Review

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Diphtheria–tetanus–pertussis vaccine: past, current & future

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The diphtheria–tetanus–pertussis (DTP) vaccine can prevent diphtheria, tetanus and pertussis. The component antigens of the DTP vaccine had long been monovalent vaccines. The pertussis vaccine was licensed in 1914. The same year, the mixtures of diphtheria toxin and antitoxin were put into use. In 1926, alum-precipitated diphtheria toxoid was registered, and in 1937 adsorbed tetanus toxoid was put on the market. The development of numerous effective DTP vaccines quickly stimulated efforts to combine DTP with other routine vaccines for infants. This overview covers the most important information regarding the invention of DTP vaccines, their modifications and the needs that should be focused on in the future.

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Strategy adopted for the literature

The authors performed a literature review of the diphtheria–tetanus–pertussis (DTP) vaccine. An objective of this literature review was to assess the development of the DTP vaccine; the current recommendations for use of the vaccine; the issues related to long-term immunity after the pertussis vaccine, in particular; and the development of new vaccines to provide better immunity. The authors focused on previously published original articles and reviews to select those that address the current investigation. Data for this review were retrieved through searches performed in Pubmed, in the online edition of *Plotkin's Vaccines* and on the website of the European Centre for Disease Prevention and Control (ECDC). The search was limited to English-language publications involving mainly animal studies and human vaccine clinical trials. To check the status of the current clinical trials, the authors used the Global Clinical Trials website.

Introduction

The DTP vaccine is one of the oldest vaccines used in humans. It prevents three serious, potentially fatal diseases that affect children and adults.

Diphtheria is an infectious, potentially fatal disease caused by corynebacteria species able to produce highly potent diphtheria toxins, including *Corynebacterium diphtheriae, C. ulcerans* and *C. pseudotuberculosis*. The disease can be transmitted through contact with infected persons as well as objects that are touched. The diphtheria toxin is regarded as the major virulence factor, while symptoms of the disease are related to the effect of the toxin [1,2].

Tetanus is an infectious, potentially fatal disease caused by anaerobic bacteria, *Clostridium tetani*. These ubiquitous, spore-forming, Gram-positive bacilli are common in the environment, especially in soil contaminated with animal feces. They produce strong exotoxins: tetanospasmin, which after infection is transmitted in the bloodstream and affects the nervous system, and tetanolysin, which plays a role solely in the infection site. Infection occurs either when bacteria or spores enter the wound or, in the neonatal form, in babies born in poor sanitary conditions when the umbilical cord becomes infected [3,4].

Pertussis, also called 'whooping cough', is a severe, infectious and highly contagious respiratory disease caused by *Bordetella pertussis*, a Gram-negative bacillus [5]. *B. pertussis* usually infects unvaccinated or incompletely immunized infants and children. Adolescents and adults, who often suffer from mild or atypical forms of the disease, play an

important role in transmitting *B. pertussis* [6]. The disease is transmitted through droplets that spread easily through coughing and sneezing by an infected person. Whooping cough can be fatal, especially in very young infants.

History of DTP vaccine development

Before the DTP vaccine was combined in 1943 and registered for the first time in 1948 in the USA, vaccines containing each component separately were developed. The vaccine containing the pertussis component was licensed in 1914; the vaccine with alum-precipitated diphtheria toxoid was licensed in 1926; and the tetanus toxoid vaccine was licensed in 1937 [7].

Diphtheria component

After the discovery of diphtheria toxin and the development of antitoxin in the 19th century, the first successful approach to active diphtheria immunization was the mixture of toxin and antitoxin, which successfully immunized both humans and animals through injection. In 1907, Theobald Smith observed an immune response against diphtheria in guinea pigs that were vaccinated with a mixture of diphtheria toxin and antitoxin. Next, von Behring began the immunization of children with the mixture of diphtheria toxin and antitoxin in large cities in Europe and the USA. In 1920, Ramon showed that the diphtheria toxin, after treatment with heat and formalin, lost its toxicity while maintaining immunogenicity properties [8]. In this way, the diphtheria anatoxin (also known as the diphtheria toxoid) has been prepared until the present day. During the next 15 years, toxin–antitoxin was replaced by diphtheria toxoid. In 1927, Ramon and Zoeller combined tetanus toxoid with diphtheria toxoid and showed that there was no antigenic competition for immune responses [3]. In 1926, Glenny and colleagues found that the adsorption of diphtheria antigen toward aluminum salts increased the immunogenicity of toxoid. In the mid-1940s, as diphtheria toxoid was combined with tetanus toxoid and a whole-cell pertussis (wP) component, the adjuvant strength of the wP component to the other components of the diphtheria–tetanus–whole-cell pertussis (DTwP) vaccine was proved [9]. The efficacy of diphtheria toxoid has never been studied in clinical trials, but the effectiveness of vaccination was confirmed by observation during an epidemic that occurred in Halifax. The number of diphtheria cases observed among the immunized population was 24.5 per 100,000 monthly compared with 168.9 per 100,000 observed among the unimmunized population [10]. In the UK in 1943, the clinical symptoms of diphtheria were observed to be 3.5-times more frequent and the mortality rate 25-times higher among nonvaccinated people [11].

Tetanus component

In 1924, a chemically inactivated tetanus toxin was developed, which is now referred to as the tetanus toxoid and is used for active immunization against *Clostridium tetani* infections. Although the tetanus vaccine became commercially available in the USA in 1937, the vaccine was widely used for the first time to immunize soldiers during the Second World War. Initially, iodine trichloride was used to inactivate the tetanus toxin, but formaldehyde is now used for this purpose [3]. The evidence of the tetanus vaccine's effectiveness has its origins in the Second World War. Among the soldiers who received two or three doses of vaccine and post-wound booster doses, only 12 cases of tetanus occurred among 2.73 million wounded soldiers (0.44 per 100,000) compared with 70 cases out of 520,000 wounded soldiers during the First World War (13.4 per 100,000) [12]. Similarly, among vaccinated British soldiers, the number of tetanus cases was four to nine per 100,000 injured soldiers, and disease occurred in the soldiers who avoided vaccination compared with 22 to 147 cases per 100,000 soldiers that occurred before introduction of the tetanus vaccination [13]. The success of tetanus vaccines in preventing tetanus during the Second World War was the inspiration for efforts to prevent neonatal tetanus in children from poor regions of the world. The first study was carried out in 1959 in New Guinea. The effectiveness of three doses of vaccine was 94% [14]. A double-blind, randomized, controlled clinical trial in rural Colombia showed that two doses of tetanus vaccine administrated to pregnant women provided tetanus neonatorum protection for more than 4 years. Neonatal tetanus did not occur in the children of mothers who received a minimum of two doses of the vaccine, whereas among the children of nonvaccinated mothers, the mortality rate was 78 per 1000 live births [15].

Pertussis component

Whole-cell pertussis vaccine (DTwP)

DTwP vaccines are suspensions of inactivated *B. pertussis* cells. The DTwP vaccines were first licensed in 1914 in the USA (to the Massachusetts Public Health Biological Laboratories), and in 1948 they became available

in combination with diphtheria and tetanus toxoids as the diphtheria-whole-cell pertussis and tetanus (DTwP) vaccine [16].

In the 1930s, many forms of vaccines were studied. Several different methods of vaccine preparation were tested, including washed or unwashed whole-cell preparations, mixed vaccines containing other bacteria from the upper respiratory track flora, fractionated vaccines (extracted vaccines), detoxified vaccines and vaccines enriched with 'toxic factors' [17].

It was demonstrated that good antibody response and clinical protection after pertussis immunization depended on the number of *B. pertussis* cells in a single dose of vaccine [17]. To improve pertussis vaccines, the number of *B. pertussis* cells in the vaccine was increased, standardized culture media were used, 'gentler' inactivating methods were applied and fresh, rapidly growing Phase I organisms were used as the inoculum [3].

Due to a lack of laboratory tests available for pertussis vaccine standardization, early pertussis vaccines were evaluated on the basis of clinical trial results [17].

One of the first clinical trials of the DTwP vaccine was conducted by Madsen during two epidemics of pertussis in 1923 and 1924 in the Faroe Islands [18]. The vaccine used in this study, prepared by the Danish Serotherapeutic Institute in Copenhagen, consisted of freshly isolated *B. pertussis* culture that had been grown for 48 h in Bordet-Gengou medium and suspended in phenolyzed saline at a concentration of 10 billion cells per ml. Madsen demonstrated some degree of protection in vaccine recipients [18,19]. The study showed that pertussis vaccine not only protected against whooping cough but also ameliorated the severity of disease in immunized persons [3,19].

In 1932, Kendrick and Eldering started a whooping cough research project in Grand Rapids. They developed and improved methods for growing *B. pertussis*, inactivating it and preparing a safe pertussis vaccine [20]. They produced autogenous pertussis vaccines for local physicians to use as treatment and prevention [21]. Kendrick and Eldering performed carefully controlled animal studies of vaccines to design safer and more potent pertussis vaccines [22]. They inactivated *B. pertussis* cells with thiomersal at cold room temperature for >1 week and performed numerous sterility and safety tests (including injecting the vaccine into their own arms to test it for safety) [20,22]. After being checked for safety, the vaccines were distributed to local physicians. They performed the first large-scale, controlled clinical trial for the pertussis vaccine [21].

In the field trial, conducted in 1934–1935, 1592 children (712 in the vaccinated group and 880 in the control group) were involved. The field trial showed that only four of the 712 vaccinated children had whooping cough (only mild cases), but 45 of the 880 unvaccinated children in the control group (90% of those exposed) contracted pertussis and suffered its full ravages [20]. In 1938, the Michigan Department of Health Biologic Products Division started large-scale pertussis vaccine production for children in Michigan, so until 1940, pertussis vaccine was widespread throughout the country [21].

Implementation of the DTwP vaccine in immunization programs had enormous benefits such as rapid decline in morbidity and mortality from pertussis. However, in some countries in the 1970s and 1980s, due to safety reasons, vaccine coverage decreased drastically, even to 10% (for example, in Japan, Sweden and West Germany), as the annual incidence of pertussis increased dramatically to the level seen before the introduction of pertussis vaccination [23]. In response to apprehension associated with DTwP vaccine safety and the rapid increase in pertussis cases, major efforts were made to develop a safer, acellular pertussis (DTaP) vaccine.

Acellular pertussis vaccine (DTaP)

Several pertussis antigens were the subject of research. Current aP vaccines are based on various combinations of five main *B. pertussis* antigens such as filamentous hemagglutinin (FHA), pertussis toxin (PT), pertactin (PRN) and two fimbria proteins (FIM2 and FIM3) [24].

In Japan during the 1980s, the first DTaP vaccines containing formaldehyde-treated FHA and formalin-inactivated PT (JNIH 6) were initially administered to children aged 2 years and older. The effectiveness of vaccines was evaluated as ranging from 78 to 92% and depending on the number of doses and the ages of the children [25]. The studies were also conducted on a monovalent vaccine containing only pertussis toxin (JNIH 7). Both vaccines were also tested in Sweden. Efficacy after two doses in infants was 69% for the two-component vaccine (FHA and PT) and 54% for the single-component vaccine (PT) [26]. Effectiveness at 71% after three doses of PT vaccine was observed in another clinical trial [27]. In 1981, the DTaP vaccine was introduced in Japan and gradually replaced the DTwP vaccine [23]. The DTaP vaccine had fewer adverse effects and was better tolerated. In 1992, the US Food and Drug Administration approved Japanese aP vaccines for booster immunization [24,28] and in 1996 one of them was introduced for primary infant vaccination [29]. The encouraging results of DTaP vaccines

in Japan stimulated other industrialized nations to design other DTaP vaccines. Many aP vaccines were developed, and they differed from each other in terms of number of components, quantity of each component, method of purification, method of toxin inactivation, adjuvants used and excipients [30].

In the 1990s, many clinical trials in various countries were conducted to assess the effectiveness of acellular vaccines. They were some of the largest and most expensive clinical trials in the history of vaccination. However, it is difficult to compare the obtained results with each other, because the trials applied different methodologies, dose schedules and case definitions. First of all, different DTwP vaccines were used as comparators, which demonstrated variable activity. Second, DTaP candidates represented different antigenic compositions [31,32]. One of the first studies was conducted in Sweden due to long-term suspension of the use of pertussis vaccine. The study qualified 82,892 babies, who were randomized to receive two-, three- and five-component DTaP vaccines or a UK DTwP vaccine. The efficacy of the British DTwP vaccine and the five-component DTaP vaccine against culture-confirmed pertussis with at least 21 days of paroxysmal cough, was similar. It was shown that both vaccines were highly immunogenic for fimbriae antigen. The lower activity of three- and two-component acellular vaccines against mild diseases suggested that fimbriae were important in preventing infection. The study showed no significant differences among the four vaccine groups in the occurrence of serious adverse events. High fever and seizures occurred more frequently after the DTwP vaccine [32]. On the contrary, in Italian studies, a US-licensed DTwP vaccine was proven to be less effective in the prevention of pertussis disease than the five-component DTaP vaccine. The effectiveness of the other two DTaP vaccines in the prevention of disease (paroxysmal coughing lasting over 21 days) was comparable, but differences in the geometric mean antibody were observed. There was a relationship between the amount of antigen per dose and the amount of antibodies produced, but this did not apply to the pertussis toxin [31]. As in the other studies [32], better immunogenicity of genetically inactivated pertussis toxin than chemically detoxified toxin, regardless of the amount of toxin per dose, was observed [31].

In developed countries, DTaP vaccines prevail over DTwP vaccines. DTaP vaccines have completely displaced DTwP vaccines in the USA, Canada, Australia, Europe (except for Poland) and some Asian and Latin American countries and are commonly used in the private sector worldwide [3]. However, WHO data show that in 64% of all countries, DTwP vaccines are still used [33], mostly in low- and middle-income countries [3].

DTaP and DTwP vaccines have different safety profiles. DTwP can elicit more adverse local reactions such as redness, swelling, pain at the injection site and systemic reactions such as fever and persistent crying [34], but no evidence was found for a relationship between the vaccination and serious, long-term sequelae such as convulsions, febrile seizures, encephalopathy, sudden infant death syndrome and Reye's syndrome [35,36].

The transition from DTwP to DTaP vaccines resulted in the reactogenicity issue but contributed to faster waning of vaccine-induced protection. Current licensed DTaP vaccines provide proper short-term protection against whooping cough but do not provide long-term protection against disease when compared with efficacious DTwP vaccines [16]. Studies provide evicence that DTaP vaccines induce an immune response fails to prevent colonization and transmission contributing to the waning of protective immunity in providing a probable explanation for the resurgence of pertussis [37]. The study performed by Olin *et al.* suggested that protection against pertussis lasted for about 5–6 years after the third DTaP dose [38]. Another study suggested that DTaP vaccines provided appropriate protection during the first years of life, which waned rapidly in young children even before the booster dose administrated between 4 and 6 years of age [39]. Pertussis epidemics among highly vaccinated school-age children who received only the DTaP vaccine may have been connected with waning immunity after DTaP vaccination. It is suggested that the main problem with DTaP vaccines is not the waning of memory responses in adolescence but the failure of inducing optimal pertussis immunity at the time of priming [40]. The study performed by van der Lee *et al.* [41] showed that adolescents primed with DTaP vaccines were less protected against pertussis compared with those being primed with DTwP vaccines.

The DTP vaccine currently

It is estimated that in 1995 DTP vaccines were manufactured by 63 producers in 46 countries worldwide [42]. In 2006, over 500 million doses of DTP vaccine were produced globally. More than 100 million doses of DTP are produced by the two largest pharmaceutical concerns, Sanofi Pasteur and GSK [3].

Thiomersal, an ethylmercury preservative, has been used in vaccines since the 1930s to ensure product sterility. Currently, some DTwP vaccines contain only trace amounts of thiomersal (less than 1 μg of mercury), or thiomersal can be included as a component of the vaccine (about 45 μg of mercury in a single human dose). DTaP vaccines do not contain thiomersal in composition [43]. The diphtheria and tetanus toxoids have been produced all over

the world in the same way. For the production of toxoids, a suitable bacterial strain able to produce large amounts of toxin in fermenter culture is used. The next stage is collection, filter sterilization and chemical-thermal detoxification of the toxin. The native toxoid is purified, concentrated, salted, sterilized by filtration and finally adsorbed on the adjuvant (most frequently on aluminum salt, usually aluminum hydroxide or aluminum phosphate). At present, various combinations of the DTaP vaccine have been licensed – for example, with Hib component, inactivated poliovirus component or hepatitis B component. The concentration of toxoid is determined in Lf flocculating units and is defined as the amount of flocculating toxoid with 1 unit of the standard reference diphtheria or tetanus antitoxin. Toxoid purity must meet the requirements of the WHO, which recommends at least 1500 Lf/mg of nondialyzable nitrogen. Assays of toxoid potency are described in the monographs of the European Pharmacopoeia [44]. Vaccines containing toxoid should always be injected intramuscularly, never subcutaneously, and should be stored at refrigerator temperature, but not frozen. In case of freezing, the vaccine should be discarded.

DTaP vaccines were initially used as toddler and school-entry booster vaccinations, then several years later were introduced for primary vaccination [30,45]. The recommendations of the Advisory Committee on Immunization Practices (ACIP) for using DTaP as a fourth and fifth booster dose of pertussis vaccine were introduced in 1992 [46]. According to the ACIP recommendations existing since 2006, all adolescents should receive the dTap vaccine as a booster dose [47]. Currently, in many countries, the primary schedule for tetanus, diphtheria and pertussis vaccination includes five doses of vaccine given in the first 2, 4, 6 and 15 to 18 months of age, with the next dose at the age of 6 years. The dTap vaccination is recommended for pregnant women to protect newborn babies against pertussis before administration of the first dose of the DTP vaccine. Studies of dTap vaccination during pregnancy showed that an infant benefits from high levels of transplacentally derived material antibody, and vaccination during pregnancy provides protection during the greatest risk period, before infant vaccination begins. Many national advisory groups have recommended 'cocooning', the vaccination of mothers and other close contacts of newborns and infants with dTap booster vaccines [47–50].

The future of the DTP vaccine

Improvements in current vaccines or the development of new vaccines that induce potent mucosal defense is needed to provide early life and long-lasting protection of infants, adults and elderly people in response to changing epidemiological situations.

Diphtheria vaccine perspectives & needs

The diphtheria vaccine is highly effective, and the disease is well controlled in countries with high anti-diphtheria vaccination coverage. However, anti-diphtheria antibody level decreases with time, and without booster vaccination, adults may again become susceptible to diphtheria. Booster vaccination of adults is especially important in countries where diphtheria cases have not been reported for many years because of reduced opportunity for natural boosting through subclinical infections [51,52]. The maintenance of a high level of diphtheria antibodies in populations is extremely important because of limited access to diphtheria antitoxin – the key medicine for diphtheria treatment [53].

Recently, the epidemiological situation of *C. diphtheriae* infections has changed, and increasing numbers of infections caused by *C. diphtheriae* strains that do not produce diphtheria toxin (non-toxigenic strains) have been recorded in some countries with high anti-diphtheria vaccination coverage. A significant percentage of these cases have covered invasive infections, including bacteremia, sepsis and endocarditis [2,51,54–57]. Invasive non-toxigenic *C. diphtheriae* infections are related to a high mortality rate, reaching over 40%. The situation of non-toxigenic *C. diphtheriae* infections should be monitored with a focus on invasive infections and the need for a new vaccine that would be effective in the prevention of not only diphtheria but also infections due to non-toxigenic *C. diphtheriae* strains that do not meet the criteria of diphtheria. The currently used anti-diphtheria vaccine contains only diphtheria toxoid and therefore protects against diphtheria toxin action – a key virulence factor for diphtheria disease – but not against infections caused by non-toxigenic strains. An initial study on potential vaccine antigens for preventing non-toxigenic *C. diphtheriae* infections was published [58]. The *in silico* analysis enabled to identify a few *C. diphtheriae* proteins which have revealed antigenic properties, have been localized on the surface of the bacterial cells, have been present in all investigated clinical isolates of *C. diphtheriae*, have had conserved amino-acid sequences and have had a crucial role in colonization of the host.

Tetanus vaccination perspectives & needs

In spite of the fact that tetanus vaccines are very effective, some factors may affect appropriate post-vaccinal immunity. These include too-short intervals between administered doses, the use of subpotent vaccine batches and improper vaccine storage conditions, especially exposure to freezing temperatures, which cause structural damage in adjuvant–antigen bonds and impair their potency [3,59]. Some immunocompromising diseases, as already mentioned, may also lead to insufficient protection after immunization. In spite of the fact that tetanus vaccines are very potent, some unexpected tetanus cases have been reported in previously immunized patients, who had mounted adequate level of antibodies, with no clear reason [60]. Attempts have been made to use novel systems for oral tetanus toxoid delivery and induction of mucosal immunological response [61]. New adjuvants for *C. tetani* toxoid have been invented with a reduction in size to nanoparticles, which enhances the immune response [62], as well as recombinant tetanus vaccines that were proved to confer stronger and more persistent antibody titers in mice, rats and monkeys. Genetically engineered protein compared with the traditional vaccine is non-pyrogenic, is easily purified and may be produced on a large scale with no need to use formaldehyde and the toxin, which also causes fewer side effects [63].

A number of studies have also been conducted to improve the stability of vaccines exposed to freeze–thawing, including tetanus–toxoid-containing ones. Usually, these efforts are achieved by the addition of compounds that are able to stabilize aluminum adjuvant adsorbed proteins, such as cryoprotectants and osmolytes (propylene glycol, glycine, sucrose, polyethylene glycol [PEG] 300 and trehalose) [64]. Glucose, PEG and trehalose used in aluminum hydroxide adsorbed tetanus toxoid vaccines were shown to retain the integrity of adjuvant–antigen bonds after exposure to freezing-induced damage [65].

Pertussis vaccine perspectives & needs

Pertussis has become the most common vaccine-preventable disease [66–68]. Recently, many countries (e.g., the UK, the Netherlands, the USA and Australia) have experienced notable increases in reported pertussis cases [68,69]. Several factors have been suggested to explain the resurgence of pertussis in highly vaccinated populations, including waning vaccine-induced immunity, pathogen adaptation to vaccination, a switch from DTwP to DTaP vaccines, decreased vaccination coverage, decreased vaccine efficacy, increased awareness of pertussis, improved diagnostics and better pertussis surveillance [67,70–72]. Different approaches have been suggested to achieve better pertussis control, including the novel formulation of DTaP vaccines, the addition of new adjuvants or the inclusion of new pertussis antigens to the current aP vaccines, as well as the development of outer membrane vesicle-based vaccines or live-attenuated pertussis vaccines.

Improvements in the DTaP vaccines include novel formulations, the use of Th1- or Th17-driving adjuvants, the addition of new antigens and the inclusion of pertussis antigens with more native structures without chemical modifications [73]. A number of new-generation adjuvants for improving the immunogenicity of pertussis vaccines were tested. The study performed by Polewicz *et al.* [74] showed that novel formulation of DTaP vaccines in microparticles containing polyphosphazene, cationic innate defense regulator peptide and cytosine-phosphate-guanosine oligodeoxynucleotides stimulated immunity faster and higher compared with anti-*B. pertussis* IgA and IgG2a antibodies and provided balanced humoral and cellular immune responses in mice when compared with vaccination with the commercial DTaP vaccine. The study performed by Agnolon *et al.* [75] showed that DTaP antigens adsorbed onto negatively charged poly(lactide-co-glycolide) nanoparticles that were combined with a synthetic immune potentiator molecule targeting Toll-like receptor 7, which resulted in enhanced IgG and IgG2a antibody levels in mice. In the study performed by Dunne *et al.* [76], a novel TLR2-stimulating lipoprotein from *B. pertussis* was used as an adjuvant in DTaP vaccines (replacing alum) and demonstrated enhanced Th1, Th17 and IgG2a immune responses. In the study performed by Agnolon *et al.* [75], two adjuvants, MF59 emulsion adjuvant and the combination of aluminum hydroxide and the Toll-like receptor 4 agonist MPLA, were tested. The results showed that both of them stimulated IgG2a/Th1 and neutralized responses in a mouse model [75].

Several new putative protective antigens have been explored. Possible new vaccine antigens include the adenylate cyclase toxin, iron-regulated *B. pertussis* proteins (especially proteins called IRP1-3 and AfuA) and the serum-resistance autotransporter protein BrkA [77]. Studies in a mouse model showed that a recombinant form of adenylate cyclase lacking enzymatic activity increased protection against an intranasal challenge of *B. pertussis* [78]. Immunization of mice with iron-regulated protein IRP1-3 induced protection against *B. pertussis* challenge [79]. Similarly, a second highly antigenic iron-regulated protein, called AfuA, also conferred protection against *B. pertussis* infection in mice [80]. The studies in a murine model showed that BrkA applied alone did not provide significant

Table 1. Pertussis experimental vaccines in clinical trials.

Name of potential vaccine	Locations	Clinical trial phase	Participants	Outcome measures	Status
BPZE1	Karolinska University Hospital Huddinge, Stockholm, Sweden	Phase Ia, single-center, dose-escalating and placebo-controlled study	48 participants, healthy adult male volunteers	General safety and local tolerability in the respiratory tract of a single ascending dose of the genetically modified *Bordetella pertussis* strain	Completed
	Karolinska University Hospital Huddinge, Stockholm, Sweden	Phase Ib, single-center, dose-escalating and placebo-controlled study	54 participants, healthy adult volunteers	The safety and immunogenicity of a higher dose formulation of the genetically modified *B. pertussis* strain	Completed
	Vanderbilt University–Pediatric–Vanderbilt Vaccine Research Center Nashville, TN, USA	Phase IIa, single-center, randomized, partially blind and placebo-controlled	50 participants, healthy adults, ages 18–49 years	The safety, tolerability and humoral immunogenicity of a single intranasal dose of either 10^7 or 10^9 colony-forming units of lyophilized vaccine	Completed
	Rapid Medical Research, Inc., Cleveland, OH, USA; DM Clinical Research, Tomball, TX, USA; Advanced Clinical Research, Inc., West Jordan, UT, USA	Phase IIb, multi-center, randomized and placebo-controlled	300 participants, healthy adults	Immunological response and safety profile of 1-dose (prime) and 2-dose (prime + boost) schedules compared with a Boostrix™ prime dose with or without a BPZE1 boost dose	Completed
Viaskin PT	Center for Vaccinology Medical Faculty UNIGE and University of Geneva (HUG), Switzerland	Phase I, randomized, double-blind and placebo-controlled	102 participants, adult male or female, ages 18–40 years	The safety and immunogenicity of a genetically detoxified pertussis toxin (PT)	Completed
Pertagen®	Pediatric Clinical Trial Platform, University Hospitals of Geneva City, Geneva, Switzerland	Phase II, randomized, double-center and observer-blind controlled pilot vaccine trial	60 participants, adolescents ages 11–15 years	The immunogenicity of the genetically detoxified pertussis toxin	Completed

protection against nasal *B. pertussis* infection. The supplement of BrkA protein to pertussis vaccine contained other *B. pertussis* antigens such as FHA and PT. The supplement of BrkA protein to current vaccines enhanced protection against colonization of respiratory mucosa after nasal *B. pertussis* challenge [81]. It was shown that genetically detoxified PT is more immunogenic than chemically detoxified PT, inducing higher neutralizing antibody titers and a Th1/Th17 response [82]. Genetically detoxified pertussis toxin (vaccine experimental name: Pertagen) was produced with a recombinant *B. pertussis* strain that was inactivated by the introduction of mutations (Arg9Lys and Glu129Gly) in the ptx operon of the S1 gene. The Global Clinical Trials website [83] provides information about clinical trials of genetically detoxified PT vaccines (details are given in Table 1). The other types of experimental vaccines (except live nasal vaccine) described in this article are only at the preclinical stage.

Outer membrane vesicles (OMVs), containing naturally incorporated bacterial surface antigenic proteins from *B. pertussis*, have been shown to be safe and highly protective against *B. pertussis* challenge in mouse models [84]. The safety profile of OMVs derived from *B. pertussis*, as evaluated in mouse models, seems to be comparable to that of DTaP vaccines, and much better than DTwP vaccines [85]. The study in a murine model performed by Raeven *et al.* [86] showed that vaccination with OMVs induces a milder inflammatory response, but with protection from bacterial colonization equal to that of DTwP. Fernandez *et al.* [87] found that a proteoliposome formulation derived from *B. pertussis* induces protection in 90% of mice against lethal infection with *B. pertussis* and reaches total clearance of bacteria after intracerebral and intranasal challenge. OMV-based vaccine is able to induce an immune response with a mixed Th1/Th17 and Th2 profile with robust humoral response [84]. Furthermore, OMV-based vaccine was shown to protect against genetically different *B. pertussis* strains, including those not expressing pertactin [88].

BPZE1 live-attenuated nasal *B. pertussis* vaccine was constructed by the genetic inactivation or removal of three *B. pertussis* major toxins: pertussis toxin, dermonecrotic toxin and tracheal cytotoxin [89]. The mouse lung colonization ability of BPZE1 was similar to that of its parent strain, with persistence for 3–4 weeks. In contrast to its virulent parent strain, BPZE1 does not induce lung pathology in mice during persistent colonization after nasal administration [89,90]. The genetic stability of BPZE1 was evaluated after serial *in vitro* and *in vivo* passages [91]. The safety of BPZE1 vaccine was confirmed in several preclinical models [92].

Studies in a murine model have provided evidence of protection against challenge after a single nasal vaccination [93]. It was shown that a single administration of BPZE1 induced comparable antibody responses to those seen after two parenteral administrations of the DTaP vaccine [89]. Nasally administrated BPZE1 vaccine induces both mucosal and systemic immune responses, which causes rapid and broader immunity than parenteral vaccine administration. It was shown that BPZE1-induced protection against lung colonization after *B. pertussis* challenge is long-lasting, significantly higher than that of DTaP vaccine [94], and provides rapid protection compared with DTaP vaccines [92]. BPZE1-induced protection in mice is vaccine dose-dependent. Anti-*B. pertussis* antibody titers, production of antigen-specific IFN-γ and colony-forming unit (CFU) reduction after challenge were directly correlated with the dose of the vaccine [93]. It was revealed that BPZE1 also protects against *B. bronchiseptica* [95].

A study performed in a baboon model of pertussis demonstrated that BPZE1 vaccine transiently colonizes the nasopharynx [84]. BPZE1 vaccine not only prevents pertussis disease in animals but also *B. pertussis* colonization, which is similar to the protection observed after recovering from pertussis. It was shown that colonization by the virulent strain in baboons vaccinated with BPZE1 vaccine was reduced by 99.998%, compared with the unvaccinated baboons [90]. This feature is very important because DTaP and DTwP vaccines do not prevent colonization by the bacteria.

A study in human preclinical *ex vivo* models showed that BPZE1 promotes human dendritic cell CCL-induced migration and drives a Th1/Th17 response [96].

Human clinical trials demonstrated that BPZE1 vaccine is safe, and now Phase II of clinical trials has been completed [83] (details are given in Table 1). The BPZE1 vaccine is able to transiently colonize the human nasopharynx and induce immune responses to several *B. pertussis* antigens in colonized individuals [97,98].

Conclusion

The long history of the DTP vaccine reveals its safety and effectiveness, but the changing epidemiological situation over the years has to be related to new challenges. The pathogens are changing in response to vaccination by producing new virulence factors or avoiding the production of factors that are present in vaccines as antigens. On the other hand, thanks to the success of the vaccinations that took place many years ago, the opportunity for natural boosting through subclinical infections has been extremely reduced in many populations, resulting in decreasing specific antibody levels with age. Moreover, the lack of serious cases of infectious disease in everyday life, successfully prevented by vaccination, has resulted in greater fear of vaccination than fear of the infectious diseases. The facts suggest that we will never be free of infectious diseases. The changeability of pathogens should be monitored continuously. Also, studies should be conducted on new vaccines that will be effective and that will not raise concerns about safety and ethical aspects of production and testing.

Future perspective

Most scientists think that the current delivered DTaP vaccine needs to be supplemented or replaced with a more effective pertussis vaccine. A lot of effort has been made to understand the mechanisms of protective immunity against *B. pertussis*, thanks to which it is possible to predict success with the pertussis experimental vaccines. The development of new-generation technologies measure antigen-specific cell subpopulations, while cytokine secretion offers high hopes for a rapid expansion of research into new potential vaccines. The phenomenon of waning immunity after DTaP vaccination, the inability to eliminate colonization and the evolution of *B. pertussis* require the improvement of pertussis vaccines. The improvement of pertussis vaccines should be focused on activating prolong effector memory B-cells and generating Th1/Th17 T-cells. Additionally, it is known that the current DTaP vaccines fail to generate CD4 T-cells and that they do not induce mucosal IgA in the lung and nasal tissue. Most vaccines are delivered by the parenteral route, mostly intramuscular. It will be more effective to replace the current vaccine administered intramuscularly with a nasally delivered pertussis vaccine, which promotes protective immunity in the nasal mucosae. It is known that pathogens infecting mucosal surfaces require effective local immunity. Due to the above, an ideal future pertussis vaccine would be administered intranasally and induce mucosal immunity, including pertussis-specific IgA. Protection would be conferred against lung disease and subsequent transmission. In the near future, the most obvious strategies involve a live pertussis vaccine. The nasal BZPE1 vaccine is now under investigation; Phase II of clinical trials has been completed [83]. It is also important that the production of a live pertussis vaccine be cheaper than production of the currently used vaccine containing purified antigens [99]. This could have a big impact in the future on its distribution to low-income countries. Vaccinologists are looking forward to the next phases of clinical trials for this vaccine. In the initial phases of clinical trials, there are now

potential vaccines containing genetically inactivated PT [83]. Other proposals for new pertussis vaccines, which, for example, are to use adjuvants or antigens that better stimulate innate immunity, are only at the preclinical stage. In our opinion, for the next 5–10 years, the implementation of new vaccines in the primary immunization program will not be easy. It will be logistically difficult due to the fact that the DTaP vaccine is administered together with other antigens such as polio, *Haemophilus influenzae* or hepatitis B. Probably, at the beginning, new DTP vaccines will be implemented within the booster vaccination program. However, the biggest challenge is to introduce a primary dose of the DTP vaccine that will induce long-term cellular response in neonates. Marketing authorization of the vaccine takes many years of preclinical and clinical study. Then a consensus is needed among vaccine manufacturers, regulators and scientists to standardize the technical aspects of control of the vaccine. On the other hand, the efficacy of the diphtheria and tetanus components of the DTP vaccine remains high. However, due to the increasing number of infections caused by nontoxigenic *C. diphtheriae* [51,54], studies concerning the development of a vaccine based on *C. diphtheriae* antigens other than diphtheria toxoid will be intensified.

Executive summary

Development & introduction of diphtheria–tetanus–pertussis vaccine

- The individual antigens of the diphtheria–tetanus–pertussis (DTP) vaccine had long been as monovalent vaccines.
- The DPT vaccine was combined in 1943 and registered for the first time in 1948 in the USA.
- The diphtheria vaccine contains diphtheria toxoid, a detoxified form of diphtheria toxin.
- The tetanus vaccine contains tetanus toxoid, a detoxified form of tetanus toxin.
- Two types of pertussis vaccine are available: whole-cell vaccines based on inactivated *Bordetella pertussis* bacilli and acellular pertussis vaccines based on one or several highly purified pertussis proteins.
- In many countries, within 10 years after the introduction of DPT vaccination, morbidity and mortality from diphtheria disease, tetanus disease and whooping cough decreased significantly in all age groups.

Why the DTP vaccine is important

- The DTP vaccine is one of the oldest vaccines used in humans.
- It prevents three serious, potentially fatal diseases that affect children and adults.
- The DTP vaccine is currently the most commonly administered vaccine in children worldwide.

Pertussis resurgence

- Whooping cough is still recognized as a major public health issue, despite the fact that pertussis immunoprevention has been available.
- Several reasons have been suggested for the return of pertussis disease in highly vaccinated populations: the switch from whole-cell pertussis vaccine to acellular pertussis vaccine associated with waning vaccine-induced immunity and the appearance of antigenically different clinical strains associated with pathogen adaptation to vaccination.
- The phenomenon of waning immunity after immunization with acellular vaccine, the inability to eliminate the colonization and evolution of *Bordetella pertussis* require the improvement of pertussis vaccines.

Challenges & future directions

- Different approaches have been suggested to achieve better pertussis control, including novel formulation, the addition of new adjuvants or new pertussis antigens and the development of outer membrane vesicle-based vaccines and live-attenuated pertussis vaccines.
- In clinical trials there is a live nasal pertussis vaccine called BPZE1, which protects against the colonization and transmission of bacteria and inducing pertussis-specific IgA.
- The medical community hopes deeply of introduction of this nasally administered vaccine for use as soon as possible.
- The DTP vaccine's long history reveals its safety and effectiveness, but the changing epidemiological situation has to be related to new challenges.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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