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Review

Nasal vaccines for pertussis

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Whooping cough, caused by *Bordetella pertussis,* is still a major cause of morbidity and mortality worldwide. Current acellular pertussis (aP) vaccines induce potent circulating IgG and prevent severe disease in children/adults and in infants born to vaccinated mothers. However, they do not prevent nasal infection, allowing asymptomatic transmission of *B. pertussis*. Studies in animal models have demonstrated that, unlike natural infection, immunization with aP vaccines fails to induce secretory immunoglobulin A (IgA) or interleukin-17 (IL-17)-secreting tissue-resident memory CD4 T (T_{RM}) cells, required for sustained sterilizing immunity in the nasal mucosa. Live-attenuated vaccines or aP vaccines formulated with novel adjuvants that induce respiratory IgA and T_{RM} cells, especially when delivered by the nasal route, are in development and have considerable promise as next-generation vaccines against pertussis.

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Introduction

The Gram-negative bacterium *Bordetella pertussis* causes whooping cough (pertussis), a life-threatening respiratory disease in young children that also affects adolescents and adults. In 2019, there were an estimated 19.5 million cases and 117000 deaths globally from pertussis, mainly in young infants [1]. Whole-cell pertussis (wP) vaccines were introduced in the 1940s but were reactogenic and were replaced by safer acellular pertussis (aP) vaccines in most developed countries in the 1990s. However, in recent years, despite high vaccine coverage, there has been a resurgence of pertussis in many countries using aP vaccines [2].

Current aP vaccines induce potent serum immunoglobulin G (IgG) responses against the 3 or 5 antigens in the vaccine. Antibodies, in particular those against pertussis toxin (PT), prevent severe disease in vaccinated infants and in newborns of mothers vaccinated during pregnancy, via transfer of antibodies through the placenta and in breast milk [3]. However, the concentration of anti-PT antibodies is lower in infants born to aP-vaccinated mothers that have been primed with aP compared with wP vaccines [2]. Furthermore, serum antibody responses wane rapidly after primary pediatric and booster aP vaccinations [4,5]. Current aP vaccines fail to prevent nasal colonization with *B. pertussis* in animal models [6,7], allowing asymptomatic transmission of *B. pertussis* from fully vaccinated individuals [8]. This has been linked with a failure of the parenterally delivered alum-adjuvanted aP vaccines to induce mucosal immunity, including secretory IgA (sIgA) and interferon-gamma (IFN-γ) or IL-17-secreting CD4 respiratory tissue-resident memory T (T_{RM}) cells, shown to be crucial for protective immunity in the lungs and nasal mucosa [9–11].

Mucosal immunity induced by infection with *B. pertussis*

Since *B. pertussis* infects respiratory mucosal surfaces, local IgA might be expected to be beneficial in controlling nasal colonization, however, evidence to support this is still limited. Passive transfer of serum containing high anti-*B. pertussis* IgG confers protection against lung infection in naive mice [12]. However, studies in $IgA^{-/-}$ mice suggested that IgA was not required for clearance of a primary or secondary infection with *B. pertussis* [13]. In contrast, intranasal inoculation of mice with a live-attenuated *B. pertussis* vaccine induced significant anti-*B. pertussis* sIgA in nasal secretions, which could transfer protection against *B. pertussis* challenge in naive mice [14].

In a human challenge model, individuals that had been immunized with wP vaccines as children showed a significant enhancement of filamentous hemagglutinin-specific IgA-secreting B cells in blood, 14 days after controlled nasal colonization with *B. pertussis* [15].

Furthermore, in individuals primed with aP vaccines as children, IgA binding to a mutated *B. pertussis* strain devoid of the vaccine antigens was significantly higher in the respiratory mucosal fluid in adolescents compared with children, suggesting that subclinical infections are increasingly common with increasing age and that these infections induce mucosal IgA responses [5]. Furthermore, administration of a booster aP vaccine enhanced mucosal IgG but not IgA responses [5].

Early studies in mice showed that T cells play an important role in protection against *B. pertussis* infection of the lungs [16]. *B. pertussis*-specific CD4 T cells, but not CD8 T cells, were able to transfer protection to T-cell-deficient mice [16]. IFN-γ-secreting Th1 cells were essential for controlling primary infection of the lungs and in protection against rechallenge with *B. pertussis* [10,17,18]. IL-17-secreting Th17 cells also play a role in preventing lung infection [10], but are indispensable for controlling infection of the nasal mucosa [6,9,19]. Studies in the baboon model showed that previous infection with *B. pertussis* induced near sterilizing immunity against re-infection, whereas immunization with aP vaccines failed to prevent nasal infection or bacterial transmission to naive baboons [7]. IL-17 was found in the nasopharyngeal washes of *B. pertussis*-infected baboons and long-lived *B. pertussis*-specific Th1 and Th17 memory T cells circulated in the blood of convalescent animals [20].

T_{RM} cells have recently emerged as key players in natural and vaccine-induced protective immunity at the mucosal surface and are major mediators of immunological memory in mucosal tissues [21]. We have reported that IL-17- and IFN-γ-secreting CD4 T_{RM} cells accumulated in the lung tissue during primary infection of mice with *B. pertussis* and expanded in situ upon rechallenge [19] (Figure 1). IL-17-producing CD4 T_{RM} cells accumulated in the nasal tissues during *B. pertussis* infection and persisted after bacterial clearance and this was linked with C-X-C motif chemokine ligand 1 (CXCL1) production and recruitment of Siglec-F^+ neutrophils to the nasal cavity [9]. IL-$17^{-/-}$ mice showed a significant delay in clearance of primary and secondary infections of the nasal cavity. Depletion of CD4 T_{RM} cells or neutrophils from the nasal tissue delayed bacterial clearance following rechallenge of convalescent mice [9]. Innate-type IL-17-secreting Vγ4$^-$γ1$^-$ γδ T cells accumulated early (hours) and pathogen-specific Vγ4 T cells, with a tissue-resident phenotype (CD69$^+$ CD103$^+$), accumulated later (days) in the lungs and expanded following reinfection with *B. pertussis* [22]. Thus, both CD4 and γδ T_{RM} cells contribute to long-term mucosal immunity to *B. pertussis*.

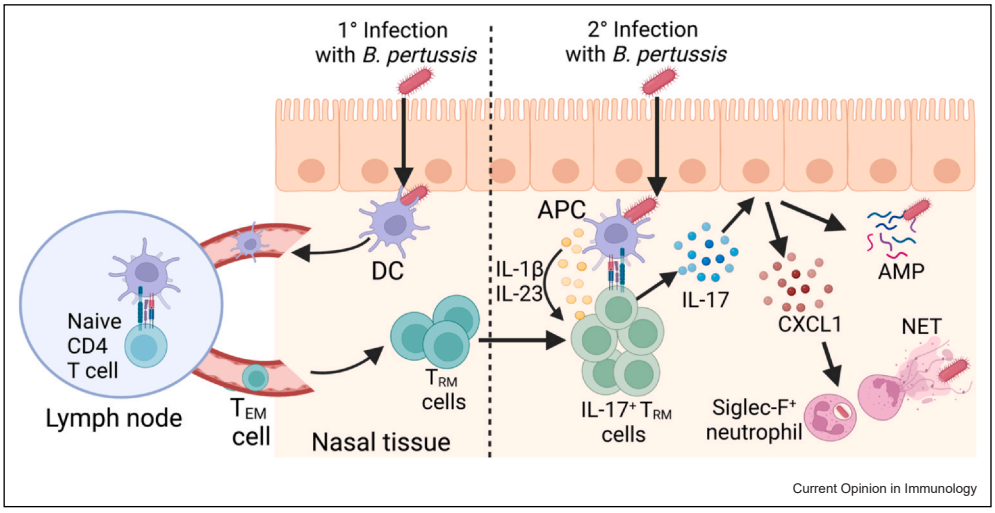
We have recently reported that IL-17-producing CD4 T_{RM} cells persist in the nasal tissue and lungs of *B. pertussis* convalescent mice, in part through bystander activation with unrelated pathogens [23]. We found that *B. pertussis*-specific CD4 T_{RM} cells can be non-specifically reactivated by *Klebsiella pneumonia* or lipopolysaccharide through induction of IL-1β, IL-18, and IL-23 production by dendritic cells (DCs) or monocytes. Furthermore, CD4 T_{RM} cells induced by a wP vaccine could also be reactivated by *K. pneumonia* and reduce the bacterial burden in the nose with this unrelated pathogen. In addition to the role of trained innate immunity [24], this may explain some of the heterologous protective effects of vaccination.

Live-attenuated pertussis vaccines

Live-attenuated vaccines mimic natural infection and when delivered by a mucosal route have potential to induce mucosal immunity and long-term protection. The most advanced candidate pertussis-attenuated vaccine, BPZE1, a Tohama-1 *B. pertussis* strain where three major toxins have been genetically modified or removed, protects mice and baboons against *B. pertussis* colonization of the nasal mucosa after a single intranasal immunization [25,26]. Protection was dependent on local sIgA and IL-17 [14]. The BPZE1 vaccine promoted accumulation of IL-17-producing CD4 T_{RM} cells in the nasal tissue, suggesting that it may induce sustained immunity in the respiratory tract [14]. The results of phase-1 clinical trials demonstrated BPZE1 was safe in adult human volunteers [27]. However, nasal colonization was not detected in around 20% of vaccinated subjects [27], which was linked to pre-existing antibodies to the antigens in the aP vaccine [28]. To address this, a pertactin (PRN)-deficient version of BPZE1 (BPZE1P) was constructed and shown to colonize mice that have been immunized with the aP vaccines [25]. Lyophilized BPZE1 appeared to be well tolerated and immunogenic in adult human volunteers [29]. A larger phase-2 clinical trial is underway (NCT03942406).

Promising data have also emerged in preclinical studies with another live-attenuated pertussis vaccine candidate, Bbvac, a *B. bronchiseptica* mutant lacking *btrS*, a regulator of immunomodulatory functions that *Bordetella* species utilize to suppress host immunity and persist in the respiratory tract [30]. A single intranasal immunization of mice with Bbvac conferred sustained protection against *B. bronchiseptica* but also *B. parapertussis* and *B. pertussis* infections of the lungs, trachea, and nasal mucosa [31]. Studies with passive transfer of serum from immunized mice showed that antibodies induced by Bbvac conferred protection against *B. pertussis* infection of the lungs and trachea and reduced the bacterial load in the nasal cavity. IL-17-secreting T cells were expanded in the lungs post *B. pertussis* challenge of mice immunized with Bbvac [31]. However, the contribution of respiratory T_{RM} cells induced with Bbvac or BPZE1 to protective immunity against nasal infection with *B. pertussis* has not been confirmed by cell

Figure 1



Role of IL-17-secreting CD4 T_{RM} cells in natural immunity to *B. pertussis*. Studies in mice have shown that following *B. pertussis* primary infection of the respiratory tract, bacteria are taken up by local DC, and once activated, they migrate to draining lymph nodes, where they prime naive CD4 T cells to become effector memory T (T_{EM}) cells, including IL-17-secreting T (Th17) cells. These T_{EM} cells can enter the nasal tissues (and lungs) and are retained as T_{RM} cells. Following reinfection of convalescent mice, respiratory T_{RM} cells expand in response to *B. pertussis* antigens presented by local antigen-presenting cells (APC). interleukin-1beta (IL-1β) and IL-23 produced by APC promote IL-17 production by T_{RM} cells, which induces CXCL1 and AMP production by epithelial cells. CXCL1 promotes recruitment of Siglec-F^+ neutrophils to the nasal tissues, which kill *B. pertussis* via phagocytosis and neutrophil extracellular trap (NET) formation. Created with BioRender.com.

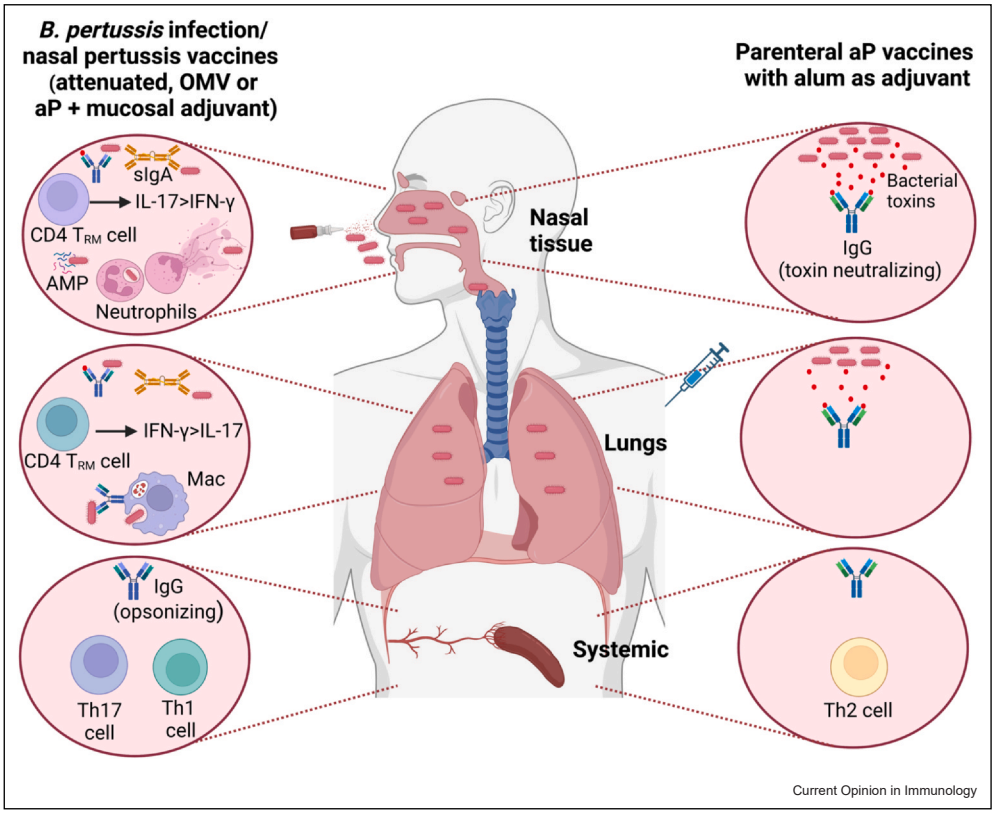
depletion or transfer studies in mice, and their induction or role has not been addressed in humans.

Nasal acellular pertussis vaccines with adjuvants

Current parenterally delivered aP vaccines, comprising 3 or 5 *B. pertussis* antigens, with alum as the adjuvant, induce potent serum IgG antibodies and a Th2-biased response but do not induce sIgA or T_{RM} cells in the respiratory tissue, required for persistent protection against *B. pertussis* infection [9,14] (Figure 2). Many of the early studies on nasal vaccines did not examine protection in the nasal cavity and/or T-cell responses [32]. The most promising candidate intranasal aP vaccines are those that promote local humoral and cellular immunity and prevent *B. pertussis* infection of the nasal cavity.

A recent study demonstrated that intranasal, oral, or intramuscular immunization of rats with a commercial diphtheria, tetanus and acellular pertussis (DTaP) vaccine (Infanrix) conferred protection against *B. pertussis* infection of the lungs and trachea and prevented *B. pertussis*-induced cough [33]. Although immunization by all routes induced systemic IgG, only intranasal immunization induced mucosal IgA [33]. Attempts to improve the immunogenicity of aP vaccines have focused on the use of novel adjuvants. Intranasal immunization of mice with pertussis antigens (PT, PRN, and fimbriae) with an adjuvant called TriAdj [poly(I:C), IDR-1002, and a polyphosphazene] formulated into cationic lipid nanoparticles induced *B. pertussis*-specific IgG in serum and sIgA in nasal secretions [34]. Furthermore, intranasal immunization of mice with pertussis aP vaccine antigens with β-glucan (curdlan) or whole-glucan particles (IRI-1501) induced strong serum IgG and mucosal IgA and conferred a good level of protection against *B. pertussis* infection of lung and nasal cavity that persisted for at least 6 months [35]. Surprisingly, intranasal immunization with the pertussis antigens alone was similarly immunogenic and protective against *B. pertussis* [35]. However, another group reported that while pertussis antigens given intranasally without an adjuvant conferred some protection against nasal infection, when combined with a stimulator of interferon genes (STING) agonist, cyclic diguanylate (c-di-GMP), the experimental aP vaccine induced stronger IgA in the nasal wash, IL-17 or IFN-γ-secreting T_{RM} cells in the lungs, and better protection against nasal infection with *B. pertussis* [36]. STING agonists act as adjuvants in part by activating innate immune responses, especially DCs that drive adaptive immunity, and when combined with a Toll-like receptor 2 (TLR2) agonist, significantly enhance DC activation and induction of T-cell responses [37]. We demonstrated that a combination of c-di-GMP and the TLR2 agonist LP1569, a synthetic peptide derived from the *B. pertussis* lipoprotein BP1569, is a potent adjuvant for an experimental aP vaccine, especially when delivered by a mucosal route [37]. Intranasal immunization of mice with the LP–GMP-adjuvanted aP vaccine promoted accumulation of respiratory IL-17-secreting CD4

Figure 2



Natural infection or nasal immunization with certain pertussis vaccines, but not parenteral immunization with aP vaccines, induces protective mucosal immunity in the lungs and nasal cavity. Studies in mice, baboons, and humans have shown that previous infection induces opsonizing and *B. pertussis*-specific IgG and systemic Th1 and Th17 responses, whereas parenterally delivered aP vaccines induce predominantly Th2 responses and IgG that can transudate into lungs and nasal mucosa, where they neutralize bacterial toxins and thereby prevent disease. However, the aP vaccines fail to induce sIgA or T_{RM} cells in the respiratory tract and do not prevent nasal infection with *B. pertussis*. Preclinical studies in mice have shown that nasal immunization with live-attenuated pertussis vaccines, pertussis OMV vaccines, or aP vaccines with mucosal adjuvants induces sIgA and T_{RM} cells in lung and nose. CD4 T_{RM} cells in nasal tissue predominantly secrete IL-17, which promotes production of AMP and recruits neutrophils that kill *B. pertussis*, whereas lung CD4 T_{RM} cells predominantly secrete IFN-γ, which activates macrophages (Mac), which together with opsonizing antibodies take up and kill *B. pertussis.* Created with BioRender.com.

T_{RM} cells and provided sustained protection in the lungs and nasal cavity for at least 10 months [37]. Collectively, these studies demonstrate that nasal delivery of aP vaccines with mucosal adjuvants induces local as well as systemic humoral and cellular immunity and, importantly, confers protection in both the upper and lower respiratory tracts (Figure 2).

Nasal whole-cell and outer membrane vesicle pertussis vaccines

Parenterally delivered wP vaccines, composed of whole *B. pertussis* organisms that have been inactivated with formalin or heat treatment, are effective at preventing whooping cough and changed the global epidemiology of the disease following their introduction in the 1940s. Although replaced by aP vaccines in developed countries, wP vaccines are still used in many low- and middle-income countries. Parenteral wP vaccines do not generate mucosal antibody responses, but they do induce circulating *B. pertussis*-specific IgG antibodies [38]. However, intranasal administration of a wP vaccine in human volunteers induced IgA in serum and nasal fluid [39], and antigen-specific T-cell responses detectable in the blood [40]. Furthermore, when delivered to mice by nasal route, the wP vaccine generated CD4 T_{RM} cells in the upper and lower respiratory tracts and conferred protection against nasal infection, superior to that induced by parenteral route [23]. It has also been demonstrated that intranasal immunization of mice with a wP vaccine adjuvanted with curdlan reduced the bacterial burden in the upper and lower respiratory tracts and enhanced Th17 responses in the spleen following challenge with *B. pertussis* [41].

To reduce the toxicity while retaining the broad range of antigens in wP vaccines, pertussis outer membrane

vesicle (OMV) vaccines are in development. Pulmonary or intranasal immunization of mice with pertussis OMV vaccine promoted mucosal IgA, Th1/Th17 responses in the spleen and lungs, and protected against infection of lungs, trachea, and nasal cavity following challenge with *B. pertussis* [42,43]. The pertussis OMV vaccine induced stronger T-cell responses, including respiratory CD4 T_{RM} cells and better protection when delivered intranasally compared with the subcutaneous route [43] [44]. Interestingly, parenteral immunization of mice with a pertussis OMV vaccine conferred substantially stronger protection than a commercial aP vaccine against infection with a PRN-deficient strain of *B. pertussis* [44], suggesting that OMV vaccines may overcome one of the challenges faced by current aP vaccines.

Conclusions and future perspectives

A consensus is emerging that current parenterally delivered alum-adjuvanted aP vaccines are suboptimal and need to be replaced with vaccines that induce mucosal immune responses in the respiratory tract, and thereby confer protective immunity against infection of the nasal cavity as well as the lungs. Although there is no agreed correlate of immunity, recent studies have demonstrated that IL-17-induced neutrophils, and possibly antimicrobial peptides (AMP), play a critical role in protective immunity in the nasal cavity, while previous studies had indicated that antibodies and IFN-γ-activated macrophages prevent disease and infection of the lungs (Figure 2). Furthermore, local mucosal immune responses will be required to confer sterilizing immunity in the nasopharynx and to sustain protection through accumulation of CD4 T_{RM} cells in the respiratory tissues.

The induction of *B. pertussis*-specific antibodies (IgG and IgA), Th1- and Th17-type respiratory T_{RM} cells can best be generated using pertussis vaccines delivered by the nasal route. Candidate nasally delivered pertussis vaccines in development include live-attenuated *B. pertussis* or *B. bronchiseptica*, pertussis OMV, and new aP vaccines formulated with novel mucosal adjuvants. Attenuated vaccines are not without risks of adverse reactions, especially in immunocompromised individuals or associated with autoimmune diseases [45], and therefore need to be developed with caution. However, it has been reported that BPZE1 is safe in severely immunocompromised mice [46]. Furthermore, a nasal influenza vaccine is currently in widespread use in humans without safety concerns. A new pertussis vaccine might be first introduced as a booster for adolescents or adults primed with aP vaccines as children, but would need to overcome the Th2-skewed immune response and potentially immunosuppressive effects of the aP vaccine on recruitment of T_{RM} cells to the respiratory tract [6,47]. The long-term solution to the pertussis problem may be a stand-alone nasal pertussis vaccine delivered to infants at 4 weeks or younger.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Kingston Mills is a cofounder and shareholder in Parvalis Tx, a Biotech start-up company involved in the development of anti-inflammatory therapeutics, and is an inventor on a patent related to a novel adjuvant for an acellular pertussis vaccine.

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