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EDITORIAL

DNA vaccination resurfaces in the struggle against melioidosis

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Burkholderia pseudomallei (*Bp*) is a gram-negative, facultative intracellular pathogen, responsible for causing melioidosis, a highly fatal disease transmitted by percutaneous inoculation, inhalation, or ingestion.[1] Disease manifestations range from pneumonia, multiple abscesses, and septicemia, with a mortality rate of up to 40% despite appropriate antimicrobial therapy and a high rate of relapse following apparent clinical resolution.[1,2] The high rate of infectivity and mortality associated with *Bp*, its intrinsic antimicrobial resistance, and potential use as a bioterrorism agent underscore the need to develop new therapeutic interventions against this organism.[3] Although many studies have been conducted to identify successful vaccine strategies that protect against *Bp*, at present, no ideal candidate has emerged for use in humans.[3]

Vaccine studies in murine models of melioidosis have shown that live attenuated mutants of *Bp* represent the most effective candidates, providing broad, long-lasting humoral and cell-mediated immunity.[4] However, the potential for reversion to virulence or establishment of a latent infection represent significant safety concerns.[4,5] For this reason, such platforms are not likely to progress further in development without extensive engineering to prevent reversion and limit the potential for persistence within host tissues.[4,5] Therefore, the ongoing challenge has been to identity non-living vaccine approaches that are able to induce effective protective immunity.[5] While killed or subunit vaccines elicit strong antibody-specific responses, these approaches do not tend to elicit strong cell-mediated immunity and therefore may not be sufficient for the clearance of intracellular bacteria, such as *Bp*.[4]

In an effort to mitigate this, DNA vaccine approaches have been evaluated in melioidosis for their ability to generate both antigen-specific antibody and cell-mediated responses, without the potential for pathogenic infection *in vivo*.[3,5-7] To date, the only DNA vaccine developed against any *Burkholderia* sp. utilizes the *Bp* flagellar subunit gene, *fliC*.[3,8-11] Over a decade ago, Chen et al. were the first to show that triple DNA vaccination with pcDNA3/FliC administered intramuscularly protected ~80% of mice up to 14 d post-intravenous (IV) infection with 10^5 CFU (~LD_{50}) of 16 different *B. pseudomallei* isolates.[9,10] The incorporation of a CpG motif into the pcDNA3/FliC platform provided a modest improvement in protection over mice vaccinated with pcDNA3/FliC alone, with a greater proportion of mice surviving to 14 d post-IV infection (93% survival), a relative decrease in splenic and liver bacterial loads, an increase in IFN-γ mRNA expression in the later stages of infection, as well as enhanced flagellin-specific IgG2a responses and proportion of IFN-γ secreting cells in the spleen.[10]

Since then, the recent work by Lankelma and colleagues[11] featured in this issue has sparked renewed interest in DNA vaccination to combat melioidosis. Their study extends on the prior work by Chen et al. 2006[9,10] with the novel assessment of DNA FliC vaccination by dermal tattoo or intranasal application, and for the first time, for protection against intranasal melioidosis. In their study, the authors developed 3 DNA FliC constructs with distinct subcellular targeting designs (pVAX-hTPA-FliC, pVAX-FliC, pVAX-FliC-KDEL) to identify a candidate with the highest potential for protection against 200–500 CFU of *Bp* 1026b (~LD_{50}) in an intranasal (IN) murine melioidosis model. Toward this end, each DNA FliC construct was initially screened in a rapid triple tattoo dermal vaccination regime for its efficacy in a 72-hour intranasal infection model. In this acute-infection study, all DNA FliC constructs were shown to elicit a significant anti-FliC IgG response,

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reduce systemic production of IL-6, MCP-1, IFN-γ, TNF-α, as well as pulmonary and liver microscopic lesions, and significantly reduce organ bacterial loads as compared with control mice. Among the tested DNA constructs, pVAX-hTPA-FliC distinguished itself in its unique ability to induce the lowest bacterial concentrations in the lung, sterile immunity in blood, and significantly reduce pulmonary concentrations of IL-6, CXCL1, TNF-α, at 72-hour post-infection. The pVAX-hTPA-FliC vaccine was, therefore, selected as the lead candidate for further testing based on its ability to induce a balanced immune response in intranasally-infected mice and significantly reduce organ bacterial burdens in the absence of severe cytokine-mediated tissue damage.

Further studies by this group showed that a single dose of the lead vaccine (pVAX-hTPA-FliC) administered by the intranasal route was similar or more effective than a single subcutaneous vaccine dose of recombinant FliC (rFliC) with Complete Freund's Adjuvant at reducing organ bacterial loads, pulmonary cytokine production and neutrophil influx, pulmonary and hepatic microscopic lesions, markers of cellular damage (ALT, AST, LDH), and systemic cytokine production at 72-hour post-intranasal infection. Interestingly, the beneficial effects associated with single, intranasal, pVAX-hTPA-FliC vaccination were noted even in the absence of a FliC-specific IgG (or IgA) response, which was in contrast very robust in rFliC-vaccinated mice, supporting a significant role for cell-mediated immunity in the protection associated with pVAX-hTPA-FliC vaccination. These encouraging data prompted additional studies exploring the impact of this vaccine candidate on the survival of intranasally-infected mice. A single, intranasal dose of this DNA vaccine was found to protect 53% of mice to 14 d post-intranasal *Bp* infection (as compared with unvaccinated mice). Altogether, Lankelma et al. report on the first, mucosally-applied, DNA vaccine, to show promise against intranasal melioidosis. An assessment of its efficacy beyond the acute phase of the disease and potential for reducing chronic infections and persistence within host tissues (a feature often lacking in current vaccine candidates) will help further advance the understanding of correlates of protective immunity associated with these vaccine approaches against *Bp*.[4] Toward this end, follow-up studies determining organ bacterial loads of IN-vaccinated mice surviving to 14 d post-IN infection will greatly bolster the large breadth of valuable data provided by this study.

Together with the studies by Chen et al., the findings by Lankelma et al. have helped provide further insights into the impact of DNA vaccines in melioidosis and have demonstrated that there is potential for DNA vaccines as a delivery system for *B. pseudomallei* vaccine antigens. These research groups have shown that continued investigation of this vaccine platform in the context of melioidosis is warranted, particularly given the ability of these vaccines to be rapidly engineered, cost-effective, thermostable, and well-tolerated, without the accompanying risks of reversion to a disease-causing state or secondary infection.[6,7] In their study, Lankelma and colleagues have combined these advantages into an easily administered, single-dose, intranasally-applied DNA vaccine that shows protection against intranasal melioidosis and therefore has applicability for biodefense and natural aerosol infections in endemic regions. The authors have also tried to mitigate well-described challenges of DNA vaccination, such as relatively poor immunogenicity in higher primates and human clinical trials (despite vigorous and effective immune responses in mice),[8,12] by 1) formulating their intranasally-administered DNA vaccine with polyethylenimine (PEI) to enhance mucosal transfection efficiency, cellular delivery, and uptake and 2) in their construct design to enhance protein secretion and augment MHC-II presentation by antigen-presenting cells. Although the use of PEI as a gene delivery system has previously been hampered by its well-known toxicity, low-molecular weight PEI derivatives (considered of lower toxicity) have been developed and, in one study in Belarus, are planned for use in humans in a Phase I clinical trial for a DNA vaccine application.[13-15] Collectively, the studies undertaken by Chen et al. and Lankelma et al. indicate that delivery of *fliC* in this way provides modest levels of protection in mice subjected to low-doses of *Bp*.[9-11] Future studies investigating the differential protective efficacy afforded by other putative virulence factors of *Bp* incorporated into an effective DNA vaccine platform that also includes components that potently stimulate immune responses (eg., CpG oligodeoxynucleotides) would be of particular interest.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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