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Pertussis vaccines and protective immunity

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Despite high vaccine coverage, reported cases of pertussis have increased steadily over the last twenty years. This resurgence has stimulated interest in host responses to pertussis infection and vaccination with the goal of developing more effective next-generation vaccines and vaccination strategies. Optimal protection against *Bordetella pertussis* appears to be multifactorial requiring both humoral and cellular responses. Natural infection and whole-cell pertussis vaccination induce Th1 and Th17-dominated responses. In contrast, acellular vaccines induce Th2-dominated responses. Available immunological data indicate that while antibodies provide protection against disease, Th1 and Th17-mediated immune responses are required for bacterial clearance and long-lasting protection. The nature of the priming in children appears to be important in modulating bias and durability of immune responses required to provide protection against *B. pertussis*. This review summarizes the current understanding of differences in immune responses and their role in protection against *B. pertussis* following infection or vaccination.

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Introduction

Pertussis is an acute respiratory disease caused by the bacterium *Bordetella pertussis*. The disease is characterized by violent coughing fits associated with an inspiratory whoop and post-tussive vomiting [1,2]. Because the first routine pertussis vaccination occurs at six to eight weeks of age, infants under two months of age are the most vulnerable and have the highest rate of serious clinical complications requiring hospitalization and the highest mortality rate [3,4]. Disease in very young infants is characterized by gagging, gasping, bradycardia, cyanosis, and vomiting [5]. Apneic episodes following paroxysmal fits are common [5]. Severe and fatal pertussis in young infants is associated with extreme leukocytosis, pulmonary hypertension, and pneumonia [5,6].

In the pre-vaccine era there were an average of 162 000 cases of pertussis per year (151 cases/100 000) in the United States with an average case fatality rate of 4% (1926 and 1929 Annual Reports of the Surgeon General of the Public Health Service of the United States). Introduction of killed, whole-cell pertussis (wP) vaccines combined with diphtheria and tetanus antigens (DTwP) in the 1940s led to a rapid decline in the incidence of reported pertussis resulting in a historic low of only 1010 cases in 1976 [7]. However, the wP vaccine was commonly associated with mild injection site pain and swelling, low-grade fever and fretfulness and less commonly with more severe reactions: convulsions and hypotonic–hyporesponsive episodes [8–11]. This reactogenicity led to reduced acceptance of the wP vaccine and declining vaccination rates in most industrialized countries [12]. In response to these concerns, less reactogenic acellular pertussis (aP) vaccines were developed consisting of purified *B. pertussis* antigens combined with aluminum adjuvant. Clinical trials confirmed aP vaccines were less reactogenic than the wP vaccines they replaced and demonstrated comparable efficacy over the first five years following vaccination [11,13–16]. High-income countries began replacing combined DTwP vaccines with combination DTaP vaccines in the 1990s. Under the currently recommended vaccination schedule in the United States, children receive the DTaP vaccine at two, four and six months of age and booster doses at 15–18 months of age, 4–6 years of age and Tdap at 11–12 years of age [17]. Approximately 95% of children receive at least three doses of vaccine by school entry and greater than 80% of children receive the adolescent booster dose by middle school enrollment [18,19]. Despite these high rates of vaccination, the United States has experienced a steady increase in reported cases of pertussis since 2000 (CDC, Pertussis Surveillance and Reporting website; URL: http://www.cdc.gov/pertussis/surv-reporting.html). Several hypotheses have been proposed to explain this resurgence including more rapid waning of protective immunity following aP vaccination, evolution of *B. pertussis* to escape protective vaccine-mediated immunity, and increased carriage and asymptomatic transmission from individuals vaccinated with the aP vaccines [20•,21••]. In this review, we summarize the current understanding of the host immune response to pertussis infection and vaccination.

Immune correlates and protection

Over one hundred years after Bordet and Gengou identified *B. pertussis* as the causative agent of whooping cough, we still lack a complete understanding of how the bacterium causes

disease or the mechanisms by which host immunity to infection or vaccination confers protection. Studies conducted during the whole-cell vaccine era had shown a correlation between measurable agglutinin titers in serum with protection against pertussis [22–24]. However, large field clinical studies that demonstrated efficacy of the aP vaccines, failed to demonstrate correlation between protection and antibody titers for any of the vaccine antigens [25]. Evidence for antibody-mediated protection was subsequently provided in household contact studies in which pre-exposure antibody levels were evaluated for cases of pertussis that occurred in two of the large efficacy trials [26•,27•]. In these household contact studies, lower attack rates were observed in children with high pre-exposure levels of anti-pertactin (PRN) antibodies, anti-fimbriae (FIM) antibodies, and to a lesser extent anti-pertussis toxin (PT) antibodies. The lowest attack rates were seen in children with quantifiable antibodies against both pertactin and fimbriae, independent of the presence or absence of anti-PT antibodies. There was no observable contribution of anti-filamentous hemeagglutinin (FHA) levels to protection [28,29]. Evidence that antibodies alone can confer protection from disease was provided by mouse studies in which high titer anti-pertussis human immunoglobulin and mouse anti-PT monoclonal antibodies protected mice from pertussis challenge even when given seven days after challenge [30••,31•]. Additional evidence includes recent studies demonstrating that vaccination of pregnant baboons with aP vaccine or mono-component PT vaccine protected newborn baboons from challenge and retrospective studies demonstrating protection in newborn children born to mothers that received Tdap in pregnancy [32–34,35•,36,37,38••]. The protection documented in these studies is reasonably assumed to be due to the trans-placental transfer of antibodies from mothers to their infants. The lack of a strong correlation between serum antibody titers and protection in the vaccine efficacy studies suggests that cell-mediated immunity and/or mucosal immunity plays an important role in establishing protective immunity (Figure 1).

Natural immunity

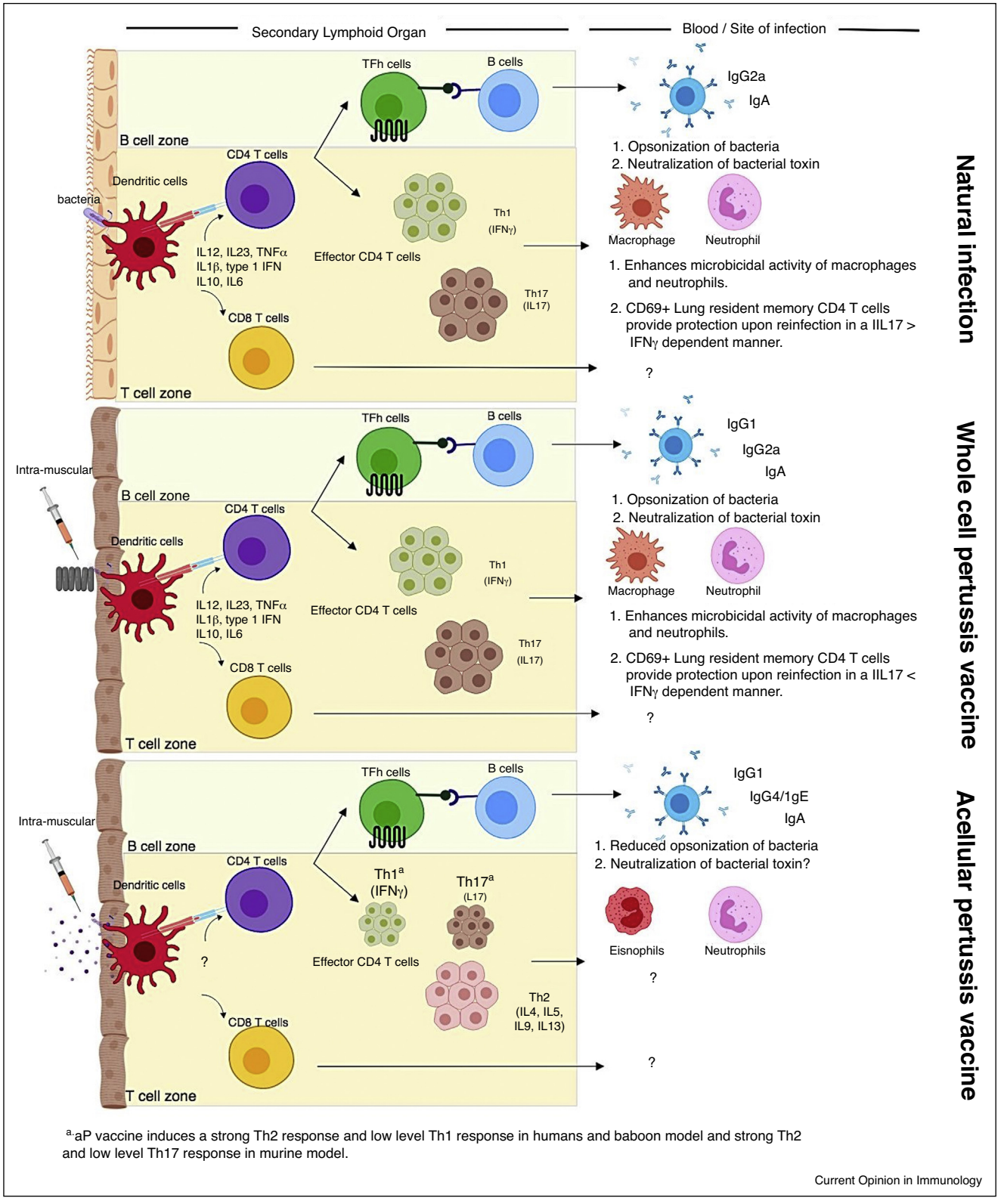
The complex etiology of *B. pertussis* is attributed to expression of multiple virulence factors that contribute directly to pathogenesis or have immunomodulatory effects. The interface between innate and adaptive immune responses is key to the recognition of *B. pertussis* and the control of the infection by the host response. The recognition of bacterial antigens by receptors on mucosal epithelial cells and innate immune cells such as macrophages and dendritic cells leads to activation of a cascade of immune responses including both pro-inflammatory (IL6, IL1β, TNFα, IL8, IL12, IL23, and IFN type 1) and anti-inflammatory (IL10) responses [39–44]. B cells and CD4 T cells were identified as the main effector cells in providing protection against *B. pertussis* infections [45,46]. It was further demonstrated that in addition to their role in antibody production, CD4 T cells provide protection against *B. pertussis* through an antibody independent mechanism [45]. Initial investigations of cytokine production by peripheral blood T cells from children recovering from whooping cough indicated that immunity generated by natural infection is mediated by IFNγ producing T cells [47]. Evidence of the relevance of these cells is provided by the observation that memory CD4 T cells clones generated from PBMCs of previously infected adults secrete IFNγ, induce anti-microbial activity in phagocytic cells and provide help to opsonizing B cells [48••]. The direct role of CD4 T cells in bacterial clearance was demonstrated by adoptive transfer from wild-type mice into immunocompromised mice [49••]. Taken together, these results suggest CD4 T cells contribute to protection from *B. pertussis* colonization through IFNγ-dependent mechanisms. Recent advances in the evaluation of Th17 responses have extended our understanding of the cellular immune response to pertussis infection. The production of the Th17-promoting cytokine IL23 by *B. pertussis*-infected human dendritic cells suggested a role for Th17 cells in anti-pertussis immunity [50••]. The detection of IL17 production in murine and baboon airways following *B. pertussis* infection and the inability of IL17A receptor knock out mice to clear *B. pertussis* infection demonstrated the importance of the Th17 response to protection following *B. pertussis* infection [51••,52•]. Further evidence of the relevance of Th1 and Th17 responses against *B. pertussis* infection and colonization was provided by the baboon model [53,54]. Following infection with *B. pertussis*, baboons exhibited strong Th1 and Th17 responses, that resulted in protection from clinical signs of disease and sterilizing immunity [21••,52•].

In addition to the proposed role of *B. pertussis*-specific IFNγ and/or IL17 responses in clearance of bacteria from the airway, the induction of IFNγ and/or IL17 producing tissue resident memory T cells observed in mouse lungs following infection has been shown to play a significant role in providing long-term memory following *B. pertussis* infection in the mouse model [55••].

Vaccine-mediated immunity

The temporal association of the switch from wP to aP vaccines with the resurgence of pertussis, combined with our expanding understanding of differences in aP and wP-induced immunity, is consistent with the hypothesis that the observed resurgence is the result of the switch from wP to aP vaccines. Comparative studies demonstrated that both wP and aP vaccines induce strong IgG responses against pertussis antigens. aP vaccines, which are formulated with a single adjuvant and a limited set of antigens, stimulate a different and more restricted immune response profile compared to wP vaccines or natural infection. Both wP vaccines and infection present a broad array of antigens and potential adjuvants. Infection also presents the signals associated with the replication of

Figure 1



[a]aP vaccine induces a strong Th2 response and low level Th1 response in humans and baboon model and strong Th2 and low level Th17 response in murine model.

Current Opinion in Immunology

Host immune response to pertussis infection and vaccination.

bacterial cells and host damage at the mucosal surface. Infants primed with aP or wP vaccines exhibit differences in the polarization of the immune response between Th1, Th2 and Th17 as shown by the *B. pertussis*-specific IgG subclass distribution observed at 4–10 years of age [56••,57••]. In children primed with wP vaccines IgG4 levels remained low despite receiving a DTaP booster at 4 and 9 years of age [56••,57••]. Increased levels of the IgG4 subclass are associated with a Th2-skewed immune response, which may influence the protection against pertussis in vaccinated children [58]. A recent study comparing the immune response between wP and aP primed individuals revealed that CD4 T cells from wP primed individuals produced high levels of PT-specific IFNγ and IL17, but no IL4, whereas CD4 T cells from aP primed individuals expressed high levels of IL4, but no IFNγ or IL17. CD4 T cells from wP primed individuals remained committed to their original skewing following boosting with aP vaccine, continuing to produce high levels of IFNγ and IL17, but no IL4 [59••]. Moreover, wP primed individuals exhibited greater expansion of pertussis-specific CD4 T effector memory and T central memory responses, and IgG and IgG1 responses, than aP primed individuals after an aP boost [59••]. Taken together these studies illustrate the importance of the priming vaccine in programing the immune response. The clinical significance of the priming vaccine was demonstrated by the observation that among 11–12-year-old children born during the transition from wP vaccine to aP vaccine in Queensland Australia, those who received only aP vaccine had the highest rates of pertussis, while those who received only wP vaccine had the lowest incidence. Of those individuals that received a mixed course of vaccination, those that received a first dose of aP followed by wP had high incidence of disease while those that had an initial dose of wP followed by aP had low incidence of disease [60].

The relative contribution of Th1, Th2 and Th17 cellular responses to vaccine-mediated protection differs to some degree depending on species. In mice, aP vaccination induces CD4+ T cells to produce IL4, IL5, IL17, and to lesser extent IFNγ, consistent with the induction of a Th2/Th17 response [51••,61••]. In contrast, wP vaccines induce IFNγ and IL17A in mice consistent with the induction of a Th1/Th17 response [51••]. Although IL4 and IFNγ expression is induced by aP vaccine in mice, aP-mediated protection was comparable in IL4$^{-/-}$, IFNγ$^{-/-}$ and wild-type mice. In contrast, aP-mediated protection was significantly reduced in IL17A$^{-/-}$ mice indicating a required role for Th17 responses in vaccine-induced protection [51••]. Protection against infection following wP vaccination was greatly diminished in IFNγ$^{-/-}$ mice with significantly higher bacterial burden in the lungs that failed to clear. Bacterial burdens in wP-vaccinated IL17A$^{-/-}$ mice are somewhat higher early after infection but the infection cleared as rapidly as in wild-type mice. These results indicate that Th1 responses are required for the protection induced with wP vaccines in mice [51••,62].

In the baboon model, vaccination with wP vaccines induced strong Th1 and Th17 responses but no Th2 response. Baboons vaccinated with wP vaccine were protected from disease and rapidly cleared infection [21••,63]. In contrast, vaccination with aP vaccines resulted in strong Th2 responses, low Th1 responses and no Th17 responses [21••,63]. Immunization with aP vaccines conferred protection against disease but failed to prevent colonization, carriage or transmission to co-housed animals [21••,63]. Taken together, these results indicate that Th2 responses are sufficient to protect against disease and Th1 and/or Th17 cells are required for the prevention of *B. pertussis* colonization in the baboon model.

Analysis of cellular responses in blood samples from children following immunization with wP vaccine or following infection revealed moderate to high levels of IFNγ, but undetectable IL5. In contrast, blood samples from children following immunization with aP vaccine demonstrated high levels of IL5 and low levels of IFNγ [64–67]. These results indicate that aP vaccination induces strong Th2 responses and weak Th1 responses in humans while wP vaccines induce strong Th1 responses. These results mirror those observed in the baboon model. Direct evidence of induction of Th17 responses following the priming series of vaccination in children is lacking. However, a recent study demonstrated high levels of IFNγ and IL17 but no IL4 following aP boosting of wP-primed children [59••]. In contrast, only IL4 was induced following aP boosting of aP-primed children [59••].

Immune memory

Although markers that correlate with duration of immunity following vaccination have not been identified and are difficult to validate, an important determinant of long-term immunity may be the induction of tissue resident memory (Trm) cells [68]. It was recently shown that *B. pertussis* infection establishes CD4+ Trm cells in lungs of infected animals [55••]. These cells expanded rapidly in the lung tissue upon re-infection and provided a protective response [55••]. The importance of Trm cells in protection upon reinfection was demonstrated by blocking the influx of lymphocytes upon reinfection and through adoptive transfer studies [55••]. Additionally, an experimental acellular pertussis vaccine adjuvanted to stimulate Th1 and Th17 responses induced *B. pertussis*-specific Trm cells in the lungs of vaccinated mice and conferred protection against infection that persisted for ten months [69••]. The protection observed in this study correlated with the number of IL17-secreting Trm cells in nasal tissue.

Conclusion

The observed rates of pertussis in high-income countries despite high rates of vaccination highlight the need for new vaccines or vaccine strategies to achieve complete control of this disease. The available evidence suggests that Th2 responses are likely sufficient to protect against disease. However, Th1 responses and/or Th17 responses targeting the bacterial cell at the mucosal surface are required to mediate clearance of bacteria from the airway and prevent asymptomatic carriage. Studies in the mouse and baboon models have shown that infection induces strong immune responses that prevent disease and result in sterilizing immunity. Killed whole-cell vaccines stimulate an immune profile similar to infection and protect against disease and colonization. Although wP vaccines do not induce sterilizing immunity, wP-vaccinated baboons were colonized at significantly reduced levels and cleared infection quickly. Although aP vaccines protect against disease, they fail to prevent carriage in and transmission from vaccinated baboons. If this observation is relevant in people, it is reasonable to hypothesize that increased asymptomatic carriage would be observed in an aP-vaccinated population leading to increased pertussis exposure in that population. Data indicate that the duration of immunity induced by aP vaccines is shorter than that induced by wP vaccines, and priming by aP vaccines in infants appears to lead to diminished duration of immunity following subsequent boosting in adolescence [70]. Despite these shortcomings, it is important to recognize that aP vaccines were developed in response to a significant need as acceptance of wP vaccination fell in high-income countries. The licensed aP vaccines have an excellent safety profile and protect vaccinated individuals from disease. With the implementation of maternal vaccination to protect newborns in their first months of life and vaccination of infants and toddlers, we can prevent severe disease in young children using the existing vaccines [71]. A comparison of pertussis rates in high-income countries today with rates in the pre-vaccine era demonstrates that we are maintaining significant levels of control of pertussis. However, pertussis remains the most common vaccine-preventable disease. Next-generation vaccines are needed that combine the safety profile and protection against disease inherent in the aP vaccines with protection against colonization and enhanced duration of immunity. A number of new approaches are being taken toward this goal. These include the development of improved aP vaccines that incorporate alternative adjuvants and antigens to induce more durable immunity and target the bacterial cell for clearance, aP vaccines based on outer membrane vesicles, live-attenuated pertussis vaccine and killed whole-cell vaccines using genetically engineered strains designed to be less reactogenic [72]. As we work toward the goal of introducing next-generation pertussis vaccines, it is important to recognize that our understanding of the host-response to pertussis infection and vaccination is incomplete. Continued efforts using powerful tools available in the mouse model and proof of concept studies with novel vaccines in the baboon model are needed to understand the mechanisms underlying vaccine-mediated protection against pertussis.

Conflict of interest statement

Nothing declared.

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