines. We now believe that we are now in a position to select promising candidates for development. These candidates need to be produced under appropriate conditions and after appropriate quality control, efficacy testing in animals and toxicity testing they could be progressed into phase 1 clinical trials in humans. These trials might be undertaken in either the UK or in the USA. Completion of these trials might then allow licensing of the vaccine for biodefence purposes. However, as outlined above, it might also be possible to carry out further clinical trials to evaluate the potential for the use of these vaccines in regions of the world where naturally occurring disease is endemic.

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Table 1. Estimated global burden of melioidosis

	melioidosis cases - thousands (95% credible interval)	melioiodosis deaths – thousands (95% credible interval)
South Asia	73 (31-171)	42 (18-101)
East Asia & Pacific	65 (28-161)	31 (13-77)
Sub-Saharan Africa	24 (8-72)	15 (6-45)
Latin America & Caribbean	2 (1-7)	1 (< 1-3)
Middle East & North Africa	< 1	< 1
Total	165 (68-412)	89 (36-227)

Adapted from [11]

Table 2. Sub units shown to provide protection against experimental *B. pseudomallei* or *B. mallei* infection

Antigen	animal model	immunization route	challenge				reference
			strain	route	dose (CFU)	dose (LD_{50} / MLD)	
capsular polysaccharide	BALB/c mice	i.p.	*B. pseudomallei* NCTC4845	i.p.	2 x 10^4	5000	[107]
lipopolysaccharide	BALB/c mice	i.p.	*B. pseudomallei* NCTC4845	i.p.	2 x 10^4	5000	[107]
capsular polysaccharide	BALB/c mice	i.p.	*B. pseudomallei* NCTC4845	inh.	12.5	2.5	[107]
lipopolysaccharide	BALB/c mice	i.p.	*B. pseudomallei* NCTC4845	inh.	12.5	2.5	[107]
lipopolysaccharide from *B. thailandensis*	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	NR	55	[67]
PotF (ATP binding cassette system)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	4 x 10^4	40	[108]

LolC (ATP binding cassette system)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	4 x 10^4	70	[108]
LolC	BALB/c mice	i.p.	*B. pseudomallei* 576	i.p.	6.6 x 10^5	8250	[108]
LolC	BALB/c mice	i.n.	*B. pseudomallei* 1026b	i.n.	NR	2	[17]
LolC	BALB/c mice	i.n.	*B. mallei* ATCC23344	i.n.	NR	2	[17]
BopA (type III effector)	BALB/c mice	i.n.	*B. pseudomallei* 1026b	i.n.	NR	2	[17]
BopA	BALB/c mice	i.n.	*B. mallei* ATCC23344	i.n.	NR	2	[17]
BimA (autotransporter protein)	BALB/c mice	i.n.	*B. pseudomallei* 1026b	i.n.	NR	2	[17]
BimA	BALB/c mice	i.n.	*B. mallei* ATCC23344	i.n.	NR	2	[17]

Omp3 (Outer membrane protein)	BALB/c mice	i.p.	*B. pseudomallei* D286	i.p.	1 x 10^6	10	[109]
Omp7 (Outer membrane protein)	BALB/c mice	i.p.	*B. pseudomallei* D286	i.p.	1 x 10^6	10	[109]
Omp85	BALB/c mice	i.p.	*B. pseudomallei* D286	i.p.	1 x 10^6	10	[110]
peptide mimotopes of exopolysaccharide	BALB/c mice	i.p.	*B. pseudomallei* NCTC4845	i.p.	4.7 x 10^4	250	[111]
Hcp 1 (integral surface-associated component of T6SS)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	5 x 10^4	50	[44]
Hcp 1	BALB/c mice	i.n.	*B. pseudomallei* 1026b	i.n.	NR	2	[17]
Hcp 1	BALB/c mice	i.n.	*B. mallei* ATCC23344	i.n.	NR	2	[17]
Hcp 2 (integral surface-associated component of T6SS)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	5 x 10^4	50	[44]

Hcp 3 (integral surface-associated component of T6SS)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	5×10^4	50	[44]
Hcp 4 (integral surface-associated component of T6SS)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	5×10^4	50	[44]
Hcp 6 (integral surface-associated component of T6SS)	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	5×10^4	50	[44]
OmpW	BALB/c mice	i.p.	*B. pseudomallei* 576	i.p.	6×10^5	7500	[112]
OmpW	C57BL/6 mice	i.p.	*B. pseudomallei* 576	i.p.	4×10^6	NR	[112]
BPSL1897 + BPSL3369 + BPSL2287 + BPSL2765	BALB/c mice	i.p.	*B. pseudomallei* K96243	i.p.	7.5 x 10^4	/100	[49]
BMA_A0768 mannitol dehydrogenase family protein	BALB/c mice	i.m.	*B. mallei* ATCC23344	i.n.	1×10^5	2	[48]
BMA_2821. ABC transporter ATP binding protein	BALB/c mice	i.m.	*B. mallei* ATCC23344	i.n.	1×10^5	2	[48]

BMA_0816 maltooligosyl trehalose synthase	BALB/c mice	i.m.	*B. mallei* ATCC23344	i.n.	1 x 10^5	2	[48]
GroEL	BALB/c mice	i.m.	*B. mallei* ATCC23344	i.n.	1 x 10^5	2	[48]

In none of these reports did the immune response to the antigen(s) indicated consistently provide sterile immunity. CFU = colony forming units. LD_{50} / MLD – lethal dose for 50% / median lethal dose. i.p. – intraperitoneal; i.m. = intramuscular; inh. = inhalation; i.n. intransal. NR = not reported.

Table 3. Immunisation and protection studies with glycoconjugates

polysaccharide	protein	immunisation	protection measured	protection profile (%) [a]			reference
				acute	chronic	survivors	
capsular polysaccharide	BSA	BALB/c mice	challenge i.p. with 14 MLD (1x10^4 CFU) of *B. pseudomallei* K96243 i.p.	0	10	90	[72]
capsular polysaccharide	BSA	BALB/c mice	challenge i.p. with 113 MLD (8.4 x10^4 CFU) of *B. pseudomallei* K96243 i.p.	0	30	70	[72]
synthetic CPS hexasaccharide	TetHc	BALB/c mice	challenge i.p. with 120-137 MLD (9x10^4 - 1x10^5 CFU) of *B. pseudomallei* K96243.	0	33	67	[70]
lipopolysaccharide	TetHc	BALB/c mice	challenge i.p. with 40 MLD (4x10^4 CFU) of *B. pseudomallei* K96243. Reduced splenic burden at 48 hr post challenge.	0	20	80	[64]
lipopolysaccharide	BSA	BALB/c mice	challenge i.p. with 54 MLD (4.05 x 10^4 CFU) of *B. pseudomallei* K96243 i.p.	NA	NA	NA	[72]

lipopolysaccharide	BSA	BALB/c mice	Reduced hepatic burden at 24hr. No overall reduction in mortality antibody enhanced opsonophagocytic uptake by RAW264 cells	NT	NT	NT	[73]
lipopolysaccharide	Tetanus toxoid	Rabbits	passive transfer of IgG or IgM antisera into streptozotocin diabetic rats. Provided ≥10^4-fold protection against i.p. challenge with *B. pseudomallei* strain 316c	NT	NT	NT	[74]
lipopolysaccharide	TetHc	BALB/c mice	challenge i.n. with 1.9 LD_{50} ($1.2x10^5$ CFU) of *B. mallei* ATCC23344.	33	0	67	[68]
lipopolysaccharide	TetHc	BALB/c mice	challenge i.n. with 6.5 LD_{50} ($4x10^5$ CFU) of *B. mallei* ATCC23344.	67	0	33	[68]
lipopolysaccharide	FliC	BALB/c mice	challenge i.n. with 1.9 LD_{50} ($1.2x10^5$ CFU) of *B. mallei* ATCC23344.	33	11	56	[68]
lipopolysaccharide	FliC	BALB/c mice	challenge i.n. with 6.5 LD_{50} ($4x10^5$ CFU) of *B. mallei* ATCC23344.	67	22	11	[68]

lipopolysaccharide	Hcp1	BALB/c mice	challenge i.n. with 1.9 LD_{50} ($1.2x10^5$ CFU) of *B. mallei* ATCC23344.	0	11	89	[68]
lipopolysaccharide	Hcp1	BALB/c mice	challenge i.n. with 6.5 LD_{50} ($4x10^5$ CFU) of *B. mallei* ATCC23344.	67	11	22	[68]
lipopolysaccharide	FliC	Rhesus macaques	challenge inh with 4.6 LD_{50} ($6.4x10^4$ CFU) of *B. mallei* ATCC23344. No overall reduction in mortality, but reduced fever and bacterial burdens in immunised animals	NA	NA	NA	[69]
O-antigen	*B. pseudomallei* flagellin	Rabbits	passive transfer of antiserum into streptozotocin diabetic rats. Provided 10^2-fold increase in LD_{50} dose of *B. pseudomallei* 316c i.p.	NT	NT	NT	[75]
O-antigen	*C. jejuni* AcrA	BALB/c mice	challenge i.n. with 10-12 LD_{50} ($2x10^3$ CFU) of *B. pseudomallei* K96243. Delayed time to death.	0	100	0	[76]

[a] The protection profile summarise the proportion of challenged mice that develop acute disease (die between day 1 and day 7 post challenge), the proportion that develop chronic disease (die between day 8 and the end of the study) and the proportion of mice that are alive at the end of the study. NA = not applicable - no difference between survival of control and immunised groups of animals. NT = not tested. CFU = colony forming units. LD_{50} / MLD – lethal dose for 50% / median lethal dose. i.p. – intraperitoneal; inh. = inhalation; i.n. intransal.

Table 4. General properties associated with lead melioidosis and glanders vaccine candidates

property	sub-unit	inactivated (whole cell, OMV)	live attenuated
route of administration	injection	injection	injection or natural
number of doses	multiple	multiple	single
need for adjuvant	yes	yes/no	no
humoral immune responses	IgG	IgG	IgG, IgA
cell-mediated immune responses	poor	poor/moderate	moderate/strong
duration of immunity	short/mid-term	short/mid-term	long-term
potential for side effects	low	low/moderate	low/moderate
use in immunocompromised individuals	yes	yes	yes/no
cost	high	moderate/low	low
shelf life	long	medium	short