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Alternative Whooping Cough Vaccines: A Minireview

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Abstract

Bordetella pertussis, the aetiological agent of an acute upper respiratory tract disease of humans, "whooping cough", can infect all age groups with adolescents and adults acting as major source of transmission of this pathogen to infants. This transmission is promoted by the fact that adolescents and adults do not exhibit the characteristic cough, the infection being either asymptomatic or manifested as a mild but persistent upper respiratory tract infection. It is established now that both antibodies and cell-mediated immune [CMI] responses are crucial for protection against whooping cough, the former being important in the early phase of the disease, with the latter being important for long-term protection. The protection offered by vaccination with the currently-marketed acellular pertussis vaccines is predominantly due to antibodies against vaccine antigens associated with a Th2-polarised immune response and has been found to be relatively short-term protection. There is an urgent need to develop alternative vaccines capable of inducing both protective antibody and CMI responses particularly given the resurgence of this vaccine-preventable disease in infants and children worldwide. While current strategies are aimed at the development of recombinant vaccines using an adjuvant that may stimulate both arms of the immune response, no discovery of a cost-effective and non-toxic adjuvant to improve protection against pertussis has been reported thus far. This review details the oral presentation on alternative whooping cough vaccines and their future potential delivered at the 2nd World Conference on Vaccines and Vaccination organised by the OMICS Publishing Group.

Keywords: *Bordetella pertussis*; Live attenuated vaccines; DNA vaccines; Biodegradable nanoparticle vaccines; Pertussis

Introduction

Whooping cough (pertussis), a respiratory disease caused by *Bordetella pertussis*, accounts for more than 3,00,000 deaths annually worldwide [1,2] and its incidence has been rising [3]. *B. pertussis* is a non-invasive pathogen which localises mainly in the upper respiratory tract and produces a large array of potential virulence factors, many of which play significant roles in the pathogenesis of pertussis [1,4]. A killed whole cell pertussis vaccine, generally given in combination with diphtheria and tetanus toxoids, has been available in many countries for over 40 years. While its use seems to have controlled pertussis epidemics, concerns over the reactogenicity, ranging from high fever, persistent crying, pain and swelling at the site of injection [4,5] led to the development of the currently marketed acellular pertussis vaccines (DTaP) which is administered to infants in Australia at 2, 4, and 6 months with a booster at 4-6 years of age [5]. In other countries like the USA, children are also vaccinated at 12-18 months of age [4]. Children under the age of 2 months of age are highly susceptible to the complications of pertussis infection but are too young to be immunised. The concern that young adults (vaccinated during their childhood) with waning immunity against whooping cough may serve as a reservoir for the pathogen for infecting infants (and children), has stimulated interest in the development of an alternative vaccine which can also be used safely in the adult population [6].

It is generally accepted that the protective efficacy of the acellular vaccine is short–term, not long-term [7]. Furthermore, given the frequency of local reactions [5,8], particularly the reported extensive limb swelling [5] that occured in a children receiving 4th booster vaccinations with DTaP, may raise an alarm, albeit undue, in the community about the safety of this vaccine. The alternatives suggested have been to either to reduce the number of booster immunisation show ever this would lead to reduced levels of immunity, use vaccines with reduced antigen content as has been done by introduction of adult acellular pertussis vaccine formulations, dTpa, for use in adolescents (http://immunise.health.gov.au), or to find a replacement adjuvant, which, unlike alum, favours the induction of Th1 responses that has been proposed to be responsible for long-term protection against whooping cough [9]. Unfortunately, no such universally acceptable adjuvant for use with DTaP approved by the Food and Drugs Administration (FDA) is available, hence the continued use of the alum-based adjuvants in pertussis vaccine formulations.

Comparison of the humoral and cellular immune responses of mice following vaccination with the killed whole cell pertussis vaccine (WCV:DTPw) versus the DTaP [10] has revealed that although the DTPw induced lower antibody titres to the pertussis toxin, filamentous haemagglutinin and pertactin, it was more effective in activating macrophages and more protective as judged in intracerebral challenge and bacterial lung clearance experiments than the DTaP [10]. These authors suggested that cell-mediated immunity might play a crucial role in eliminating bacteria that escape the humoral defence mechanisms. This suggestion is further supported by the fact that *B. pertussis* can survive within mammalian cells including macrophages [11,12]. It has also been suggested that circulating antibodies may play a role in toxin neutralisation and prevention of bacterial attachment to respiratory epithelial cells particularly in the early phase of infection [10]. There is now evidence that whole-cell pertussis priming in infancy may be more effective than DTaP priming on subsequent protection in childhood [13].

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The perceived fears of potential adverse reactions to whole cell pertussis vaccines, and the cost-ineffectiveness of DTaP formulationsfor socially or financially disadvantaged populations worldwide, investigations aimed at developing novel but affordable pertussis vaccines are essential and are constantly in progress in different relevant organisations. The 1st approach that is being tested is the development of biodegradable nanoparticle-encapsulated whooping cough vaccine. *B. pertussis* subunit antigens [PTxoid, FHA, PRN] administered intranasally or parenterally to mice following encapsulation in biodegradable micro- or nanoparticles was reported to induce CMI (Th1-polarised) or antibody (Th2-polarised) responses but the protection was no better than that induced by pertussis antigens administered in solution [14,15].

The 2nd approach has been the development of DNA vaccines for prevention of infection with *B. pertussis* has revealed that the arm of the immune response induced depended on not only the eukaryotic vector used for expression of the viruence antigens but also application of prime boost strategy invloving purified recombinant potential protective antigens or select cytokines. Li et al. [16] reported that use of prime boost strategy involving co-administration of Granulocyte Macrophage Colony Stimulating Factor [GMCSF]. Kamachi et al. [17] reported protection against *B. pertussis* challenge in mice vaccinated using a gene gun with a DNA vaccine comprising the full S1 subunit of pertussis toxin expressed in pcDNA 3.1 vector. However, because of the potential logistical difficulties associated with the method of immunisation, Fry et al. [18] reported that immunisation of mice by the intramuscular route with a DNA vaccine constructed using the pcDNA 3.1 vector expressing pertussis toxoid induced a significant cell-mediated immune response but only little antibody response. Although mice were protected upon challenge with the virulent parent, the rate of bacterial clearance was better in DTaP- vaccinated mice. Further studies revealed that booster vaccination of DNA vaccine-immunised mice with purified recombinant pertussis toxoid produced significantly higher level of antibodies and cell-mediated immunity than vaccination with the DTaP vaccine (Fry, Chen, Daggard and Mukkur, unpublished), rendering such a vaccine unaffordable for majority of the population potentially because of the potential increase in manufacturing costs and concerns regarding safety of DNA vaccines.

Another promising option is the development of live attenuated whooping cough vaccine delivered by the nasal route, so as to mimic the natural route of infection, potentially leading to induction of long lasting immunity. Roberts et al. [19] developed an aromatic-dependent mutant (aroA) of *B. pertussis*, but found to the vaccine strain to persist in the lungs of mice for only a short period of time (4 days at reasonable numbers) thus casting doubt on its ability to stimulate effective cell-mediated immunity (CMI) considered to be essential for long-term protection against whooping cough. This result was unexpected given previous reports regarding the success of the aroA mutant of Salmonella species [20,21]. as a successful vaccine. On the other hand, the aroA mutants of Shigella species were found to be poorer vaccines than the aroD deletion mutants of the same species [22,23]. Mielcarek et al. [24] developed *B. pertussis* vaccine candidate [BPZE1] in which PT was attenuated by genetic detoxification, dermatonecrotoxin (dnt) was deleted and ampG gene replaced with the orthologous *E. coli* ampG gene, with the aim of modulating the activity of tracheal cytotoxinand reported to be immunogenic in young [3 week-old] mice and adult mice. We provide a review of the immunobiological properties of aromatic-deficient [aroQ] *B. pertussis* [hereafter referred to as aroQBP] developed in our laboratory, including humoral and cellular immune response pre- and post-challenge with the aroQBP vaccine candidate [25] and its future potential.

Methods and Materials

Method of construction of the aroQBP vaccine candidate, immunisation, measurement of the antibody isotype and cell-mediated immune responses, using IL-2 and IFN-γ as indirect indicators, and protection against challenge with virulent *B. pertussis* have been described in detail elsewhere [25].

Results

The aroQ *B. pertussis* mutant (aroQBP) vaccine candidate [25] was found to survive longer in mice than the aroA *B. pertussis* mutant [19] and found to be detectable until days 10-12 post-immunisation (Figure 1).

Immunisation of mice with the aroQBP vaccine resulted in the induction of anti-filamentous haemagglutinin (FHA), anti-pertussis toxin (PT) and anti- *B. pertussis* killed whole cells (BPWC) antibodies of the IgG isotypes, IgG1 and IgG2a (Figures 2-7) in the serum [24]

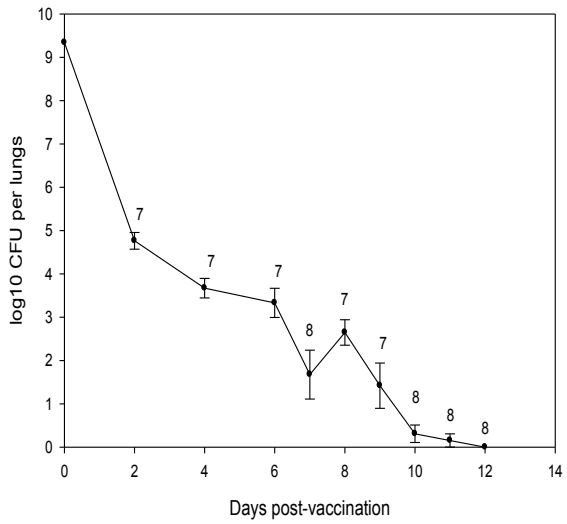

Figure 1: Persistence of aroQ *B. pertussis* [aroQBP] in the lungs of vaccinated mice (data from reference [25] re-plotted for enhanced clarity).

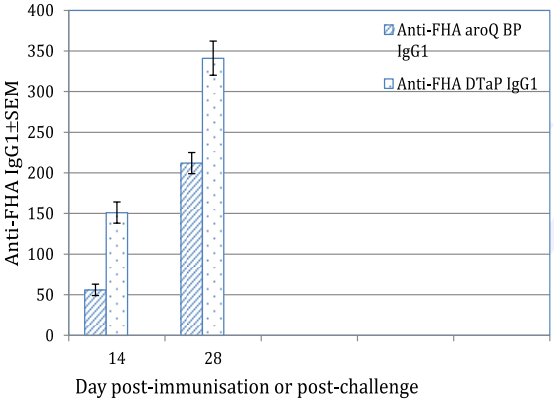

Figure 2: Anti-FHA IgG1 response of mice vaccinated with a single dose of aroQBP versus 0.1 mL of a single standard human dose pre- and post-challenge with virulent *B. pertussis* (data from reference [25] re-plotted for enhanced clarity).

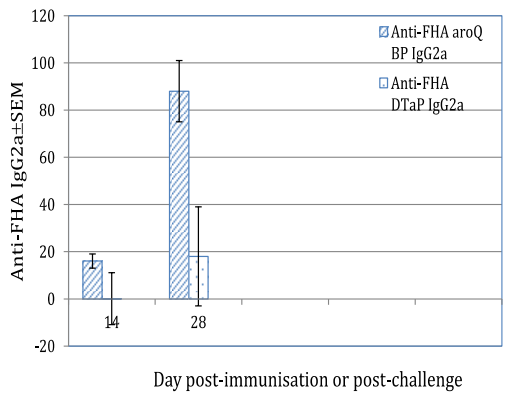

Figure 3: Anti-FHA IgG2a response of mice vaccinated with a single dose of aroQBP versus 0.1 ml of a single standard human dose pre- and post-challenge with virulent *B. pertussis* (data from reference [25] re-plotted for enhanced clarity).

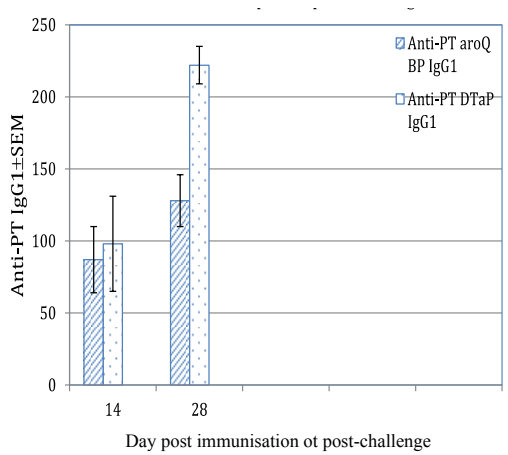

Figure 4: Anti-PT IgG1 response of mice vaccinated with a single dose of aroQBP versus 0.1 ml of a single standard human dose pre- and post-challenge with virulent *B. pertussis* (data from reference [25] re-plotted for enhanced clarity).

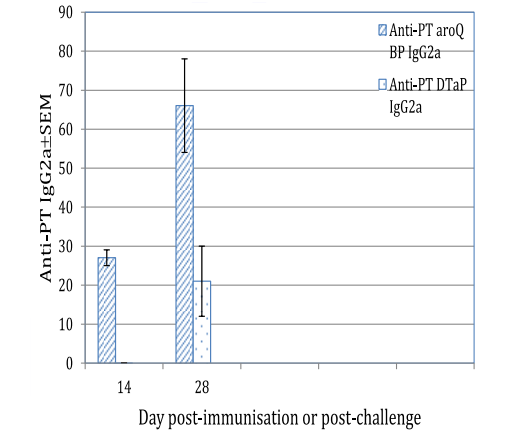

Figure 5: Anti-PT IgG2a response of mice vaccinated with a single dose of aroQBP versus 0.1 ml of a single standard human dose pre- and post-challenge with virulent *B. pertussis* (data from reference [25] re-plotted for enhanced clarity).

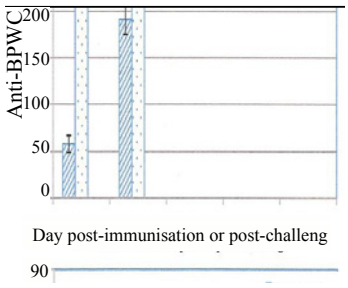

Figure 6: Anti-BPWC IgG1 response of mice vaccinated with a single dose of aroQBP versus 0.1 mL of a single standard human dose pre- and post-challenge with virulent *B. pertussis* (data from reference [25] re-plotted for enhanced clarity).

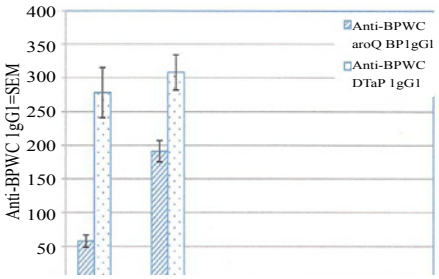

Figure 7: Anti-BPWC IgG2a response of mice vaccinated with a single dose of aroQBP versus 0.1 ml of a single standard human dose pre- and post-challenge with virulent *B. pertussis* (data from reference [25] re-plotted for enhanced clarity).

Vaccine	IgG1	IgG2a
aroQ *B. pertussis*	50	80
DTaP	220	0

Values shown represent reciprocal endpoint titre

Table 1: IgG1 and IgG2a antibody titres against whole-cell *B. pertussis* [BPWC] Tohama I of pooled lung homogenates of mice vaccinated with a single dose of aroQ *B. pertussis* or 3 doses of DTaP vaccine.

and/or lung homogentes. In contrast, neither IgG2a nor CMI-indicator cytokines were detectable.

While IgG1 and IgG2a were produced in respiratory secretions [lung homogenates] of mice immunised with the aroQBP vaccine (Table 1), only the IgG1 isotype was detectable in the lung homogenates of mice vaccinated with DTaP.

In addition, cell-mediated immunity (CMI) against *B. pertussis*, as judged by production of IL-2 and IFN-γ, was induced only in mice that were immunized with the live aroQBP vaccine candidate by the intranasal route (Table 2).

Mice immunised with a single dose of the aroQBP vaccine were protected against challenge infection with the virulent parent up to the dose of 4.2×10^{10} colony forming units [CFUs] whereas protection in mice with one dose of the DTaP were only partially protected when challenged with a dose of the same dose (Table 3).

Discussion

In order to ensure maintenance of herd immunity against whooping cough, a minimum of five vaccinations with either a whole cell pertussis

Parameter	Antigen			
	FHA	PTd	BPWC[a]	Concanavalin A
IL-2 [pg ml^{-1}]	360	970	1,310	>6,000
IFN-γ [pg ml^{-1}]	730	4,310	3,000	> 6,000

[a]BPWC denotes killed Bordetella pertussis whole cells; [b]Antibody titer was determined using indirect dot blotting assay (data extracted from reference #25).

Table 2: IFN-γ (pg ml-1) in antigen-stimulated pooled splenocyte supernatants of mice immunised with a single dose of aroQ *B. pertussis* at day 28 post-challenge with the virulent *B. pertussis*.

Vaccination Group	Percent survival of immunised or sham-immunised mice post- intranasal challenge [CFU/20 µL/mouse] with virulent *B. pertussis*			
	4.2×10^{7}	4.2×10^{8}	4.2×10^{9}	4.2×10^{10}
aroQBP [i/n*- 1 dose]	100	100	100	100
DTaP [s/c**-1 dose]	100	100	100	67
PBS[s/c-1 dose]	67	33	0	0
PBS [i/n*-3 doses]	50	33	17	0

i/n denotes intranasal; s/c* denotes subcutaneous.

Table 3: Protective efficacy of a single dose of aroQ *B. pertussis* against intranasal challenge of immunised mice with virulent B pertussis at day 28 post-immunisation.

vaccine (DTwP) or acellular pertussis vaccine (DTaP), depending upon the country, are required. In Australia, vaccination is recommended at 2, 4 and 6 months of age, 4 years and 15-17 years, followed by a single booster dose of reduced antigen content vaccine (dTpa) for all adults planning a pregnancy, for both parents, grand parents and carers as soon as possible after delivery of the infant. The major problems asociated with the use of these vaccines is the relatively short duration of immunity imparted by the DTaP due potentially to predominant induction of Th2 polarised immune response and lack of CMI induction after primary immunisation with the DTaP vaccine as demonstrated in studies using the pertussis mouse model [1,26] and the perceived fear of serious side reactions despite the fact that the encephalopathy/encephalitis, febrile seizures/provocation of convulsions, and sudden deaths have been reported to be significantly lower with acellular pertussis vaccination than with whole cell pertussis vaccination [27].

The live attentuated vaccines, on the other hand, may require one or 2 booster vaccination at best to provide potential longer term protection as already reported for a different live attenuated vaccine candidate, BPZE1 [24], using the pertussis mouse model. In the latter vaccine candidate, the pertussis toxin gene has been modified to render the pertussis toxin non-toxic, dermonecrotoxin (DNT) gene deleted and the ampG gene replaced with an *E. coli* counterpart with the aim of modulating/reducing the potential toxicity of tracheal cytotoxin (TCT). More recently, a phase trial in adult humans was carried out with this vaccine candidate, results of which have not been published as yet. It will be interesting to determine the immunological properties of the BPZE1 vaccine candidate following deletion of an essential gene such as the aroQ gene. Regardless of the type of attenuated vaccine candidate however, it is important to determine the level of protection afforded by these vaccine candidates against challenge with the clinical isolates showing polymorphisms in different virulence antigens [28]. It is therefore desirable to ensure that all live attenuated vaccine strains including BPZE1 and aroQBP vaccine candidates are non-reverting completely non-toxic and can also impart protection against the newly emerging *B. pertussis* isolates.

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