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Chapter 8

Immunization against Pertussis: An Almost Solved Problem or a Headache in Public Health

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Abstract

Whooping cough or pertussis is a serious infectious disease of the human respiratory tract, caused by Gram-negative bacteria *Bordetella pertussis* and *Bordetella parapertussis*$_{HU}$. The current pertussis vaccines may consist of dead cells of *B. pertussis* (whole cell pertussis vaccines—wPs) or purified antigens from the bacterium (acellular pertussis vaccines—aPs). The aPs are less reactogenic and have been widely used in developed countries for more than two decades, but their high cost of production makes them prohibitive for developing countries, and the accelerated rate of epidemic outbreaks has led to the hypothesis that aPs are less effective than the wP ones. Considering cost-effectiveness, some authors have pointed out questions about the possibility of reintroduction of wP vaccines into the primary doses of pertussis vaccination. The Butantan Institute in São Paulo, Brazil, developed a wP vaccine with low endotoxicity (Plow) obtained by chemical extraction of the lipooligosaccharide (LOS) fraction from the outer membrane of the bacterial cell, showing to be less reactogenic and equally immunogenic and protective as the traditional wP vaccine. The Plow may possibly be introduced into the vaccination schedule for immunization of adolescents and young adults in Brazil, an important epidemiological contribution to reducing the circulation of *B. pertussis*.

Keywords: pertussis, *Bordetella pertussis*, whole cell pertussis vaccine, acellular pertussis vaccine, resurgence of pertussis

1. Introduction

Pertussis or whooping cough is an acute and serious infectious disease of the respiratory tract, directly transmitted from human to human through respiratory aerosols [1]. The World Health Organization (WHO) estimates the annual incidence of 16 million cases of pertussis, with 195,000 deaths per year, one of the main causes of mortality for vaccine-preventable diseases in children less than five years [2, 3]. The main causative agent is the Gram-negative bacterium *Bordetella pertussis*, but although *Bordetella parapertussis*$_{HU}$ leads to a milder disease [4, 5], it has also been associated with more severe episodes, such as pneumonia and bronchopneumonia in children, with possible lethal consequences [6, 7]. The disease is exclusively human, with characteristics that differentiate it from other respiratory diseases [8, 9] and was widely disseminated in pre-vaccine era, mainly affecting children from 1 to 9 years of age. [10]. There is evidence that *B. pertussis* and *B. parapertussis*$_{HU}$ were adapted to restricted niches of hosts, which possibly allowed a more effective infection [11–14]. Pertussis toxin (PT), considered the main virulence factor of *B. pertussis*, is not produced by *B. parapertussis*, where the PT gene is transcriptionally silent [5, 15], which could be a reason for the frequently milder symptoms following infection by *B. parapertussis* [4, 5, 16, 17].

The classical manifestations of pertussis are divided into three phases: catarrhal, paroxysmal, and convalescent [18], and are particularly serious in unprotected newborns and young infants (<1 year old), with bacteria disseminating into the lungs causing necrotizing bronchiolitis, intra-alveolar hemorrhage, and fibrinous edema. In the most severe cases, there is usually intense lymphocytosis, correlated with pulmonary hypertension, respiratory failure, and death [19]. Older children, adolescents, and adults can also be affected [20], and although in these age groups the clinical manifestations may vary from the classic symptoms to moderate or even absent cough [21], high rates of the bacteria have been found in this population [9], who act as reservoirs and can transmit the infection to at-risk groups, such as neonates and infants [22].

2. Pertussis vaccines: an almost solved problem?

The initial attempts to develop a vaccine against *B. pertussis* occurred in a completely empirical way, after the culture of this bacterium in the laboratory by Jules Bordet and Octave Gengou, of the Pasteur Institute in Brussels in 1906 [23]. The first effective pertussis vaccine was developed in the 1930s by Pearl Kendrick and Grace Eldering using killed whole *B. pertussis* cells [24]. The introduction of such whole cell pertussis vaccines (wPs) in the late 1940s, right after combined with the diphtheria and tetanus toxoids for the formulation of the triple bacterial vaccine (DTP) [25], greatly reduced the incidence of the disease [9], leading to its almost eradication in the early 1970s [10]. However, although effective, wPs were associated with undesirable side effects, which led to a decrease in the acceptance of these vaccines and the rapid increase of pertussis incidence in several

countries [26, 27]. In Great Britain, by 1977, the vaccination coverage rate for pertussis fell from 77 to 33%, and up to 9%, in some districts [28]. In Japan, the government suspended pertussis vaccination in February 1975, due to widespread publicity of two deaths in children, allegedly related to the vaccine, leading to a whopping cough peak two years later, accounting for 13,000 reported cases and 40 deaths [29, 30]. The reactogenicity of wPs was extensively evaluated in DTP, and the pertussis component proved to be mainly responsible for the toxicity of these combined vaccines. Summarizing the findings from these analyses, some authors report a prospective study conducted in Los Angeles from January 1978 to December 1979 in children of 0–6 years old, involving 15,752 doses of DTP and 784 doses of DT. The children were evaluated for local and systemic reactions occurring within 48 hours of immunization. Overall, all local and systemic reactions were significantly more frequent in children who had taken DTP vaccine than DT. At the site of application, redness, swelling, and pain occurred in 37.4, 40.7, and 50.9%, respectively, in those receiving DTP, but only 7.6, 7.6, and 9.9%, respectively, in those who received DT. The percentage of these reactions in DTP vaccinated increased from the first to the fifth dose [9].

The global consequence of the refusal to accept the wP vaccines resulted in the development of the first acellular pertussis vaccine (aP), the Japanese vaccine of Sato et al. [31], containing purified antigens from the bacterium. As there was an ongoing pertussis epidemic at that time, DTaP vaccines were very rapidly developed in Japan and immediately incorporated into their vaccine calendar in 1981 [32, 33]. In the late 1990s, the wPs were gradually replaced by aPs in many developed countries [34]. Current aP vaccines contain 1–5 purified pertussis proteins: inactivated PT, filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2 and 3 [35].

Nowadays, DTP vaccines are available in various formulations, containing whole cell (wP) or acellular (aP) pertussis component combined with diphtheria toxoid (D/d) and tetanus toxoid (T) to produce either full-strength— diphtheria/tetanus/wP (DTwP) or aP (DTaP) vaccines—or reduced antigen-content (Tdap) vaccines, which are used for primary (DTwP or DTaP) or booster (Tdap) immunization. Whole cell pertussis vaccines are not indicated for individuals over seven years of age, and WHO only recommends formulations with lower concentrations of diphtheria toxoid and pertussis (Tdap and Td) in order to reduce their reactogenicity [36, 37]. More recently, DTP is presented as the basis for combined vaccines containing additional antigens added alone or in combinations, such as *Haemophilus influenzae* type b (Hib), hepatitis B, and inactivated poliovirus [38–43], allowing the administration of multiple vaccine antigens in a single injection, leading to the induction of simultaneous immunity for multiple diseases [44, 45]. These combined vaccines were approved by the World Health Organization's Expanded Program on Immunization (EPI) [46–48], which substantially reduced the number of injections required in the childhood vaccine schedule. Clinical studies using the DTPa-HBV-IPV/Hib hexavalent vaccine have shown that it is safe and effective [45, 49], and the incidence of local and systemic adverse reactions was comparable to those observed after administration of single vaccines or other DTaP-based vaccines [50–53], always less reactogenic than the combinations using whole cell pertussis vaccine (DTPw) [52].

3. Pertussis resurgence: a multifactorial problem

The preliminary clinical trials comparing DTaP with DTwP in the 1990s suggested comparable efficacy and immunogenicity [54–58]. However, more recent data have shown that the disease is not adequately controlled and outbreaks have occurred, even in countries with extensive vaccine coverage [59–61]. The reasons for the apparent ineffectiveness of current vaccines and vaccination programs in the control of infection and transmission are unclear, but there are likely to be a number of factors contributing to the short-lived immunity after vaccination [62–64]. DTaP vaccines were licensed and recommended as a booster in the USA in 1992 and introduced as primary immunization in newborns in 1997. Currently, even with 95% vaccine coverage in newborns and use of booster dose in adolescents, pertussis is the least immunopreventable disease, with the highest incidence rates already reported in the post-vaccination era [65]. Possible reasons for the resurgence of pertussis include a reduction in vaccine efficacy, with rapid waning immunity, improvement in the epidemiological surveillance and diagnostic methods, and genetic changes in the pathogen [66].

DTaP vaccination induces excellent, but not durable, immune response [67–69]. The higher antigenic load of the wPs may explain the epidemiological evidence that supports the longer lasting protection induced by these vaccines, in relation to the aPs. Potentially protective antigens may be absent or may be in insufficient quantity in the aP formulations, or may exhibit poor cross-match with antigens present in the circulating bacterial strains [70]. The immunity conferred by DTaP drops every year after the fifth dose, so that 5 years after the last dose the probability of a child vaccinated with this vaccine to acquire the disease is four to fifteen times greater than that after the initial doses [63, 64, 71, 72], and 80% of these children are no longer protected at the time of Tdap booster [73]. The efficacy conferred by Tdap was 75.3% in a pertussis outbreak in Wisconsin in 2012, falling after 2 years to 34.5% [74].

Differences between wP and aP vaccines, related to its antigenic load and presentation of antigens to antigen presenting cells, lead to a different balance of Th1/Th2/Th17 response. The role of Th1 and Th17 cells has been demonstrated in the protective immunity induced by *B. pertussis* infection or immunization with wP, and on the other hand, immunization with an aP vaccine administered with alum as adjuvant induced Th2 and Th17 cells, but poor Th1 response [75]. The multiple virulence factors of *B. pertussis*, many of them efficiently maintained in the wP vaccines, presuppose a better stimulation of innate immunity, leading to the generation of effective Th1/Th17-skewed adaptive immunity [76].

The probability of contracting the disease of humans primed with DTwP is lower than those primed with DTaP [62, 63, 68, 69]. Adolescents vaccinated with three doses of DTaP were 3.3 times more likely to contract pertussis than children vaccinated only with DTwP [46, 62]. These data were recently confirmed in baboons, an animal model that reproduces the characteristics of human infection [77–79]. Baboons immunized with DTaP and challenged with a clinical isolate of *B. pertussis* are heavily colonized and do not control the infection until 4–5 weeks, transmitting the bacteria to naive animals. Those vaccinated with DTwP are colonized, but without leukocytosis, and control the infection in 2–3 weeks, faster than those not previously vaccinated [78].

There is evidence that circulating strains of *B. pertussis* are evolving to evade the vaccine-conferred immunity [80]. In fact, pertactin-deficient *B. pertussis* strains were identified in 85% of the isolates obtained from eight US states between 2011 and 2013 [81]. These samples emerged rapidly and did not express the PRN contained in the DTaP vaccine, and suggesting selective advantage, individuals previously vaccinated against pertussis had higher chance of infection with the PRN-deficient strains than with the strain expressing that protein [82–85]. Besides that, an increase in the incidence of vaccine alleles of *B. pertussis* could also suggest an evolutionary epitope-mediated vaccine pressure [86–89], contributing to the reemergence of pertussis in humans, and in this sense, it is also not clear how the *B. parapertussis* can answer to the selective pressure exerted by large-scale vaccination against *B. pertussis*.

Although highly effective in reducing the incidence of pertussis infections, the acellular pertussis vaccines have little or no efficacy against *B. parapertussis* [17, 90, 91]. Some authors have postulated that vaccination with aPs can interfere with the "clearance" of *B. parapertussis*, facilitating the adaptive performance of this pathogen, which could lead to the emergence of more susceptible hosts to *B. parapertussis* infection [92]. Accordingly, a gradual increase in the prevalence of *B. parapertussis* has been observed as a result of epidemiological pertussis immunization with vaccines that are less protective against *B. parapertussis* than the natural infection with *B. pertussis* [93]. Similar to the serum specificity observed in other infectious diseases, pertussis vaccines may have led to epidemiological pressure, with an increase in the prevalence of *B. parapertussis*. Since the differential diagnosis would not affect clinical procedures, the vast majority of pertussis studies are not directed to the identification of *B. parapertussis*, which probably has led to unreported cases. However, studies aimed at the differential diagnosis showed that *B. parapertussis* comprise from 2 to 36% of the cases [94].

In August 2015, the World Health Organization published its position on pertussis vaccines [95], in an attempt to provide substantiated information for immunization and public health programs, in a document that replaces the previous one published in 2010 [35]. The main goal of this position paper was to guide the choice of pertussis vaccines—wP or aP—to the most current strategies to reduce the risk of pertussis in infants and young children. In this document, it was established that the goal to be achieved in all countries is the maintenance of high vaccination coverage (higher than 90%). High levels of safety and protection can be obtained by the wP and the aP vaccines, after the primary series of immunization with three doses, ideally completed by the sixth month of life (**Table 1**). However, although systemic and local reactions are more commonly associated with wP, the duration of protection conferred by these vaccines is longer [96–98]. The pertussis vaccination schedule should maintain protection for at least six years in countries using wP, but the protection may suffer a marked decline before the age of six years when aP is used (**Table 1**) [95]. Vaccination of pregnant women has been recommended by WHO as the best strategy for disease prevention in infants too young to be vaccinated or with incomplete immunization schedule, and the change from wP to aP in primary immunization should only be considered in countries that are able to maintain a schedule with periodic reinforcement and sustainable maternal immunization. If this is not the case, immunization with wP should be maintained and in national programs using aP, consideration should be given to the introduction of additional booster doses in the case of pertussis reemergence [95]. The production cost of the aP vaccine is considerably higher than that of the wP (difference of more than 5 US$ per dose with PAHO's revolving

Balance of benefits and costs	Acellular pertussis vaccine (aP)	Whole cell pertussis vaccine (wP)
Quality of evidence for benefits	Highly effective	Highly effective
Intervention effects	• Primary series reduces the risk of severe pertussis • Lower incidence of adverse reactions than with wP vaccine [96, 97]	• Primary series reduces the risk of severe pertussis • Primary series not associated with serious adverse effects
Duration of protection	• May decline before 6 years	• At least 6 years • Longer than that induced by aP [98]
Resource implications	• Significantly more expensive than the wP vaccine	• Significantly less expensive than the aP vaccine

Table 1. WHO evidences to pertussis vaccines [95].

fund price), which has a direct implication in health systems, especially in underdeveloped and developing countries.

Considering cost-effectiveness in the implementation of national vaccination programs, some authors have pointed out questions about the possibility of reintroduction of wP vaccines into the primary doses of pertussis vaccination [76, 99, 100], which could again lead to the problems with the reactogenicity of these vaccines.

4. Back to the past: whole cell pertussis vaccine as a new alternative

In Brazil, mandatory notification of all outbreaks began in 1975 when the pertussis entered the list of notifiable diseases. In the early 1980s, more than 40,000 cases were reported per year, with an incidence rate >30/100,000 inhabitants. With the introduction of the DTP vaccine in the Brazilian scheme of childhood vaccination in 1983, this number fell sharply [101]. In 2002, the first three doses of the DTwP were replaced by the tetravalent vaccine DTwP + *H. influenzae* type B (DTwP-Hib), that in 2012 was replaced by the pentavalent DTwP + *H. influenzae* type B + hepatitis B (DTwP-Hib-HBV). The first three doses of the pentavalent vaccine are administered at 2, 4, and 6 months of age, followed by DTwP booster at 15 and 48 months of age. DTaP vaccine is recommended for children at increased risk of developing or who have developed severe adverse events to the DTwP, and the vaccine is available at the Special Immunobiological Reference Centers. After 2014 the Brazilian National Immunization Program began to offer Tdap to pregnant women [102]. Despite the high vaccination coverage (>95%) since 2011, a significant increase in the number of reported cases of pertussis in Brazil has been observed, with an incidence rate in 2013 of 14,058 confirmed cases/100,000 in infants under one year of age, the majority of cases and deaths in unvaccinated children younger than 4 months old [103].

The Butantan Institute in São Paulo, Brazil, produces DTwP vaccine since 1953. Currently, more than 90% of Brazilian children are vaccinated at the age of 2/4/6 and 15 months life,

which are about 250 million doses annually. Over the past 20 years, the Institute has been investigating new pertussis vaccines, less reactogenic and at low cost [104].

Although effective, wP vaccines contain a significant amount of lipooligosaccharide (LOS), an endotoxin of Gram-negative outer membrane that may be involved in the local and systemic adverse vaccine reactions. The introduction of procedures that increase the safety of wP vaccines maintaining its effectiveness remains a very important aspect, especially for developing countries that do not have access to currently available aP vaccines. In this sense, a whole cell pertussis vaccine was developed with low endotoxicity (Plow) obtained by chemical extraction of the LOS fraction from the outer membrane of the bacterial cell [105]. This vaccine was evaluated as DTwP vaccine, combined with tetanus and diphtheria toxoids in a Phase I field trial in infants, showing to be less reactogenic and equally immunogenic and protective as the traditional DTP vaccine [106].

Many developed countries using acellular pertussis vaccines in infancy have introduced a booster dose for adolescents [107], preventing the carrier state, an attempt to block the spread of the disease to infants not immunized or with incomplete immunization schedule. Due to its low reactogenicity, the Plow vaccine may possibly be introduced into the vaccination schedule for immunization of adolescents and young adults in Brazil, an important epidemiological contribution to reducing the circulation of *B. pertussis*.

Preliminary studies in our laboratory have shown that the Plow is able to protect mice against *B. parapertussis* (unpublished data), suggesting an important role in the control of this pathogen, which has not been reached by vaccination with acellular pertussis vaccines [17, 90, 91, 108, 109].

The cost to produce the Plow vaccine is the same as the conventional whole cell pertussis vaccine, which makes its use feasible in developing countries, such as Brazil [89, 110], as an alternative for use in different strategies for the control of pertussis resurgence, including vaccination of adolescents and adults, due to their lower reactogenicity.

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