Review Article

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Next-generation Pertussis Vaccines with Special Focus on Intranasal Vaccination

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ABSTRACT

Pertussis is a highly contagious respiratory disease caused by the gram-negative bacterium Bordetella pertussis (Bp). The disease is most severe in infants and young children, whereas adolescents and adults typically experience milder symptoms but serve as important reservoirs for transmission. Despite widespread vaccination efforts, pertussis continues to pose a significant public health challenge. Historically, the first generation of pertussis vaccines, formulated as inactivated whole cell pertussis (wP) vaccines, were associated with notable side effects, prompting the development of safer acellular pertussis (aP) vaccines. The second generation of pertussis vaccines contains purified components of Bp and provides protection comparable to that of the older whole-cell vaccines. However, recent studies have reported a resurgence of pertussis, attributed to several factors, including improved diagnostic methods, waning immunity following vaccinations, and the emergence of antigenically divergent or vaccine-adapted strains. To address these challenges, researchers are developing next-generation pertussis vaccines using various approaches, such as transitioning from intramuscular to intranasal administration, formulating outer membrane vesicle (OMV)-based vaccines, designing live attenuated pertussis vaccines, and exploring nucleic acid-based vaccines and novel adjuvants aimed at inducing long-lasting mucosal and systemic immunity. This review primarily focuses on assessing the efficacy of the next-generation intranasally administered pertussis vaccines in both pre-clinical and clinical settings.

Keywords: Bordetella pertussis, Whooping cough, Pertussis vaccine, Acellular vaccine, Intranasal vaccine

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INTRODUCTION

Pertussis, commonly referred to as whooping cough, is a highly contagious respiratory disease caused by the gram-negative bacterium Bordetella pertussis (Bp) (1, 2). The most severe and potentially fatal cases occur primarily in infants and young children (3). In contrast, adolescents and adults often present with mild or asymptomatic infections, yet they play a critical role in the transmission of Bp within the population (4). Pertussis spreads primarily via respiratory droplets expelled during coughing or sneezing by infected individuals (5).

Today, despite the widespread global vaccination efforts, pertussis remains inadequately controlled. In 2019, an estimated 19.5 million cases were reported worldwide, resulting in approximately 117,000 deaths among affected individuals (6).

Following the isolation of BP in pure culture by Jules Bordet and Octave Gengou in 1906, the first generation of pertussis vaccines, formulated as inactivated whole cell pertussis (wP) vaccines, were licensed in the United States in 1914 (7). These vaccines became publicly available in combination with diphtheria and tetanus toxoids in 1948 (Fig. 1) (7, 8). The introduction of the combined Diphtheria, Tetanus, and whole-cell Pertussis (DTwP) vaccine in the United States led to a dramatic decline in pertussis incidence, decreasing from an estimated 115,000–270,000 cases annually in the pre-vaccine era to approximately 1,200–4,000 cases per year by the 1980s (8). The whole-cell DTwP vaccine has played a pivotal role in controlling pertussis infections globally (9, 10). However, studies conducted during the 1970s and 1980s identified several adverse reactions associated with its use in infants, including high fever, persistent crying, febrile seizures, whole limb swelling, and hypotonic hyporesponsive episodes (HHE) (8). In response to these safety concerns, most developed countries transitioned from the DTwP to the acellular pertussis vaccine (DTaP) during the 1990s (11-13). The second generation of pertussis vaccine, known as (DTaP), contains diphtheria toxoid, tetanus toxoid, and one to five components derived from the virulence factors of Bordetella pertussis. These components include Pertussis toxoid (PT), pertactin (PRN), filamentous hemagglutinin (FHA), and fimbriae serotypes 2 and 3 (FIM 2 and 3) (14-16).

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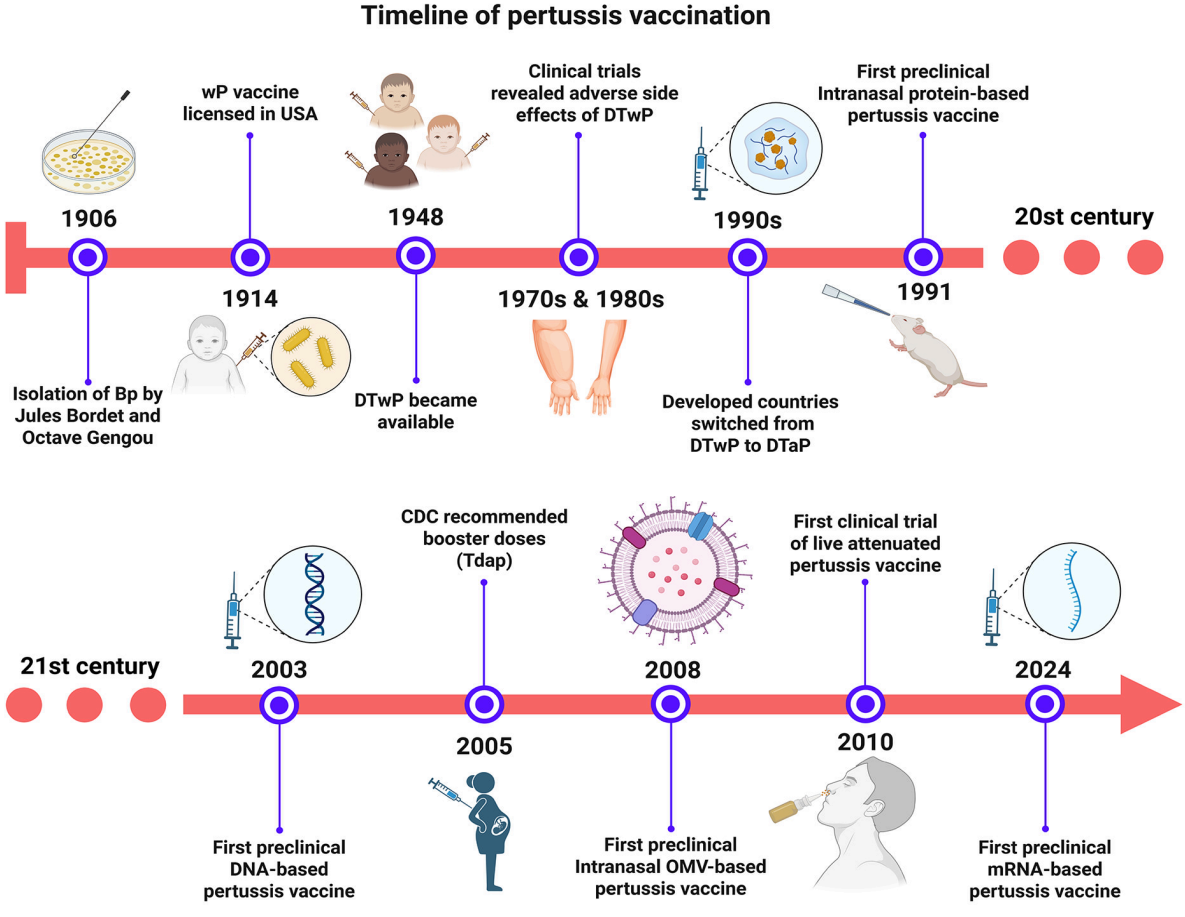

Fig. 1. Timeline of major milestones in pertussis vaccine development, beginning with the isolation of Bordetella pertussis in 1906 and extending through the introduction of whole-cell (DTwP) and acellular (DTaP) vaccines, as well as advances in next-generation pertussis vaccines in the 21st century.

Research on the effectiveness of whole-cell and acellular pertussis vaccines shown that immunity against pertussis persists for over 15 years in convalescent individuals and approximately 10 years in those vaccinated with the whole-cell formulation. In contrast, individuals vaccinated with the acellular pertussis vaccine typically maintain protection for only 3 to 5 years, reflecting a pattern of waning immunity (17). Consequently, in 2005, the Centers for Disease Control and Prevention (CDC) incorporated booster doses of the acellular pertussis vaccine (Tdap) into the immunization schedule for adolescents and pregnant women. The CDC also recommends Tdap administration to healthcare workers and adults every ten years (18, 19). Despite widespread vaccine coverage and the implementation of booster strategies since 2005, epidemiological studies across multiple countries have documented a resurgence in pertussis incidence with several outbreaks reported over the past two decades (20-23).

The resurgence of pertussis may be attributed to multiple contributing factors. First, advancements in diagnostic techniques have enhanced case detection. Second, the lack of booster doses in the immunization schedules of many developing countries has led to diminished population-level immunity. Third, protective immunity conferred by the DTaP vaccine wanes over time. Fourth, DTaP and Tdap vaccines may not induce robust mucosal immunity in respiratory tract, permitting Bp colonization. This increases the prevalence of asymptomatic carriers and facilitates community transmission, thereby creating opportunities for Bp to mutate and evade immune responses targeting acellular vaccine antigens. Fifth, the effectiveness of DTaP and Tdap vaccines is reduced against emerging Bp strains that lack key antigens (24, 25).

The forthcoming third generation of pertussis vaccines is currently undergoing pre-clinical and clinical evaluation and has not yet been released for public use. These vaccines are designed to overcome the safety concerns and limited duration of protection associated with earlier formulations (26). To achieve this, researchers have explored several innovative strategies, including altering the route of administration from intramuscular (IM) to intranasal (IN), incorporating novel adjuvants into existing acellular pertussis vaccines, and developing outer membrane vesicle-based and live attenuated pertussis vaccines to promote durable mucosal immunity in the respiratory tract (27). In the subsequent sections of this review, we will focus on the pre-clinical and clinical studies of intranasally administered next-generation pertussis vaccines, as well as recent advances in nucleic acid-based BP vaccine platforms.

Intranasal Protein-based Pertussis Vaccines

Currently available acellular pertussis vaccines (DTaP and Tdap), administered intramuscularly with alum adjuvant, elicit a strong systemic IgG response and a Th2-biased immune profile. However, they do not induce secretory IgA (sIgA) or Th1/Th17 tissue-resident memory (TRM) cells the respiratory mucosa, which are critical for mucosal immunity against Bp (6, 27). However, they do not induce secretory IgA (sIgA) or Th1/Th17 tissue-resident memory (TRM) cells in the respiratory mucosa, which are critical for mucosal immunity against Bordetella pertussis (Bp) (6, 27). As a result, while DTaP and Tdap vaccines are effective in preventing severe clinical manifestations of pertussis, they fail to prevent bacterial colonization of the upper respiratory tract and do not block asymptomatic transmission of Bp (28). To overcome these limitations, numerous pre-clinical studies have investigated alternative strategies. These include shifting the route of administration from IM to IN and replacing alum with novel adjuvants that promote Th1- and Th17 responses, thereby enhancing durable protective immunity in the respiratory mucosa.

The first investigation into the intranasal administration of protein-based pertussis vaccines was conducted by Lipscombe et al. in 1991.

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Table 1. Summary of recent preclinical studies (2020-2025) investigating intranasal delivery of protein-based pertussis vaccines.

Study No.	Antigens	Intranasal adjuvants	Experimental groups (except control groups)	Animal	Main findings	References
1	-PT -PRN -FIM 2/3	-TriAdj (Poly I:C+IDR1002+ PCEP) -Lipid nanoparticle (DDAB + DOPE)	-Ags only (IN) -Ags + TriAdj (IN) -Ags + TriAdj + lipid nanoparticle (IN)	BALB/C	- The highest level of serum IgA and serum IgG and sIgA in nasal wash against PRN and PT was seen in Ags + TriAdj + lipid nanoparticle (IN) group compared to the other groups.	(31)
2	-PT -PRN -FHA	-c-di-GMP -2',3'-cGMP	-Ags + Alum (IP) -Ags (IN) -Ags + 2',3'-cGMP (IN) -Ags + c-di-GMP (IN)	BALB/C	- The serum level of IgG against PRN, FHA and PT antigens was higher in the Ags + c-di-GMP (IN) and Ags + Alum (IP) groups than the other groups. - The level of sIgA in nasal wash and BALF against PRN, FHA and PT antigens in the Ags + c-di-GMP (IN) group was higher than the other groups. - Ags + c-di-GMP (IN) group displayed stronger Th1 and Th17 responses, whereas Ags + Alum (IP) induced stronger Th2 response in spleen and lung than other groups. - The lowest amount of CFU was observed in nasal homogenate of Ags + c-di-GMP (IN) group.	(32)
3	-PT -PRN -FHA	-Alum -Curdlan -IRI1501	-Convalescent -Ags (IN) -Ags + Alum (IN) -Ags + curdlan (IN) -Ags + IRI1501 (IN)	BALB/C	- There was a significant decrease in the amount of CFU in the lungs and nose of all groups compared to the control group, but there was no significant difference between the vaccinated groups. - Serum IgG and IgA levels in nasal wash against whole Bp in Ags + Alum (IN) and Ags + IRI1501 (IN) groups were higher than other groups. - The level of IL-17 after bacterial challenge was higher in the lung homogenate of the Ags + IRI1501 (IN) and Ags + Alum (IN) groups than the other groups. - The CFU count after bacterial challenge was lower in Ags + Alum (IN), Ags + IRI1501 (IN) and Convalescent groups compared to the other groups.	(33)

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Study No.	Antigens	Intranasal adjuvants	Experimental groups (except control groups)	Animal	Main findings	References
4	-PT -PRN -FHA	-Alum	-Whole cell pertussis (IM) -Ags + Alum (IM) -Ags + Alum (IN) -Ags + Alum (Oral)	Sprague-Dawley rats	- Serum IgG levels against PT increased only in Ags + Alum (IN) and Ags + Alum (IM) groups, whereas IgA was increased in the lung of the Ags + Alum (IN) group. - All vaccinated groups displayed lower CFU count in lung homogenate and nasal wash after bacterial challenge.	(34)
5	-PT -PRN -FHA	-Alum -BcfA	- Ags + Alum (IM priming; IM boosting) - Ags + Alum + BcfA (IM priming; IM boosting) -Ags + Alum (IM priming; IN boosting) -Ags + Alum + BcfA (IM priming; IN boosting)	C57BL/6J	- The Th1 response in the lungs and the Th17 response in the lungs and nose of the Ags + Alum + BcfA group (IM priming; IN boosting) were stronger than the other groups. - The level of pulmonary IgA against PRN, FHA and PT antigens in the Ags + Alum + BcfA group (IM priming; IN boosting) was higher than the other groups. - The CFU count in the nasal homogenate in the Ags + Alum + BcfA group (IM priming; IN boosting) was lower than the other groups.	(35)
6	-PT -PRN -FHA	-Alum -BECC438b	- Ags + Alum (IM) - Ags + Alum + BECC438b (IM) -Ags + Alum (IN) -Ags + Alum + BECC438b (IN)	CD-1 mice outbred	- The CFU count in the lung 3 days post-challenge was the lowest in group Ags + Alum + BECC438b (IN). - The serum levels of IgG against Bp, FHA and PRN were higher in group Ags + Alum + BECC438b (IM) than the other groups.	(36)
7	-PT -FHA -Recombinant PRN (rPRN) -Recombinant adenylate cyclase (RTX) -rPRN-RTX	Alum	- Ags + Alum (IN) - Ags + rPRN + Alum (IN) - Ags + RTX + Alum (IN) - Ags + rPRN + RTX+ Alum (IN) - Ags + rPRN-RTX+ Alum (IN)	BALB/C	- Serum IgG titer against PRN increased in the Ags + rPRN + Alum, Ags + rPRN + RTX + Alum, and Ags + rPRN-RTX + Alum groups. - Saliva IgA titer against PRN increased in the Ags + rPRN + RTX + Alum and Ags + rPRN-RTX + Alum groups. - Serum IgG and saliva IgA titers against RTX increased in the Ags + RTX + Alum, Ags + rPRN + RTX + Alum, and Ags + rPRN-RTX + Alum groups. - While the CFU counts in the lungs of the Ags + rPRN + Alum, Ags + rPRN + RTX + Alum, and Ags + rPRN-RTX + Alum groups were significantly reduced, it was decreased in the nose of only the Ags + rPRN-RTX + Alum group.	(37)

TriAdj, triple adjuvant (Poly I:C+IDR1002+PCEP); Poly I:C, Polyinosinic:polycytidylic acid; IDR1002, innate defense regulator peptide 1002; PCEP, Poly[di(sodium carboxylatoethylphenoxy)phosphazene]; DDAB, Dimethyldioctadecylammonium bromide; DOPE, Dioleoylphosphatidylethanolamine; c-di-GMP, Cyclic di-Guanosine Monophosphate; 2',3'-cGMP, 2′,3′-cyclic Guanosine Monophosphate; IRI1501, purified whole beta-glucan particles; BcfA, Bordetella colonization factor A; BECC438b, modified lipid A structure designed as a TLR4 agonist; RTX, recombinant adenylate cyclase; Alum, aluminum-based adjuvant; Ags, antigens; IN, intranasal; IP, intraperitoneal; IM, intramuscular; BALB/C, Bagg albino laboratory-bred substrain c; C57BL/6J, C57 black 6 Jackson laboratory substrain; CD-1, Cesarean derived 1; IgG, immunoglobulin G; sIgA, secretory immunoglobulin A; BALF, bronchoalveolar lavage fluid; CFU, colony forming unit; TH1, T helper 1 cells; TH2, T helper 2 cells; TH17, T helper 17 cells; IL-17, interleukin-17; IFN-g, interferon-gamma.

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In this study, the researchers genetically fused the gene encoding the B subunit of the Escherichia coli heat-labile enterotoxin (LTB) with the gene encoding PRN from Bp. The resulting recombinant fusion protein was expressed and formulated as a vaccine, which was subsequently administered intranasally to female BALB/c mice to assess its immunogenicity (29). A follow-up study by Shahin and colleagues in 1992 involved the administration of FHA from Bp via nasal and oral routes to mice. The study aimed to assess the immune response induced by the vaccine and its protective efficacy against pertussis challenge (30). Pre-clinical research in this area havs continued to date, and key findings from both recent and earlier studies are summarized in Tables 1 and 2, respectively.

Intranasal Live Attenuated Pertussis Vaccines

One promising strategy to induce durable mucosal immunity in the respiratory tract is the intranasal administration of the wP vaccine, which mimics the natural infection route of Bp. In a preclinical study conducted by Berstad et al in 1999, a killed wP vaccine was administered intranasally to 6 adult human volunteers (43-45). The study reported robust immunogenicity, with high titers of serum IgG and nasal sIgA specific to Bp antigens. Additionally, antigen-specific T cells were detected in the PBMC of vaccinated individuals. However, one participant experienced epistaxis (nosebleed) following vaccination, which may have contributed to the discontinuation of intranasal wP vaccine administration in subsequent clinical trials.

Table 2. Summary of earlier preclinical studies (before 2020) on intranasal protein-based pertussis vaccines.

Study No.	Antigens	Intranasal adjuvants	Experimental groups (except control groups)	Animal	Main findings	References
1	-PT -PRN -FHA	-c-di-GMP -LP1569	-Ags + LP1569 (IP) -Ags + c-di-GMP (IP) -Ags + LP1569 + c-di-GMP (IP) -Ags + Alum (IP) -Ags + LP1569 + c-di-GMP (IN)	C57BL/6J	- The lowest bacterial colonization rate in nose after pertussis challenge was seen in Ags + LP1569 + c-di-GMP (IN) group. - The lowest bacterial colonization rate in lung after pertussis challenge was seen in Ags + LP1569 + c-di-GMP (IP) group. - The levels of IL-17 and IFN-g against FHA, PRN and whole Bp antigens in the spleen of Ags + LP1569 + c-di-GMP (IN) and Ags + LP1569 + c-di-GMP (IP) groups were higher than the other groups. - The level of pulmonary IgA against PRN was higher in the Ags + LP1569 + c-di-GMP (IN) group, whereas IgG2c was higher in the Ags + LP1569 + c-di-GMP (IN) and Ags + LP1569 + c-di-GMP (IP) groups compared to the other groups.	(38)
2	-PT -FHA	Recombinant B subunit of cholerae toxin (rCTB)	-Ags (IN) -Ags + rCTB (IN)	BALB/C	- Serum IgE titer against PT was higher in Ags + rCTB (IN) group. - The CFU count was undetectable in the Ags (IN) group later than the Ags + rCTB (IN) group post-challenge.	(39)

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Study No.	Antigens	Intranasal adjuvants	Experimental groups (except control groups)	Animal	Main findings	References
3	-PT -PRN -FHA	-Alum -Bacterium-like particle (BLP)	-Ags (IP) -Ags + BLP (IP) -Ags + Alum (IP) -Ags (IN) -Ags + BLP (IN) -Ags + Alum (IN)	BALB/C	- The level of serum IgG against PRN, FHA and PT antigens in the Ags + Alum (IP) group was higher than the other groups. - The level of IgA against FHA and PT in nasal wash was higher in Ags + BLP (IN) group than other groups. - The Ags + BLP (IN) group displayed the lowest CFU count in the lung homogenate.	(40)
4	-PT -PRN -FHA	-Alum -Curdlan	-Ags + Alum (IP) -Ags + Alum + Curdlan (IP) -Whole cell pertussis (IP) -Ags + Alum (IN) -Ags + Alum + Curdlan (IN)	CD-1 mice outbred	- The level of IgA in the lung was higher in the Ags + Alum + Curdlan (IN) and Ags + Alum (IN) groups than in the other groups. - The level of IgA in the nose was increased only in the Ags + Alum (IN) group. - In lung homogenate and nasal wash, the lowest CFU count was associated to the whole cell pertussis (IP), Ags + Alum (IP) and Ags + Alum (IN) groups.	(41)
5	-PT -FHA	-Alum -N-trimethyl chitosan	-Ags (IN) -Ags + Alum (IP) -Ags + N-trimethyl chitosan (IN)	BALB/C	- The levels of serum IgG against PT and FHA antigens in the Ags + Alum (IP) group and IgA in nasal wash in the Ags + N-trimethyl chitosan (IN) group were higher than the other groups. - The expression levels of IL-17 and IFN-g against PT and FHA antigens in supernatant of cultured spleen cells of the Ags + N-trimethyl chitosan (IN) group and IL-4 in the spleen of the Ags + Alum (IP) group were higher than the other groups.	(42)

c-di-GMP, Cyclic di-Guanosine Monophosphate; LP1569, synthetic TLR2 agonist based on the lipoprotein BP1569 from Bp; Alum, aluminum-based adjuvant; BLP, bacterium-like particle; Ags, antigens; IN, intranasal; IP, intraperitoneal; IM, intramuscular; BALB/C, Bagg albino laboratory-bred substrain c; C57BL/6J, C57 black 6 Jackson laboratory substrain; CD-1, Cesarean derived 1; IgG, immunoglobulin G; sIgA, secretory immunoglobulin A; BALF, bronchoalveolar lavage fluid; CFU, colony forming unit; TH1, T helper 1 cells; TH2, T helper 2 cells; TH17, T helper 17 cells; IL-17, interleukin-17; IFN-g, interferon-gamma.

An alternative approach for intranasal pertussis vaccination involves the use of live attenuated Bp strains that either lacks key virulence factors or has been inactivated. In 2006, Locht and colleagues successfully engineered an attenuated variant of Bp known as BPZE1 by inactivating or deleting three major toxins from the BPSM parental strain (46). BPZE1 was engineered by deleting the gene encoding dermonecrotic toxin, replacing the mutated and enzymatically inactive pertussis toxin gene, and substituting the Bp ampG gene with its functional counterpart from Escherichia coli. These genetic modifications significantly reduced the overall toxicity of BPZE1 compared to wild-type Bp strains (47). Tracheal cytotoxin (TCT), a degradation product of peptidoglycan found in the cell wall of gram-negative bacteria, is typically transported into the cytosol by the AmpG permease for recycling during cell wall synthesis.

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However, due to the inefficiency of the Bp AmpG gene, TCT remains in the extracellular environment, where it contributes to host tissue damage. By replacing the Bp ampG gene with the more efficient E. coli ampG, the engineered BPZE1 strain produces less than 1% the TCT levels observed in unmodified Bp strains (46).

Pre-clinical studies conducted by Locht et al. demonstrated that BPZE1 exhibits high stability both in vitro and in vivo, and like its virulent parental strain, BPZE1 efficiently colonizes the respiratory tract; however, it does not induce lung tissue damage (48, 49). Furthermore, these studies revealed that BPZE1 stimulates robust mucosal immune responses, including the production of sIgA and IFN-γ and IL-17-secreting TRM cells in both the lung and nasal mucosa, and therefore provides long-term protection in upper and lower respiratory tracts (50).

Another live attenuated pertussis vaccine, developed by the Gamaleya Research Institute and referred to as GamLPV, has successfully completed Phase 1 clinical trials, demonstrating a favorable safety profile. An additional Phase 1/2 trial has been initiated, although its current status remains unreported (51). While the genetic composition of the vaccine strain has not been disclosed, preclinical studies have confirmed its safety, immunogenicity and protective efficacy (52). A summary of clinical trial data related to intranasal live attenuated pertussis vaccines is presented in Table 3.

Intranasal Outer Membrane Vesicle-based Pertussis Vaccines

An innovative strategy proposed by researchers for intranasal pertussis vaccination involves the use of outer membrane vesicles (OMV) derived from Bp. OMVs are nanoscale, spherical structures ranging from 20 to 250 nm in diameter that bud off from the outer membrane of gram-negative bacteria. These vesicles are released either spontaneously during bacterial growth or in response to stress conditions induced in vitro. OMVs play a critical role in bacterial survival by facilitating immune evasion, resistance to bacteriophages, and antimicrobial peptides, and promoting inter-bacterial communication (53, 54).

OMVs of Bp contain lipids and proteins from the bacterial outer membrane, as well as components of the periplasmic region. They carry a diverse repertoire of Bp antigens, including both aP and additional non-aP antigens such as GroEL, Vag8, and BrkA. Moreover, OMVs incorporate membrane pathogen-associated molecular patterns (PAMPs), which enhance their immunostimulatory capacity. Collectively, these features suggest that OMVs may provide superior protection compared to the current aP vaccines (27).

Table 3. Clinical trials investigating intranasal administration of live attenuated pertussis vaccines.

Study No.	Vaccine name	NCT number	Phase of study	Number of enrolled subjects	Age of participants (Years)	Status	Starting date	Completion date
1	BPZE1	NCT02453048	1	54	18-32	Completed	2015-09	2017-12
2	BPZE1	NCT01188512	1	48	19-31	Completed	2010-08	2012-01
3	BPZE1	NCT03541499	2	50	18-49	Completed	2018-10	2020-05
4	BPZE1	NCT05461131	2	53	18-50	Completed	2022-06	2023-10
5	BPZE1	NCT03942406	2	300	18-50	Completed	2019-06	2020-06
6	BPZE1	NCT05116241	2	367	6-17	Completed	2021-10	2024-05
7	GamLPV	NCT03137927	1	36	18-40	Completed	2017-06	2017-12
8	GamLPV	NCT04036526	1/2	50	18-40	Unknown	2019-06	-

NCT: National clinical trial

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The first study to utilize OMVs derived from Bp as an intranasal vaccine in a murine model was conducted by Roberts and colleagues in 2008 (55). Since then, preclinical investigations have continued to explore this approach. Results from these studies have demonstrated that intranasal administration of OMV-based vaccines can elicit robust humoral immunity, characterized by the production of all types of IgG subclasses (IgG1/IgG2a/IgG2b/IgG3), as well as a mixed Th1/Th2/Th17 cellular immune responses, without triggering excessive inflammatory reactions (56).

Nucleic Acid-based Pertussis Vaccines

Another platform explored in the development of pertussis vaccines involves nucleic acid technologies, specifically DNA- and RNA-based vaccines. In 2003, Kamachi and colleagues were the first to demonstrate both immunogenicity and protective efficacy of a DNA-based pertussis vaccine in a murine model (57). Subsequent studies further investigated the potential of this strategy, with DNA-based vaccines administered via intradermal and intramuscular routs; however, none evaluated intranasal delivery (58-63). Owing to persistent concerns regarding the safety of DNA-based vaccines, these candidates have not yet progressed to human clinical trials (64, 65).

The global deployment of SARS-CoV-2 mRNA vaccines, such as those developed by Pfizer and Moderna, during the COVID-19 pandemic has heightened interest in extending this vaccine platform to other infectious diseases, including pertussis (66). In 2024, Damron and colleagues reported promising results on the immunogenicity and protective efficacy of an mRNA-based pertussis vaccine formulated with lipid nanoparticles. This candidate vaccine was administered intramuscularly and evaluated in both BALB/c mouse and Sprague-Dawley rat models. .Immunization utilized ten mRNA constructs developed by Moderna, each encoding one of the following antigens including PT, PRN, FHA, FIM2/3, BrkA (Bordetella-resistant-to-killing gene A), SphB1 (a subtilisin-like serine protease autotransporter), TCFA (tracheal cytotoxin factor A), the RTX domain of adenylate cyclase toxin, diphtheria toxoid, or tetanus toxoid. The results demonstrated that this mRNA-based pertussis vaccine was capable of eliciting strong humoral immunity together with a balanced Th1/Th2 cellular immune response (66, 67).

DISCUSSION

It has been seven decades since the initiation of global pertussis vaccination; nevertheless, transmission of the infection remains insufficiently controlled, resulting in the death of numerous infants each year (6). This persistent burden has driven researchers to develop next generation pertussis vaccines aimed at overcoming the limitations of the two earlier vaccine generations.

The optimal strategy for next-generation pertussis vaccination is to mimic the protective immune response triggered by a natural infection (68). Fig. 2 illustrates the interplay between different components of the immune system in response to natural infection, intranasal pertussis vaccines, and systemic DTaP and DTwP vaccines. The principal elements mediating protective immune response in the upper respiratory tract against Bp include Th17 cells, neutrophils, and secretory IgA (sIgA). In contrast, immune defense in the lower respiratory tract is primarily driven by Th1 cells, alveolar macrophages, and IgG antibodies (27, 69). Following infection, a distinct population of tissue resident memory T cells (TRM) specific to Bp, persists within the respiratory tract. Among these, Th1 and Th17 TRM cells play a critical role in long-term protection against pertussis. Experimental evidence demonstrates that depletion of TRM cells from nasal tissue leads to a significant delay in bacterial clearance upon rechallenge in convalescent mice.

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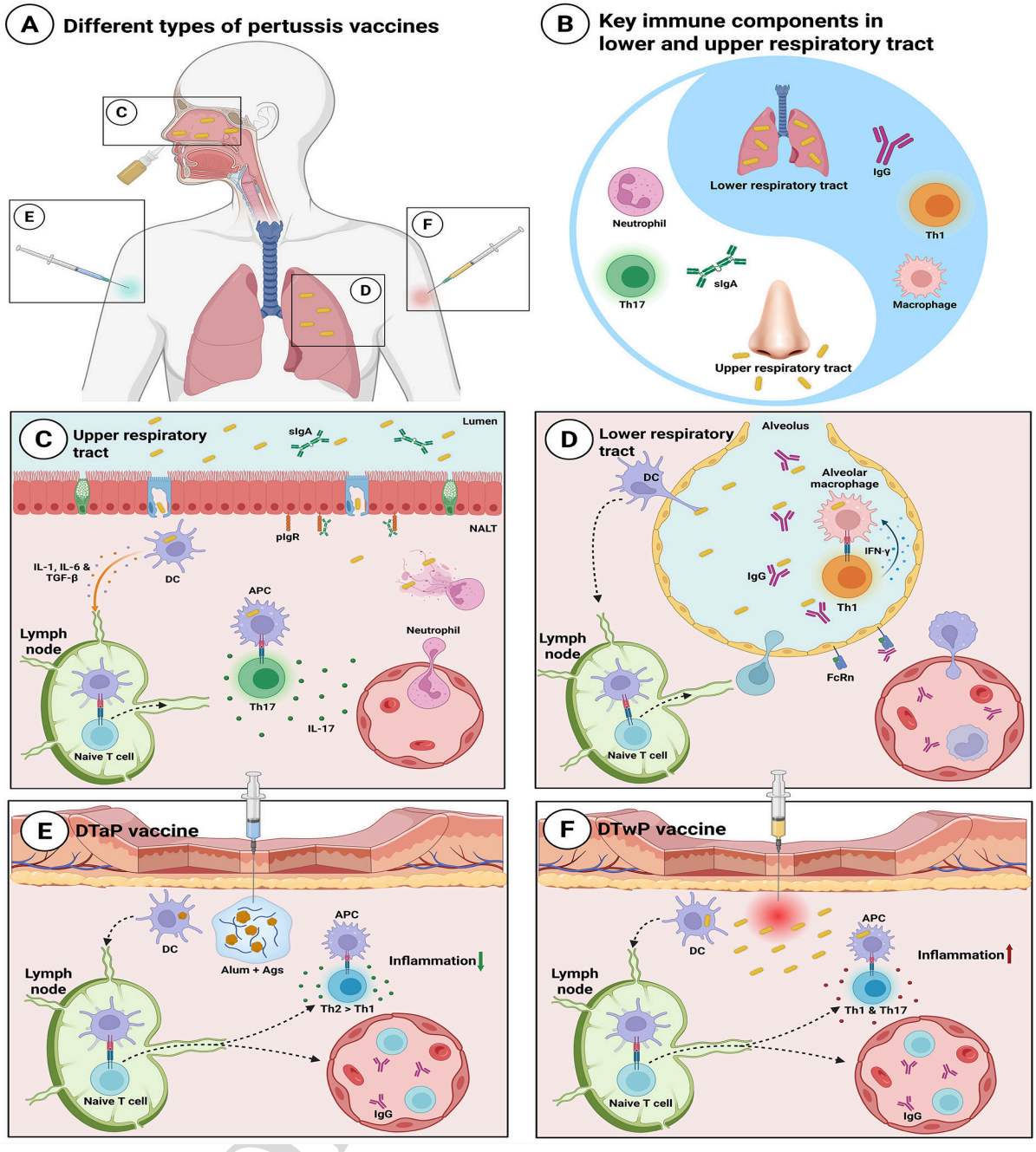

Fig. 2. Interplay between different components of the immune system in response to natural infection, intranasal pertussis vaccines, and systemic DTaP/DTwP vaccines (A). Key immune components involved in protection against Bp in the upper and lower respiratory tract (B). Naturally, after colonization of the upper respiratory tract, DCs in the nasal mucosa capture Bp antigens and present them to naive T cells in the cervical lymph nodes in the presence of IL-1, IL-6, IL-23 and TGF-β. These signals drive differentiation into Th17 effector and memory cells, which then reside in the nasal-associated lymphoid tissue (NALT). IL-17 production of by Th17 cells induces cytokines and chemokines that recruit neutrophils and to lesser extent monocytes, leading to clearance of Bp from the upper respiratory tract. IL-17 also increases the expression of defensins and the poly-Ig receptor, which are essential for the secretion of sIgA. By mirroring this route of entry and the inducing Th17 TRM cells in NALT, intranasal vaccines provide long-term protection against Bp in the nasal cavity (C). When natural Bp or intranasal pertussis vaccine antigens reach the lower respiratory tract, DCs capture antigens and present them to naive T cells in the mediastinal lymph nodes (D). Vaccination with TdaP and DTaP, administered intramuscularly with alum adjuvant, induces systemic Th2 responses, limited Th1 responses, and serum IgG production (E). DTwP vaccination elicits systemic Th1 and Th17 responses along with serum IgG, is associated with excessively vigorous inflammatory reactions

In addition, sIgA and serum IgG produced in response to Bp infection effectively inhibit bacterial adherence to respiratory epithelial cells, thereby preventing disease progression (68-71).

Since DTaP and Tdap vaccines induce systemic IgG and, to some extent, Th1 responses, they are effective in preventing Bp colonization in the lungs. However, due to their inability to generate sIgA and Th17 responses, these vaccine fail to eliminate Bp from the nasal mucosa. Several studies have shown that, following the challenge with live Bp, experimental animals immunized with DTaP exhibited delayed clearance of Bp from the upper respiratory tract compared to naive controls. This delay is likely attributable to systemic Th2 cells induced by DTaP, which compete with naïve T cells that would otherwise differentiate into Th17 cells within the lymph nodes (32, 38, 72-75). By contrast, the DTwP vaccine can prevent bacterial colonization in the lungs and, to some extent, in the nasal passages by inducing systemic IgG, Th1 and Th17 responses (75, 76).

The new generation of protein-based pertussis vaccines which are administered intranasally with Th1/Th17-driving adjuvants, mimic the natural route of pathogen entry. This results in an immune response profile resembling that of a natural infection. Overall, these vaccines have demonstrated a strong safety record in both infants and adults. However, a key limitation is their restricted antigen repertoire, which diminishes t their effectiveness against circulating mutated strains of Bp (14, 77-80). Additionally, protein-based vaccines require purification and the incorporation of suitable adjuvants, which increases production complexity and cost when scaled to large volumes (81). Future formulations must therefore prioritize broader antigen coverage and scalable manufacturing processes to improve accessibility. Intranasal OMV-based pertussis vaccines offer an advantage over protein-based platforms by presenting a diverse array of Bp antigens. However, the presence of PAMPs and native virulence factors within OMVs has restricted their clinical application (82). Ongoing research is necessary to focus on further detoxifying these vesicles while preserving their immunostimulatory properties to ensure both efficacy and safety.

Live attenuated pertussis vaccines most closely mimic natural infection and are significantly less expensive than protein-based and outer membrane vesicle-based formulations. The safety of the BPZE1 vaccine as a booster dose in adults has been confirmed up to Phase 2b clinical trials, with no vaccine-related serious adverse events reported. However, its use as a primary vaccination in infants remains uncertain due to the presence of Bp PAMPs (83). Long-term clinical data and carefully designed trials will be essential to establish safety in infants, particularly within primary immunization schedules.

Nucleic acid-based vaccines represent a promising platform for the prevention of pertussis. DNA-based vaccines utilize double-stranded DNA plasmids that must enter the nucleus for transcription, a requirement that theoretically increases the risk of genomic integration and thus limits their clinical applicability (84). In contrast,, mRNA-based vaccines utilize single-stranded mRNA molecules that are translated directly within the cytoplasm, effectively bypassing the risk of host genome integration (85). Despite this advantages, mRNA vaccines are inherently unstable and require storage at ultra-low temperatures, creating significant logistical and financial challenges for widespread distribution (86). Another limitation lies in the mucosal delivery of nucleic acid-based pertussis vaccines. Obstacles such as degradation by extracellular nucleases, poor penetration of the mucus layer, and inefficient uptake by epithelial cells must be addressed before effective intranasal administration can be achieved (87, 88). Recent advances in mRNA delivery technologies offer potential solutions. In particular, thermostable formulations

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currently under development may overcome t the cold-chain barrier, thereby enhancing accessibility in low-resource settings (89, 90). Advances in lipid nanoparticle chemistry have enabled more efficient delivery and targeted expression within mucosal tissues, opening the possibility of intranasal mRNA-based pertussis vaccines (91). Antigen selection remains a critical factor for achieving broad protection against circulating Bp strains. Vaccines that rely solely on classical antigens such as PT, FHA, or PRN may be less effective against emerging vaccine-resistant strains, underscoring the importance of developing multivalent formulations. A principal limitation of nucleic acid vaccines targeting bacterial pathogens, such as Bp, arises from the expression of bacterial antigens within eukaryotic host cells. This process can result in aberrant glycosylation, protein misfolding, and altered tertiary structures that deviate from the native conformation of bacterial proteins (92). Consequently, the capacity of these vaccines to induce neutralizing antibodies against conformational epitopes may be reduced compared to traditional platforms. Nevertheless, nucleic acid-based vaccines are well recognized for eliciting strong cellular immune responses, which may contribute substantially to protection against infection. Importantly, preclinical evaluations of nucleic acid vaccines for pertussis have not yet investigated intranasal administration. Further studies are therefore needed to evaluate the efficacy and safety of this rout. Taken together, nucleic acid vaccines represent a promising next-generation platform for pertussis, but their success will depend on rational antigen design, optimized delivery systems, and rigorous safety assessment in clinical trials.

While pre-clinical studies in murine and rat models have generated important insights into the development of intranasal pertussis vaccines, these models also carry notable limitations. The immune system of small rodents, particularly the relative contributions of Th1/Th17 responses and the capacity to generate mucosal sIgA, do not fully reflect human immune function (93). Consequently, vaccine candidates showing strong protection in these animals may not yield comparable efficacy in humans. Species-specific differences in dosing requirements and adjuvant responsiveness further complicates direct translation of preclinical results (93). An additional consideration involves the method of vaccine delivery. Most studies assessing pertussis vaccine efficacy in murine and rat models have applied intranasal formulations as liquid droplets using a laboratory pipette (31-42). In humans, however, intranasal vaccines are typically delivered as aerosolized sprays designed to distribute the vaccine more uniformly across the nasal mucosa. This methodological variation can substantially influence antigen deposition, mucosal absorption, and both the magnitude and quality of the ensuing immune response (94-96). Collectively, these species-specific and methodological discrepancies underscore the need for cautious interpretation of animal data and emphasize the importance of rigorously designed clinical trials to establish efficacy and safety in human populations.

Several novel adjuvants, including cholera toxin B subunit and c-di-GMP, have demonstrated promising immunogenicity in preclinical models of pertussis vaccination. However, their safety profiles in humans remain a significant concern. For instance, cholera toxin derivatives carry the risk of reactogenicity, which limits their direct clinical application (97-99). Likewise, c-di-GMP, a potent inducer of both mucosal and systemic immunity, has yet to undergo comprehensive safety evaluation in human trials (100). Thus, while these adjuvants hold considerable potential for enhancing vaccine efficacy, careful translation from preclinical studies to clinical application and rigorous safety assessment are essential before considering their incorporation into licensed pertussis vaccines.

Because pertussis infection in adults rarely leads to severe complications and

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given the dose-sparing characteristics of current formulations, separating the pertussis component from the combined Tdap booster vaccine and administrating pertussis and tetanus-diphtheria vaccines as two injections is not economically justified. For this reason, future next-generation pertussis vaccine formulations should incorporate protein or nucleic acid components corresponding to diphtheria and tetanus toxoids. An intranasal diphtheria-tetanus vaccine was evaluated by Aggerbeck et al. in a phase 2 clinical trial in 1997 and demonstrated adequate immunogenicity, but also raised serious safety concerns. Specifically, 3 of the 221 adults who received the intranasal vaccine experienced epistaxis (101). In 2006, Pour-Dounighi et al. further evaluated intranasal diphtheria-tetanus vaccine using several formulations in a phase 1 clinical trial and reported satisfactory immunogenicity and acceptable safety results (102). Accordingly, assessing the safety of next-generation intranasal pertussis vaccines as a booster dose coformulated with tetanus and diphtheria toxoids should be prioritized in future clinical trial studies. Additional research on less reactogenic intranasal formulations and optimized mucosal adjuvants will also be essential for the successful development of intranasal combination boosters.

Perspectives

In order to advance next-generation pertussis vaccines, researchers should prioritize designing multivalent formulations capable of protecting against emerging vaccine-resistant strains. They should also focus on developing safe and effective intranasal delivery systems that maximize Th1/Th17 and sIgA responses through aerosolized formulations that mimic human nasal immunology. Efforts must also focus on improving the feasibility of nucleic acid vaccines by stabilizing mRNA formulations and optimizing mucosal delivery strategies. It is essential to conduct rigorous long-term safety studies in both infants and adults. Finally, policymakers and funding agencies should prioritize investment in next-generation vaccines as a critical measure to reduce global infant mortality.

AUTHORS' CONTRIBUTION

Y.E. drafted the main manuscript text and prepared figures. F.G.S. and M.M.A. Conceptualized the study and contributed to manuscript review and editing. F.S. supervised, reviewed and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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