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COMMENTARY

Prospects for subunit vaccines: Technology advances resulting in efficacious antigens requires matching advances in early clinical trial investment

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ABSTRACT

With the continued march of antimicrobial resistance, a renewed impetus for better vaccines has been heralded. Identification of potent subunit vaccines has been greatly facilitated by recent developments in reverse vaccinology and proteomics strategies. There are a range of antimicrobial resistant bacterial pathogens that could be targeted by potent vaccine antigens identified within the coming years. However, cost is a significant hurdle in progressing lead antigen candidates to clinical trials. In order for novel vaccine technologies to realize their clinical potential, there is a requirement to improve investment and incentives to expedite the development of vaccines that are apparently efficacious in preclinical trials.

The global problem of antimicrobial resistance (AMR) is fast becoming one of the major scientific and health issues of modern times. Globally, drug-resistant infections are responsible for several hundred thousand deaths annually. By 2050 that figure could be more than 10 million. Of particular concern is the mounting prevalence of infections caused by multidrug resistant (MDR) Gram-negative bacteria. In particular, the human pathogens *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii* have been singled out as "*serious threats to human health*" by the EU, the US Centers for Disease Control and Prevention, and the World Health Organization due to the existence of extremely drug resistant strains. Other multidrug resistant pathogens, such as members of the genus *Burkholderia* also cause chronic life-threatening infections and their eradication is very challenging.

In the final report prepared by the Review of Antimicrobial Resistance,[1] one of the key recommendations for reducing the demand for antibiotics was the promotion of the development of vaccines and alternatives. In particular, a renewed impetus for early stage vaccine research was recommended. It is widely accepted that vaccines do not induce selection pressures on the environment and consequently do not contribute to antimicrobial resistance.[2] Their impact on public health has been tremendous, with global reductions in both mortality and morbidity of infectious diseases. For example, recent data from the World Health Organisation on polio eradication highlights the effectiveness of vaccination (http://www.polioeradication.org/Dataandmonitoring/Poliothisweek.aspx). This paralysing and potentially fatal infectious disease is now endemic in only 2 countries, Afghanistan and Pakistan, and the number of countries that reported no cases of polio in 2015 increased on previous years. When compared with data from 30 y ago, the impact of vaccination is unequivocal. It is estimated that there were over 394,000 cases of poliomyelitis worldwide in 1987, before the global eradication effort began, while there were only 29 cases of wild-type polio reported globally in 2015 (www.polioeradication.org). The efforts of the Global Alliance for Vaccines and Immunisation (GAVI) contribute to this effort directed toward reducing childhood mortality by increasing access to immunisation in poorer countries.

Recent advances in subunit vaccine identification

Despite these unquestionable successes to date, there is a need for vaccines that specifically target antibiotic resistant bacterial infections. Subunit vaccine antigens have potential as safe, scalable, efficacious vaccine candidates. A number of strategies have been devised to identify novel vaccine antigens. Rappuoli et al. pioneered the use of reverse vaccinology to identify novel antigens against *Neisseria meningitides* serogroup B. They sequenced the genome, identified 350 surface proteins and administered them to mice to identify those that were immunogenic.[3] This predictive approach which has revolutionised vaccine antigen discovery is based on the assumption that proteins that induce protective immunity are located outside the cell membrane and therefore possess signal sequences.[4] The recently licensed MenB vaccine (Bexsero®) was developed following this approach and should have a real impact on meningococcal disease caused by MenB, which had not been covered in previous meningococcal vaccines.[5] Immunoproteomics approaches are also being used to identify novel antigens that elicit potent immune responses, but when used in isolation, they have limitations and may miss some key antigens.[6] For example, the prophylactic *Bordetella pertussis* antigen FHA

(filamentous hemagglutinin), a component of all licensed whooping cough vaccines, was undetectable in 2 immunoproteomic studies.[7, 8]

Historically, efficacious vaccine antigens are involved in host cell attachment, such as the *B. pertussis* acellular vaccine. The current approved vaccine contains 5 *B. pertussis* antigens: pertussis toxoid, fimbriae 2 and 3, pertactin, together with the aforementioned FHA. These antigens are all virulence factors involved in bacterial attachment,[9] thereby highlighting the potential of adhesins and bacterial proteins involved in interactions with host cells as potent vaccine antigens. Indeed, the 4 main components of the MenB vaccine are also involved in the virulence of *N. meningitides*, including host cell attachment and serum resistance.[10,11] Identification of previously undiscovered adhesins or other proteins that are used by bacteria to attach to host tissue has the potential to unearth efficacious vaccine subunit antigens. Recent advances in proteomics have enabled the rapid identification of proteins that are involved in the direct interactions between the host cells and bacterial pathogens. In particular the sensitivity of mass spectrometry (MS) based techniques has been invaluable in the identification of adhesins that had not been previously identified. We developed a proteomic platform technology to identify bacterial adhesins involved in host cell attachment (see below) and have proven our approach in preclinical studies.[12,13] Lead candidates demonstrated efficacy in preclinical trials against 2 recalcitrant infections.

The proteomic approach

We have a particular interest in pathogens in the genus *Burkholderia*, which are highly antibiotic resistant and cause challenging, difficult to treat infections. One subgroup of species within this genus, *Burkholderia cepacia* complex (Bcc), causes opportunistic infections in people with cystic fibrosis and in certain immunocompromised patients. Bcc is very difficult to eradiate due to both inherent and acquired antibiotic resistance mechanisms and consequently is a suitable model pathogen for investigating our subunit vaccine discovery approach. Despite it having a complex genome of 8Mb, relatively few adhesins had been identified using mutagenesis methods.[14-17] We initiated a proteomic examination of the adhesins involved in attachment to host lung epithelial cells.[12] This involved probing 2-dimensional blots prepared from bacterial membranes with lung epithelial cells to facilitate interaction of the human host cells with the separated bacterial proteins. The bacterial proteins which were highlighted as interacting with host cell proteins were identified by Matrix-assisted laser desorption/ionisation (MALDI-ToF) MS. In this way, we reproducibly identified 14 proteins used by *Burkholderia* to attach to host epithelial cells.[12] Most of these proteins had not previously been identified as having involvement in host-pathogen interactions involving *Burkholderia* species. The outer membrane protein OmpW was among those identified as being involved in the attachment of 2 Bcc species, *B. multivorans* and *B. cenocepacia*. This protein had been previously identified as an adhesin in *Vibrio cholera*[18] but was not previously associated with pathogenesis in Bcc. Another protein reproducibly identified in our analysis was the 29 k_D protein, Linocin, which had been previously shown to be immunogenic in *Mycobacterium*

tuberculosis, stimulating potent T-cell responses,[19] but, again, had not been previously identified as having a role in host cell attachment or Bcc pathogenesis. We had previously shown that 9 of the proteins identified in our Cell-blot were immunoreactive in an earlier study, in which 2-dimensional blots of bacterial membrane preparations were probed with serum from Bcc infected cystic fibrosis patients.[20] This was an important point to establish as it highlighted that these proteins, identified from microbial cultures, were indeed expressed during human infection and were immunoreactive. As a proof of concept study to test our Cell-blot strategy, we selected 2 of the proteins involved in host cell attachment for pre-clinical vaccine efficacy assessments. We recombinantly expressed both proteins, Linocin and OmpW in *E. coli*, purified them and removed endotoxin. Immunisation with the individual proteins showed protection against challenge, as measured by several log reductions in bacterial lung counts for the 2 most clinically relevant Bcc species, *B. multivorans* and *B. cenocepacia*.[12]

Burkholderia pseudomallei is another member of the *Burkholderia* genus and is the causative agent of melioidosis, a tropical disease endemic in Southeast Asia and Northern Australia. As with Bcc, these infections are difficult to treat and cause chronic and often fatal infections in the host.[21] Vaccination of susceptible populations would have much more potential in terms of reducing the morbidity and mortality of this life-threatening infection. To date, there are no approved vaccines to protect against melioidosis. As a further evaluation of our proteomic Cell-blot strategy, we assessed whether *B. pseudomallei* OmpW would have potential as a vaccine candidate against *B. pseudomallei*. A closely related homolog of *B. pseudomallei* OmpW (*B. thailandensis* OmpW) had previously been shown to be immunoreactive in an immunoproteomic analysis [22] and, with this in mind we examined the potential of *B. pseudomallei* OmpW to protect mice against lethal infection. Immunisation with endotoxin-free OmpW protein, adjuvanted with monophosphoryl lipid A, protected challenged mice with 75% survival relative to 25% in unimmunised controls. Furthermore, the protection in a genetically resistant mouse model, C57BL/6, against a lethal *B. pseudomallei* infection lasted for up to 80 days, surpassing the efficacy of the live attenuated strain (2D2) confirming that the protection by this protein was not restricted to a single MHC haplotype.[13] This level of protection from a single antigen is notable. Multiple subunit antigens would be likely to give optimal long-term protection in the human population and additional studies are warranted to identify additional antigens which might be components of a multi-subunit *B. pseudomallei* vaccine. Nevertheless, the fact that these antigens identified by our proteomic Cell-blot strategy showed considerable protection against 2 distinct *Burkholderia* infections substantiates our strategy to identify potent subunit antigens.

Future prospects

Antigen identification is the first step in a lengthy development process to obtain a licensed vaccine. Their safety, stability, formulation and identification of optimal adjuvant are other noteworthy steps in the developmental path. Recent advances such as a systems biology approach or "systems vaccinology"

approach to investigate immunity will help elucidate the adaptive responses to vaccination in immunised cohorts[23] and should both enhance and expedite vaccine development. Ultimately, all vaccines, despite their potential in preclinical models and/or known correlates of protection, must be evaluated in human trials. Progression of vaccine antigens that have shown good efficacy and safety in animal models to human trials is a substantial hurdle. In particular, the leap in costs from preclinical to clinical trials is considerable and beyond the resources of many of the academic researchers engaged in the identification of potent vaccine antigens. There are considerable risks associated with the development of vaccines, as with any new drug, making investors understandably cautious. Indeed, efficacy trials with vaccines can take much longer than traditional clinical trials for new therapies, as a proportional reduction in infection rate between vaccinated and non-vaccinated people must be determined. In rarer infections, this can lead to a considerably prolonged developmental path. Due to these risks, most investors or potential licensees are reluctant to engage with any vaccine development project in advance of Phase 1 human data. Yet, by our estimation, the most limited phase 1 study on a subunit vaccine antigen would cost in the region of €400 K, including GMP manufacture. This results in a very tight bottle neck, with the consequence that the process of identifying better, more potent antigens is at risk of becoming a mere academic exercise, unless there is a real global will to support more early stage clinical trials. These supports might include an international fund specifically targeted at early vaccine clinical trials. The Strategic Multi-Attribute Ranking Tool for Vaccines or "SMART Vaccines" tool developed by the Institute of Medicine can help decision makers prioritize vaccines in development which is welcome.[24] If vaccines are to be one of the weapons in the armoury against drug resistant infections, companies could also incentivised to invest in early stage clinical trials in order to bridge the gap between preclinical vaccine research and human efficacy and safety data. These kinds of incentives at government level could include tax incentives, enhanced patent protection and marketing rights or financial subsidisation of SMEs or pharmaceutical companies involved in early stage vaccine clinical trial research. Furthermore, a national or international agency that streamlines GMP manufacture and clinical trial testing of vaccines would be a significant vehicle for the expedient development of vaccines against antimicrobial resistant bacteria.

In summary, there have been substantial advances in the identification of efficacious vaccine candidates in the past decade. Progression of the best of these toward clinical trials will be the next major challenge in developing better human vaccines.

Disclosure of potential conflicts of interest

There are no conflicts of interest to disclose.

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