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Novel approaches to reactivate pertussis immunity

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Abstract

Introduction: Whole cell and acellular pertussis vaccines have been very effective in decreasing the deaths of neonates and infants from *Bordetella pertussis.* Despite high vaccine coverage worldwide, pertussis remains one of the most common vaccine-preventable diseases, thus suggesting that new pertussis vaccination strategies are needed. Several candidates are currently under development, such as acellular pertussis vaccines that use genetically detoxified pertussis toxin, acellular pertussis vaccines delivered with new adjuvants or new delivery systems, or an intranasally delivered, live attenuated vaccine.

Areas covered: This review discusses the different possibilities for improving current pertussis vaccines and the present state of knowledge on the pertussis vaccine candidates under development.

Expert opinion: Until there is a safe, effective and affordable alternative to the two types of existing vaccines, we should maintain sufficient childhood coverage and increase the vaccination of pregnant women, adolescents and young adults.

Keywords: *Bordetella pertussis*, vaccine-preventable, vaccine, vaccine effectiveness, neonates, delivery routes

Article highlights

- Increasing rates of pertussis worldwide, despite high vaccine coverage, emphasizes the need for new vaccine strategies.
- New pertussis vaccines should have low reactogenicity as aP vaccines and suitable cell-mediated immunity as wP vaccines.
- Th2-type immune responses induced by aP vaccines are sufficient to protect against disease, but the induction of Th1- and Th17-type immune responses is required for the clearance of bacteria from the airways and the prevention of asymptomatic carriage.
- New vaccine candidates are explored, such as less reactogenic live vaccines or improved aP vaccines that contain genetically-detoxified pertussis toxin, or those containing new adjuvants and antigens or combined with new delivery systems, or aP vaccines based outer membrane vesicles.
- Until a better pertussis vaccine is found, it should be recognized that adults are the main reservoir for the ongoing circulation of *B. pertussis* and the source of infections of infants and efforts must be focused on the vaccination of pregnant women, a cocooning strategy and vaccinating regularly young adults, as well as maintaining sufficient childhood vaccine coverage.

1. Introduction

1.1. Epidemiology and the burden of disease

Bordetella pertussis causes acute respiratory disease that manifests as a spasmodic, paroxysmal cough in young adults. In very young children, it is associated with apnea and cyanosis and can lead to very severe disease and death in infants under the age of one (1). This disease severity and frequency led to the development of a whole-cell pertussis vaccine, which was first licensed in the US in 1914, and was combined with tetanus and diphtheria toxoids (DTP vaccine) in the 1940s and introduced large scale into the pediatric immunization schedule in 1948, resulting in a marked drop in pertussis cases (2). Nevertheless, concerns about the reactogenicity of whole-cell vaccine led to a refusal by many parents to vaccinate their children (3), which motivated manufacturers to develop in the 1980s new, less reactogenic pertussis vaccines. Sato and colleagues designed the first purified component acellular pertussis vaccine in Japan in 1981 (4). Many other acellular vaccines were then developed, and tested extensively in the 1990s (5). These vaccines were composed of various pertussis antigens, such as the pertussis toxin (PT), filamentous hemagglutinin adhesin (FHA) and pertactin (PRN). aP vaccines showed a better safety profile when compared with wP vaccines and were effective in preventing pertussis disease, at least in the short term (6). As a result, these new vaccines were introduced in the pediatric immunization schedules of many high-income countries (7).

However, over the past decades, there has been a rise in pertussis incidence, first in young adults solely vaccinated with the aP vaccine, but also in the rest of the population. In a cross-sectional study in Spain published in 2000, period during which pertussis immunization was only given during childhood with aP vaccine, it was observed that the prevalence of antibodies to PT and FHA reached 35% and 65% respectively in the 5-12 age group and 52% and 80% respectively in the 30-39 age group (8). As pertussis immunity is not long-lasting, it was presumed that the increase in antibodies in older age group was due to repeated natural exposure to *B. pertussis*, and ongoing circulation of the bacteria in the general population(8), with pertussis infection increasingly frequent in adolescents and young adults who now represent the majority of cases. Although hospitalization, complications and mortality in adolescents and adults are rare, these populations serve as a reservoir for *B. pertussis* and play an important role in the transmission to very young infants (9).

Many factors are responsible for the resurgence of pertussis in countries using aP vaccines, such as the laboratory methods used to diagnose *B. pertussis*, waning of immunity with time, genetic modification of *B. pertussis*, etc. (all discussed in Ref. (10)). However, the most important is likely to be the inability of aP vaccines to prevent colonisation with *B. pertussis* due to the different immune responses generated (see below).

Pertussis continues to be an important cause of infant death worldwide and remains a public health concern, even in countries with high vaccine coverage (11). In 2013, the World Health Organization estimated that pertussis was still responsible for 63,000 deaths in children aged <5 years, despite global vaccination coverage. Coverage with the 3-dose, pertussis-containing vaccine during infancy was estimated at 86% in 2014 (11) and also confirmed in 2018. Additionally, according to a modelling study with data from 2014, it was estimated that there

were around 24 million cases of pertussis worldwide and approximately 160,700 deaths directly attributable to the disease in children <5 years, with the largest proportions in the African region, representing 33% of cases and 58% of deaths. Furthermore, it was reported that 5.1 million (21%) of estimated cases and 85,900 (53%) of estimated deaths were in infants <1 year (12).

It is also important to note that with the exception of a couple of countries, most low and middle income countries use wP, but yet carry the largest estimate of burden of disease and mortality from pertussis, for various reasons, such as low access to PCR-based diagnostic tools to improve surveillance, and the fact that disease burden is increased due to HIV infection and exposure (13).

During COVID19 pandemics, the incidence of pertussis has decreased due to the lockdown measures, however, maternal and childhood vaccination have also slightly diminished (14). Therefore, it will be important to monitor pertussis cases and vaccine coverage in the coming months-years because there is an important potential for resurgence of pertussis due to lack of population immunity.

There has been a few recent reviews discussing the current pertussis problem, a.nd new vaccines approaches (15-17) In this review, I will discussed new vaccine candidates including acellular pertussis vaccines that use genetically detoxified pertussis toxin.

1.2. Protection against *B. pertussis*

Protection against *B. pertussis* is mediated by both humoral and cell-mediated immunity (18, 19). Indeed, protection has been shown to persist among children whose antibody levels drop below the level of detection over time (20). Immune correlate of protection are usually established in randomized, placebo-controlled efficacy trials in which the development of post-vaccination levels of antibodies or other components of the immune response, correlate with disease prevention "vaccine efficacy". In other words, vaccine efficacy is the degree to which a vaccine prevents disease, and possibly also transmission, under ideal and controlled circumstances – comparing a vaccinated group with a placebo group. Between 1997 and 1999, an NIH-sponsored study, the APERT trial, evaluated the efficacy of an acellular pertussis vaccine in adolescents and adults. After vaccination, they followed the volunteers for 2 years, for pertussis illness with phone calls every 2 weeks and serum were obtained at routine interval, as well as nasopharyngeal aspirates in case of cough (21). However, it is unethical to perform a placebo controlled trial for a disease for which we have a vaccine. Few correlate of protection have emerged such as anti-PT-IgG levels >5 IU/mL (25).

In contrast, vaccine effectiveness meanwhile refers to how well a vaccine performs in the real world (22). Estimates of vaccine efficacy also profoundly vary with case definition, ranging from 5% efficacy in preventing spasmodic cough lasting 1 day or longer, to 100% efficacy in preventing culture-confirmed pertussis with spasmodic cough lasting 28 days or longer (23) .

An active mouse protection test, which measures protection following intracerebral challenge of *B. pertussis*, was traditionally considered as the gold standard to predict vaccine protection

after wP vaccine, but it was unsuitable to predict acellular vaccine protection (24). It was then replaced by a murine respiratory challenge test model.

Even if we do not have a good correlate of immune protection after vaccination, we now better understand the mechanisms leading to protection against *B.pertussis*. First, it is now clear that the native bacteria produces a number of toxins, such as pertussis toxin (PT), tracheal cytotoxin (TCT), adenylate cyclase toxin (ACT), heat-labile toxin, and endotoxin or lipopolysaccharide (LPS), and a range of other critical receptor-binding virulence factors, including filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae. All these components are antigenic targets that activate innate cells, and adaptive cells.

Other human studies have shown that recovery from whooping cough in children is associated with the induction of interferon (IFN)- secreting T helper type 1 (Th1) cells (26 , 27). In addition, murine respiratory challenge models have allow to better clarify the roles of the various components of the immune system for clearing the bacteria ; A murine study has shown that higher amounts of proinflammatory cytokines in the lungs were associated with an induction of selective Th1-cells and the secretion of cytokines, such as IFN-ϒ (Th1) and IL-17 (Th17) (28). It is known that cytokines secreted by Th1 cells, particularly IFN-ϒ, favor the production of opsonizing antibodies and the activation of macrophages and neutrophils to kill or mobilize intracellular *B. pertussis* (29). Indeed, studies in mice have shown that Th1 cell responses are required for bacterial clearance following infection or immunization with wP (29, 30). By contrast, it has been observed that immunization of mice with aP induces Th2-type responses, which promote humoral immunity, but do not appear to be as effective as Th1 cells in clearing *B. pertussis* from the respiratory tract. Other clinical studies in humans have confirmed these findings showing that immunization with wP induced selective , Th1-type immune responses, even in infants (26), while aP vaccines induced strong antibody and Th2 responses (31, 32, 33).

Other studies in baboon and mouse models have shown that aP vaccines protect against disease (lung) but do not protect against infection/transmission (nasal) (26, 34, 35). Indeed, primary infection with *B. pertussis* in mice induces tissue resident memory T (TRM) cells that secrete IL17 into nasal tissue, which help with the clearance of the bacteria from the nasal mucosae by the mobilization of neutrophils, with a strong neutrophil extracellular trap (NET) activity (34) Further it has also been observed that immunization with wP vaccine in mice protects against lung and nasal colonization, whereas aP vaccine do not protect the nose. This is due to the induction of nasal TRM cells that secrete IL-17 (35).

In summary, wP vaccines induce Th1/Th17 responses with the induction of bactericidal and opsonizing antibodies, while aP vaccines induce preferentially Th2 responses with the induction of antibodies which are not opsonizing and not bactericidal, because the chemically detoxified PT loses its immunological properties and intrinsic adjuvant capacity (33, 36). Th2 cells are known to help B cells to secrete IgE and IgG1 antibodies that neutralize toxins and prevent the adherence of bacteria in the respiratory tract (31-33). In parallel, both wP and aP vaccines induce protection from disease but aP vaccines do not impair the colonization and therefore transmission. So aP vaccines have probably little effect on herd immunity. All these observations are key information to develop new vaccines that are effective in preventing pertussis infection but also transmission. Finally, it would be very important to demonstrate how clearance of infection is induced with all these new vaccines candidates. Traditionally, this was only assessed in baboon and mouse models (37). However, the recently developed

human challenge model by de Graaf et al. will certainly help to design and evaluate new vaccines against B. Pertussis (38).

1.3. Pertussis immunity and the need for new vaccine strategies

Pertussis immunity acquired through immunization or even infection appears to be short-lived and several studies have reported that a second pertussis infection can occur some years after the first event. It has been estimated that natural pertussis infection offers a protection for 7 to 20 years, although in some circumstances (depending on the specific pertussis epidemiological context) it can last only 3.5 years (39, 40). According to a modelling study, immunity induced by natural infection with pertussis lasts on average more than 30 years, while 10% of individuals lose immunity after 10 years (41). As a comparison, protection following wP vaccination appears to last between 4-10 years according to a study in England reporting that 85% of children were still protected 4 years after immunization, and 50% children were still protected after 7 years (42). By contrast, aP vaccination appears to offer a shorter protection. In a Canadian study, children vaccinated with aP vaccines in infancy were susceptible to develop pertussis infection already during the first 4 years of life, while those vaccinated with wP vaccine in infancy remained protected until 5-9 years of age (43). Furthermore, a case-control study performed in the USA after a pertussis outbreak in 2010-2011 reported that most of the pertussis adolescent cases occurred in those previously immunized in infancy with the aP vaccine, rather than in those with the wP vaccine (44). Therefore, the maintenance or reactivation of immunity against pertussis requires repeat boosting. In 2014, the World Health Organization specified that countries currently using an aP vaccine could continue to use this vaccine, but they should consider the need for additional booster doses and strategies to prevent early childhood mortality in case of the resurgence of pertussis (45).

In 2013, Chiappini *et al*. listed various vaccination strategies, which included cocooning, vaccination of pregnant women and newborns, and administering booster doses of aP vaccines to preschool children, adolescents, adults and healthcare workers (46). A puzzling observation was that the efficacy of the current aP was higher in infants and children than in adolescents in whom vaccine efficacy appeared to be reduced and of short duration. A study of the 2012 Washington State pertussis epidemic in the USA showed that protection waned within 2 to 4 years after adolescent boosting and vaccine effectiveness decreased to 34% (47). The resurgence of pertussis is considered as resulting largely from the limited durability of aP-vaccine-induced immunity in adolescents. This may derive – at least in part – from priming and repeat immunizations with aP vaccines containing chemically-detoxified PT and thus the induction of antibodies specific for the chemically-detoxified PT, but unable to efficiently recognize the native PT expressed by *B. pertussis* (48, 49).

Therefore, new vaccine strategies should be developed, such as the re-introduction of less reactogenic wP vaccines, or the development of optimized aP vaccines, which include antigens with a similar immunogenicity profile as native PT, but deprived of its toxic properties. Further, new vaccines will have to protect against infection as well as disease in order to induce herd immunity. It is unlikely that aP vaccines will help in a cocooning strategy as they do not prevent colonization/infection.

It is important to add that new vaccines will need to protect against infection as well as disease to provide herd protection. The current aP vaccines will not help in a cocooning strategy as they do not prevent colonisation/infection. They will need to be formulated with novel adjuvants to induce cellular immune responses in the respiratory tract, especially when delivered by the intranasal route (35, 50).

2. New wP vaccines

2.1. Development of wP vaccines without lipopolysaccharide

Wp vaccines have the advantage to induce a broader immune response that mimics a natural infection with pertussis, with the induction of a Th1- and Th17-type immune responses, with the production of opsonizing and bactericidal antibodies, which have been shown to be required for bacterial clearance in the respiratory airways and long-term immunity (29, 33). The high reactogenicity of wP vaccines has been associated with proinflammatory cytokines, in particular IL-1beta and IL-1-associated signalling components (51). As lipopolysaccharide (LPS) possesses endotoxic activity and also has powerful adjuvant activity, LPS was considered to be one of the most important factors that contribute to the reactogenicity of wP (52). Indeed, LPS is recognised by TLR complex TLR4/MD-2 with subsequent activation of NF-κB (53). Therefore, many manufacturers have worked on the generation of a pertussis vaccine with a reduced quantity of LPS (54). In a mouse model, Bannatyne *et al.* observed that the activity of the endotoxin contained in the diphtheria tetanus wP (DTwP) vaccine could be reduced after exposure to polymyxin B, as assessed in a limulus-lysate test, without decreasing the effect of the vaccine (55). Others have managed to remove LPS by chemical extraction (52). However, as said earlier, LPS activates dendritic cells through TLR4, and induces a strong Th1 and Th17 responses, which is important for long-term protection, among other through the induction of bactericidal antibodies. Therefore, it appears that it is not a good idea to remove the LPS from wP vaccine and new strategies should rather focus upon ways to modify LPS in order to keep its advantages, but lose its reactogenicity (56, 57).

2.2. Development of live molecularly-attenuated pertussis vaccine delivered intranasally

The observation that natural infection with *B. pertussis* can induce a strong and long-lasting immunity compared to vaccine-induced immunity (39) has led to the development of live vaccines deliverable by the intranasal route in order to better mimic a natural infection stimulating mucosal immunity, together with the systemic immune response (58). Mucosal immunization against pertussis has already been tested. Oral delivery of a wP vaccine was administered to newborns during the first days of life, with an additional dose at 6 weeks, and resulted in the induction of specific salivary IgA and systemic IgG and cellular immune responses (59). Another study assessed the intranasal delivery of a single dose of a wP vaccine in adults and showed that this immunization route could induce antibodies in nasal secretions, but not in serum (60).

Locht *et al.* have developed a live candidate vaccine through genetic attenuation of *B. pertussis* comprising the deletion of a dermonecrotic toxin gene, reduction of tracheal cytotoxin to a background level, and complete inactivation of PT. This novel genetically

attenuated strain, named BPZE1, showed a reduced pathogenicity, while retaining its ability to colonize the mouse respiratory tract and induce better protection than aP vaccines in infant mice after a single intranasal administration (58, 61, 62). Other advantages of the use of BPZE1 are the relatively low production costs, rendering it also affordable for developing countries, its needle-free administration, which would increase its acceptability, and its capacity to induce mucosal and systemic immunity. Furthermore, it could also be used for the presentation of other vaccine antigens against many respiratory pathogens, e.g., in a multivalent vaccine (63). Importantly, this method could be used for very young children, even at birth (58, 64). Phase I clinical trials with the BPZE1 vaccine have started already and have shown very promising results (65, 66). Jahmatz *et al*. assessed the safety, colonization and immunogenicity of the BPZE1 pertussis vaccine when given intranasally in a Phase 1b double-blind, randomized, placebo-controlled, dose escalation study in 48 adults in Sweden and reported that the vaccine was safe, induced a high colonization rate, and was immunogenic at all dose levels (65). However, further studies are needed to confirm if this vaccine could be used as a priming vaccine for young infants and a booster vaccine for adolescents and young adults.

3. New aP vaccines

3.1. Development of new aP vaccines with more antigens

In contrast to the wP vaccines that contain much more *B. pertussis* antigens, current aP vaccines contain only up to five antigens, i.e., detoxified PT, FHA, PRN, and the serotype 2 and 3 fimbriae (FIM 2/3). It has been suggested that the limited number of antigens could result in a narrower range of specific immune responses (66). It appears also that aP vaccines have a suboptimal balance of antigens as antibody to PRN or FIM leads to an effectiveness of 70%, while stronger antibody responses to PT seem to decrease the effectiveness of antibody to FIM (67). This incorrect balance in the antigens in aP vaccines could participate to the decreased effectiveness of the vaccine. A Swedish study assessed the response to PT, FHA, PRN and FIM 2/3 in serum of convalescent children who had been primed or not with an aP vaccine and found that infants primed with a PT toxoid vaccine had a blunted response to the non-vaccine antigen FHA compared to those who had received a PT/FHA vaccine(68). Similarly, infants who had pertussis and who had received a PT/FHA vaccine had a blunted response to the non-vaccine antigens PRN and FIM 2/3 compared to those who had received a PT, FHA, PRN and FIM 2/3 vaccine (68). These observations are explained by the fact that memory B cells out-compete with naïve B cells for access to the *Bordetella* epitopes because their frequency is higher and their B cell receptor has a stronger antigen affinity. This “linked epitope suppression phenomenon” suggests that upon exposure and infection, previous vaccinees have more robust antibody responses to the antigens contained in the vaccine they had received than to *Bordetella* antigens not in the received vaccine, thus underlying the importance that the aP vaccine should contain multiple antigens. However, it appears that having the correct number and balance of antigens may be difficult to achieve.

It has also been suggested that the limited number of antigens contained in aP vaccines could facilitate the immune selective pressure that could lead to the emergence of new strains of *B. pertussis*, such as the PRN negative strains (56, 69, 70). Indeed, it has clearly been shown

that in Europe the longer the period since the introduction of acellular vaccines containg PRN, the higher the frequency of circulating PRN-deficient isolates (71). Thus, the addition of new antigens to aP vaccines could increase protection and reduce the immune selective pressure. Several researchers have looked at additional protective antigens that could be included in aP vaccine, such as the BvgAS, system, which includes two components, i.e., the histidine kinase BvgS, and the response regulator BvgA, (72). Another candidate antigen includes the *Bordetella* resistance to killing A (BrkA), which is a virulence factor activated by phosphorylated BvGA that has an autotransporter function and plays a role in the adherence of bacteria to lung epithelial cells and in the resistance to complement- mediated phagocytosis (73). However, preliminary studies in mice models reported that the administration of BrkA did not induce a significant protection against nasal challenge with *B. pertussis*, while the addition of BrkA to FHA and PT was shown to increase the protection over FHA and PT alone (73).

In another mouse infection model, the effect of the addition of antigens into the aP vaccine to prevent colonization of nasal mucosa by *B. pertussis* was assessed. For example, type 2 and type 3 fimbriae (FIM2/3) with outer membrane LPS (LOS) and/or of the adenylate cyclase toxoid (dACT), which induces neutralizing antibodies against the CyaA toxin. However, it was found that the addition of these antigens only modestly increased the aP vaccine to prevent lung infection. However, a striking observation was that irrespective of FIM2/3 with LOS and dACT addition, the aP vaccine promoted a prolonged colonization of the nasal mucosa by the bacteria (74, 75, 76).

Sera from convalescent individuals infected by *B. pertussis* were also found to have antibodies specific for another pertussis protein, IRP1-3. Antibodies against IRP1-3 can induce opsonization and neutrophil uptake of iron-starved *B. pertussis*. This protein has been shown to induce protection in a mouse model after nasal challenge with iron-starved *B. pertussis* (77). So far, the inclusion of IRP-3 to the currently used aP or wP vaccines has not been studied.

Another study, has found no differences between aP vaccines with ≥3 components and those with 1 component (78). Globally, the inclusion of additional antigens to the existing aP vaccines administered parenterally may not be sufficient to induce sufficient protection in the upper respiratory tract.

3.2. Development of aP vaccines delivered with new adjuvants

The replacement of the first generation wP vaccines by the aP vaccines has reduced the occurrence of systemic adverse reactions observed with wP vaccines as they do not contain lipopolysaccharide. While the current aP vaccine contains alum as adjuvant, which favors a Th2-type immune response, it appears essential to also induce a strong Th1/Th17 immune cellular response for the clearance of *B. pertussis* as observed following wP vaccination or infection, and which prevents infection and disease and provides longer protection (18, 26, 37, 79). Several preclinical studies in mice have tested the current alum-absorbed aP vaccines delivered with new adjuvants that promote a Th1/Th17 immune response, such as Toll-like receptor (TLR) agonists, e.g. Alum-TLR7a (alum adsorbed SMIP 7.10), LP-GMP (TLR2 agonist LP1569 plus STING agonist cyclic dimeric guanosine monophosphate (c-di-GMP), which is an intracellular receptor stimulator of interferon genes). The Alum-TLR7a together

with a 3-component pertussis vaccine has been shown to induce a Th17/Th1 immune response in a mouse model compared to the control aP vaccine (80). The LP-GMP showed similar results (81). Other candidates vaccines have used the *Bordetella* colonization factor A, or the curdlan, a 1,3-β-glucan polysaccharide, with very encouraging results (82). Another adjuvant, the CpG motif-containing oligodeoxynucleotides (ODN), that can induce a Th1-type immune response, has also been studied for pertussis vaccines. The addition of CpG ODN to the current alum-adjuvanted DTaP vaccines has been shown to induce a Th1-type immune response in mice (83).

Dynavax conduct a phase 1 randomized, participant-blinded, active-controlled, dose escalation, clinical trial to evaluate the safety, tolerability, and immunogenicity of an investigational tetanus/diphtheria/pertussis (Tdap) booster vaccine utilizing CpG 1018 adjuvant compared to a licensed Tdap vaccine in healthy volunteers between 10 and 22 years of age, in Australia (ACTRN12620001177943p).

Similarly, the addition of CpG ODN with other immunostimulatory molecules, such as polyphosphazenes and cationic innate defense regulator peptides, was shown to be very immunogenic in adult and neonatal mice (84).

3.3. aP vaccines delivered in microparticle delivery systems

A new vaccine formulation consisting of microparticle, co-encapsulating pertussis toxoid, polyphosphazene, CpG ODN 10101 and synthetic cationic innate defense regulator peptide 1002 given subcutaneously, was able to induce better protection than these components given in a soluble formulation in mouse models (28). Indeed, in mouse studies, the co-encapsulation of the adjuvants and the antigen in the microparticle resulted in a strong shift towards Th1/Th17 responses in the lung, despite a lower systemic humoral response, which appears to be essential for long-term immunity (28, 85). It has been shown that multicomponent vaccine formulations need an effective delivery system for the co-delivery of all components to the immune cells and tissues to induce a good immune response. Microparticle delivery allows for a better vaccine stability and uptake of te antigen to the MHC-class I and II compartments, resulting in both cell-mediated and humoral immune responses (86). So far, none of the new vaccine formulations are undergoing further development.

3.4. aP vaccines delivered with outer membrane vesicles

After observing that outer membrane vesicles (OMV) of Gram -negative bacteria are strongly immunogenic, this technology was developed for pertussis vaccine (87). In mouse models, immunization with pertussis OMV vaccine has been shown to induce a strong antibody response with high opsonizing activity and a Th1-type immune response compared to the aP vaccines (50, 88)Administration of OMV pertussis vaccine via the pulmonary route in mice induced local and systemic Th17- and Th1-type responses and local IgA (89). In comparison, intranasal administration of the OMV vaccines was able to stimulate mucosal IL17 and IFNϒ, nasal and lung IgA, and high Th17 systemic responses (89). The OMVs of *B. pertussis*

contain many antigens, including the lipooligosaccharide. Further research is needed on this new vaccine candidate.

3.5. aP vaccines containing genetically-detoxified PT

The observations that current aP vaccines, which contain chemically-detoxified PT, induce a higher immune response in infants and children than in adolescents in whom vaccine efficacy is limited and rapidly wanes (90, 91) may result largely from the short duration of aP-vaccine-induced immunity in adolescents. Indeed, it has been suggested that priming and repeated boosting with aP vaccines containing the chemically-detoxified PT could induce antibodies and memory B cells specific for the chemically-detoxified PT, but unable to efficiently recognize the native PT expressed by *B. pertussis* (92, 93). Research has been also focused on developing optimized aP vaccines that include antigens with a similar immunogenicity profile as native PT, but without the toxic properties. This has been shown to be best obtained through genetic, rather than chemical detoxification of PT. Indeed, PT chemically detoxified reduces binding of the holotoxin to target cells and impairs key activities such as antigen-presenting cells activation and cytokine secretion, loosing therefore its adjuvanticity (31). In contrast, genetically detoxified PT is safe due to the loss of its ADP ribosylating activity (94). However, it maintains an intact quaternary holotxoin structure and cell binding capacity, conserving it ajuvanticity (31). Both native PT and genetically detoxified PT can activate APCs, such as dendritic cells, as well as TLR- 2 and 4 and secretions of multiple cytokines such as IFN-Y, IL-1β, IL-12, IL-23 and IL-6 (31). The different cytokines drive differentiation of naïve T cells toward Th1, Th2 and Th17 phenotype, which have all a specific role in the immune response against *B. pertussis*.

In adults, children and infants below one year, the safety and immunogenicity of a 9K/129G genetically-detoxified r-aPT was first demonstrated in a monovalent r-aPT vaccine and in a combined Td-r-aPT vaccine. In infants, vaccines containing r-aPT were shown to be safe, highly immunogenic, efficacious and able to elicit antibodies and protection which persisted up to 6 years (95, 96). The superior immune response of r-aPT-containing vaccines was associated with the conservation of 75-80% of native PT, allowing efficient binding of immune cells to B and T cell epitopes (92, 93). The development of this vaccine was unfortunately interrupted by patent issues.

Later, following expiration of the patents, BioNet-Asia (Bionet) developed a new *B. pertussis* strain expressing a recombinant PT (97) that retains the functional antigenic properties of native PT, but with loss of its toxicity. BioNet has developed two investigational products that have been tested in clinical studies : 1) BioNet aP: A stand-alone recombinant acellular pertussis vaccine containing a proprietary genetically-detoxified PT (PT-9K/129G; PTgen), filamentous hemagglutinin (FHA) and pertactin (PRN) antigens. This vaccine was manufactured, formulated and filled by BioNet in Thailand. 2) BioNet TdaP: A combined vaccine containing the three *Bordetella pertussis* antigens produced by BioNet and formulated with the bulk of tetanus toxoid (TT) and diphtheria toxoid (DT) supplied by a World Health Organization (WHO) pre-qualified vaccine manufacturer, PT Bio Farma, Indonesia. TdaP was then formulated and filled by BioNet in Thailand. In the new formulation only the PT (PTgen) and filamentous hemagglutinin (FHA) are contained in the

BioNet TdaP and aP vaccines (5 μg of each antigen). Both vaccines contain aluminum hydroxide as adjuvant and are presented as a single dose suspension of 0.5 mL per dose in a pre-filled syringe (PFS).

A phase I/II randomized controlled trial in wP-primed Thai adults showed similar safety, but a significantly higher antibody response to the r-aP vaccine than to the standard aP vaccine (98). These findings were subsequently confirmed in phase II/III in 450 wP-primed Thai adolescents (99). Compared with the chemically-detoxified PT-containing Tdap vaccine, the higher PT-specific and neutralizing PT antibody responses persisted one year post-immunization (100). Further, a phase 2 randomized-controlled safety and immunogenicity trial evaluating different doses of the recombinant acellular pertussis vaccine containing genetically-inactivated pertussis toxin (PT_{gen}) was conducted in women of childbearing age in Thailand and showed that all the vaccines were safe and immunogenic.

A study using the same vaccine in aP-primed Swiss adolescents showed that the total PT-antibodies, PT-neutralizing antibodies and PT-memory B cells were higher following r-aPT-containing vaccination compared with the standard aP vaccine, while the response to the FHA was similar in both groups (101). However, at one year post-immunization, only the total anti-PT antibodies remained higher in the r-aPT vaccinated adolescents (101). These observations suggest that r-aPT vaccines may need to be given repeatedly, earlier and with novel Th1/Th17-inducing adjuvants to exert a significant effect in aP-primed adolescents.

A monovalent r-aPT vaccine would be useful for boosting neonates, pregnant women and adults in contact with young infants. The r-aPT monovalent vaccine has been shown to be highly immunogenic in adolescents (100, 102), which has supported licensure in Thailand. Further, a study using the baboon model found that a monovalent aP vaccine had equivalent effectiveness than Tdap during pregnancy (103). Another study in Australia, showed that a monovalent aP vaccine is immunogenic and safe in human neonates (104), and therefore, could potentially be used for newborns whose mothers did not receive the Tdap vaccine during pregnancy.

Based on the data from phase I/II and phase II/III clinical studies of BioNet, marketing authorization was approved for both BioNet aP (Pertagen®) and TdaP (Boostagen®) vaccines (both containing 5 μg each of PT and FHA) in September and October 2016, respectively (registration no. 2A 2/59 (NBC) for Pertagen®; registration no. 2A 1/59 (NBC) for Boostagen®). Subsequently, Pertagen® and Boostagen® were released from the safety monitoring program (SMP) of Thailand Food and Drug Administration (FDA) and have become fully licensed vaccines on 24 April 2019 for Boostagen® with new registration number 2A 1/59 (NB) and on 12 January 2022 for Pertagen® with new registration number 2A 2/59 (NB). A single dose of Pertagen® and Boostagen® is intended for active booster immunization for the prevention of tetanus, diphtheria and pertussis disease in adolescents and adults. Further, active pharmacovigilance confirms that this recombinant pertussis vaccines aP_{gen} (Pertagen®) and $TdaP_{gen}$ (Boostagen®) are safe in adolescents and adults, including pregnant women vaccinated in the second or third trimester of pregnancy (105).

The r-aPT vaccine developed by BioNet, has also been combined with an epicutaneous patch (Viaskin®, DBV Technologies, Paris, France), used for desensitization to peanut allergy. It was tested first in mice as a single application and was shown to efficiently recall memory responses in aP-primed mice (88). This r-aPT-coated-Viaskin® epicutaneous patch has also

been tested to recall memory responses in healthy Swiss adults in a phase I double-blind, placebo-controlled randomized trial (106). It was administered on days 0 and 14 using the Viaskin® patches applied directly or after epidermal laser-based skin preparation. It was observed that the Viaskin-PT applied after laser-based epidermal skin preparation induced similar anti-PT antibodies to the standard Tdap (106). These results are very encouraging and should guide further research on this novel approach as this might be a very interesting option for needleless and adjuvant-free vaccination, which could facilitate vaccine acceptance and also vaccine accessibility.

4. Conclusions

Pertussis remains highly endemic and continues to rise and to cause infant death, despite high vaccination coverage worldwide. Several strategies have been implemented to control the situation, including maternal immunization, neonatal vaccination and cocooning, but with limited effects, thus emphasizing the importance of developing new vaccine candidates. However, current aP vaccines do not generate protective immunity against nasal colonization and do not prevent asymptomatic transmission in vaccinated individuals. New vaccine approaches should focus on inducing humoral and Th1- and Th17-type immune responses in the respiratory tract, which are important for clearance of infection and prevention of re-infection. New challenge models such as the recently developed human challenge model by de Graaf et al. (38) will also be important to assess the effect of each vaccine on clearance of infection, and maybe also to define new correlate of protection. The development of new pertussis vaccines are underway, with the most advanced candidates including the new live attenuated BPZE1 pertussis vaccine delivered intranasally and an acellular vaccine using a genetically-detoxified pertussis toxin. It is possible that recombinant vector vaccines and mRNA vaccines that have been developed against the present SARS-CoV-2 pandemic could be adapted to make new pertussis vaccines.

5. Expert opinion

Pertussis remains responsible for infant deaths, despite high vaccine coverage worldwide. This can be explained by the widespread circulation of the bacteria in the population, especially among adolescents and young adults, who have been vaccinated with the aP vaccine. This group represents the major reservoir of the infection. The hypothesis is that repeated doses of the aP vaccine containing chemically-detoxified PT induces antibodies specific for this vaccine but unable to efficiently recognize the native PT expressed by the natural bacteria (24). In contrast, the wP vaccine induces a longer-lasting immunity, but is associated with strong reactogenicity, causing high vaccine resistance among parents. For this reason, new vaccines against whooping cough are needed.

Increased understanding of immunity against *B. pertussis* in order to induce long-lasting immunity and prevent colonization and transmission of the bacteria is crucial for the development of good candidate vaccines, particularly the important role of the Th1- and Th17-type immune responses in addition to the humoral responses. These include aP vaccine using a genetically-detoxified PT, which appears to be among the most promising short-term candidates. Indeed, this vaccine is already licensed in several countries, such as Singapore and Thailand, and has been used in several clinical trials in a monovalent form. This vaccine could be used to boost pertussis immunity in adolescents, young adults and pregnant women

at the time of each pregnancy, as well as young fathers, and all other persons in close contact with infants.

We have shown in a recent randomized controlled trial (RCT) in adolescents that this vaccine can induce, at least in the short term, a stronger anti-PT neutralizing and binding antibody and memory B cell responses, compared to the currently licensed aP-vaccine containing chemically-detoxified PT. However, this superior immunogenicity was found to be transient, as it was less clear after one year (71). Although genetically-detoxified PT is expected to induce more Th1 responses than chemically-detoxified PT, both vaccines are absorbed on aluminium salts and generate preferential Th2 responses. Therefore, the genetically-detoxified PT may have to be given repeatedly or earlier in the pediatric immunization schedule before too many memory B cells specific to the chemically detoxified-PT-containing vaccine are elicited. Currently, we are conducting a new RCT to establish if repeated doses of the genetically-detoxified PT-containing vaccine could induce stronger antibody and memory B cell responses.

Alternatively, the genetically-detoxified PT vaccine could be combined to a Th1/Th17-inducing adjuvant to promote a stronger inflammatory response that could increase the immunogenicity of this vaccine. However, it remains to be assessed whether the monovalent form of the vaccine (pertussis without the concomitant Td administration) is also effective.

Another promising vaccine is the live attenuated pertussis vaccine administered intranasally, the BPZE1, which has been tested in clinical studies.

Other alternatives include the use of novel adjuvants and vaccine delivery systems, however, these other candidates still require further assessment in pre-clinical studies. Therefore, the first step may be to use an aP vaccine for booster vaccinations with a genetically-detoxified PT, which could be later combined with a new adjuvant or delivery system. Then, in the longer term, the objective could be to use an easily administered, nasally delivered, live attenuated pertussis vaccine to induce long-lasting immunity in the nasal mucosa and the lungs from early life.

5.1 Five-year view

We should aim towards the approval from the regulatory organs of a Tdap vaccine using a genetically-detoxified PT, to be used first as booster vaccines in adolescents and adults previously primed with the DTaP vaccine, and then for regular Tdap vaccines in infancy.

This will help to decrease the circulation of *B. pertussis* in young adults who are the principal reservoir. In parallel, we should maintain sufficient childhood coverage with the DTaP vaccine and ensure that all pregnant women receive the Tdap vaccine between 27 and 36 weeks' gestation with each pregnancy. A monovalent aP vaccine using the genetically detoxified PT would also be useful for boosting neonates, pregnant women and adults in contact with young infants. The advantage of such monovalent vaccine is that it could be used in high-income countries (where tetanus and diphtheria boosting is not needed) and would also facilitate the option of neonatal vaccination.

Finally, the countries that use wP vaccines should probably continue to do so, at least in the primary childhood immunization schedule.

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