

The Diphtheria and Pertussis Components of Diphtheria-Tetanus Toxoids–Pertussis Vaccine Should Be Genetically Inactivated Mutant Toxins

John B. Robbins,¹ Rachel Schneerson,¹ Birger Trollfors,² Hiroko Sato,^a Yuji Sato,^a Rino Rappuoli,³ and Jerry M. Keith¹

¹National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; ²Department of Pediatrics, University of Göteborg, Sweden; ³Chiron Vaccines, Siena, Italy

Replacement of cellular with acellular pertussis (aP) vaccines has considerably reduced the systemic reactions observed with diphtheria-tetanus toxoids–pertussis vaccine but has not eliminated the extensive swelling (sometimes involving an entire limb) observed after the fifth injection of diphtheria-tetanus toxoids–aP (DTaP) vaccine. This local reaction, which is likely an Arthus hypersensitivity reaction caused by high levels of antibodies reacting with DTaP vaccine, could discourage its use in adults, who serve as the major reservoir of pertussis for infants. That a critical level of antibodies to pertussis toxin is both essential and sufficient to prevent infection with *Bordetella pertussis* is derived from data from animal and clinical studies, including data showing the similarities between the immunity induced by diphtheria and pertussis toxoids. The genetically inactivated diphtheria and pertussis mutant toxins are more immunogenic and, therefore, induce comparable levels of antitoxin at lower protein levels than do the formalin-treated native toxins. Replacement of the diphtheria and aP components with these improved antigens will reduce the amount of protein in DTaP vaccine and, most likely, the incidence and severity of local reactions in teenagers and adults.

Prompted by the local adverse reactions to the fifth injection of diphtheria-tetanus toxoids–acellular pertussis (DTaP) vaccine, their potential for negative publicity, and reduced acceptance of the acellular pertussis (aP) vaccine in adults, we propose that the diphtheria and pertussis components be replaced with the more immunogenic, and theoretically safer, genetically inactivated mutant toxins [1–8]. These local reactions most likely are Arthus hypersensitivity reactions mediated by high antibody levels induced by primary immunization with diphtheria-tet-

anus toxoids–pertussis (DTP) and DTaP vaccine. Reduction of the protein content of DTaP vaccine can be achieved by (1) replacing the current toxoids with the genetically inactivated and more immunogenic diphtheria and pertussis mutant toxins or (2) using monocomponent pertussis toxoid for the aP.

This proposal is not controversial for the diphtheria component. However, no consensus exists as yet with respect to what the aP component should be, as there are licensed pertussis vaccines composed of 1, 2, 3, and 5 components. Consensus exists that pertussis toxoid is essential, but protective antibody levels for the non-pertussis toxin (PT) components (filamentous hemagglutinin [FHA], pertactin, and fimbriae) have not been established. Two aspects of pertussis vaccine-induced immunity contribute to this dilemma: (1) the efficacy of multicomponent aP vaccines

has been overestimated, because the antigens used for serologic diagnosis are also vaccine components, and (2) herd immunity requires vaccination of at least one-half of the population, and, after the recent trials of multicomponent aP vaccines, the incidence of pertussis in the entire community was not determined.

Although the 2 diseases are different, the similarities between toxoid-induced immunity to diphtheria and pertussis provide insight. First, Tox⁺ *Corynebacterium diphtheriae* and *Bordetella pertussis* are pathogens for and inhabitants of people only; they have no animal vectors or reservoirs [9–11]. Second, both diseases result from colonization that is limited to the respiratory epithelium; no other tissues are invaded. Third, both pathogens secrete an exotoxin (ADP ribosyl transferase) that is essential for disease. Fourth, vaccination with their respective toxoids

Received 26 March 2004; accepted 21 July 2004; electronically published 30 November 2004.

^a H.S. and Y.S. are retired.

Reprints or correspondence: Dr. John B. Robbins, National Institutes of Health, Bldg. 31, Rm. 2A-25, Bethesda, MD 20892 (robbsjjo@mail.nih.gov).

The Journal of Infectious Diseases 2005;191:81–88

© 2005 by the Infectious Diseases Society of America. All rights reserved.

0022-1899/2005/19101-0013\$15.00

prevents diphtheria and pertussis and inhibits colonization with and transmission of Tox⁺ *C. diphtheriae* and *B. pertussis*, resulting in herd immunity [10, 11].

DIPHTHERIA

There has never been a randomized, double-blind, controlled study of diphtheria toxoids—in the past, diphtheria was too common and too severe to delay implementation of improvements in prophylaxis. In the United States of 1910, 0.1% of children <5 years of age died of diphtheria annually [12]. Diphtheria has been virtually eliminated in countries that have a high level of routine vaccination of infants and children [11].

The efficacy of diphtheria toxoid against disease is only ~75%, but it is close to 100% protective against death [11, 13]. Diphtheria may occur in patients with antitoxin levels considered to be protective (>0.01 IU/mL) [13]. At least one-half of adults in developed countries do not have “protective” antitoxin levels, although most received diphtheria toxoid during childhood [11].

So how was virtual elimination in the developed world achieved when the toxoid vaccine is only ~75% protective, diphtheria can occur in individuals with protective levels of antitoxin, and at least one-half of the population does not have protective antitoxin levels? Prompted by outbreaks after World War II, the World Health Organization (WHO) sponsored trials of diphtheria toxoid in Romania [14]. Between 1958 and 1972, most of the population was vaccinated. The number of cases decreased significantly after 5 years, when ~60% of the population was vaccinated; after 7 years, tox⁺ *C. diphtheriae* was no longer detectable. This is the basis for toxoid-induced herd immunity—the pathogen is no longer present.

A genetically inactivated, nontoxic, fully immunogenic diphtheria toxin—denoted “CRM₁₉₇”—has been isolated [15, 16]. CRM₁₉₇ differs from the native toxin by a substitution of C52 glycine with glutamic

acid and requires only 1/100 of the formalin needed for inactivation of the wild type [17]. Safety is assured by the extensive experience with CRM₁₉₇ as a carrier of *Haemophilus influenzae* type b and pneumococcal polysaccharide conjugates. CRM₁₉₇ would provide a more immunogenic diphtheria component of DTP, with less protein and fewer adverse reactions [18].

PERTUSSIS

Pertussis is a coughing disease caused by *B. pertussis*. Although the 3 major species of the genus *Bordetella*—*B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*—have the PT structural gene, it is expressed only by *B. pertussis* [19–22]. The 1949 observation of the highest incidence in children 3–5 years of age, with most deaths in children <1 year of age, still stands [23, 24]. DTP vaccination was designed to prevent this high death rate in infants.

Pertussis is a highly contagious infection of the respiratory epithelium. After inhalation, *B. pertussis* adheres to the cilia. Within 7 days, a mild, nonfebrile, non-specific upper respiratory tract infection occurs. The coughing becomes more frequent, prolonged, and, in 2 weeks, paroxysmal. The paroxysms may last as long as 3–5 min, exhaust the patient, and cause apnea with cyanosis. The resultant hypoxia and hypercapnia stimulate gasps or whoops, often provoke vomiting, and may result in aspiration pneumonia.

During the first week of illness, when the symptoms are mild and nondiagnostic, nasopharyngeal cultures are positive >80% of the time. As the paroxysmal coughing becomes manifest, *B. pertussis* gradually disappears from the respiratory tract—that is, the symptoms of pertussis occur in the absence of the pathogen [25]. This explains why the quarantine period was only 4 weeks, even though the paroxysmal coughing continues for 6–12 weeks, and why antibiotics do not affect the duration or severity of paroxysmal coughing [26, 27]. Macrolide antibiotics will prevent pertussis in case contacts and

patients who have mild cough of <1 week's duration, and antibiotics will treat secondary infections.

Intracerebral (ic)–challenge potency assay. Vaccine development followed the principles expounded by Pasteur—iso-late, inactivate, and inject the causative organism. The first vaccines were difficult to standardize, because neither the protective antigen nor the host immune factors were known. Kendrick et al.'s ic challenge of mice was quantitated by Pittman [28, 29], by use of the following assay. Mice are immunized intraperitoneally and are challenged ic 2 weeks later. Mice that die within 2 days of the ic challenge are omitted from the assay. Two weeks later, survival is compared with that afforded by a vaccine standard. It was found that immunization with *B. parapertussis* and *B. bronchiseptica*, which do not express PT, does not confer protection [30]. The limitations of this assay are that mice do not cough and there is no spread of *B. pertussis* from infected mice within litters. But the pathogenesis of the ic challenge does mirror the events on the pulmonary epithelium. *B. pertussis* adheres to the cilia of the cerebral ventricles; there are no positive blood cultures or direct invasion of the brain [31]. Of the *Bordetella* species, only *B. pertussis* causes mice to die; toxin-deficient mutants are not lethal [32], and only PT antibodies, actively induced or passively administered as monoclonal or polyclonal antibodies, protect against ic challenge [33, 34]. The incidence and severity of coughing caused by *B. parapertussis* are less than those caused by *B. pertussis* [35, 36], and we have not found an instance in which *B. parapertussis* infection has caused hospitalization or death. In summary, the ic-challenge potency assay is a test for anti-PT IgG.

Pertussis in the United States. Beginning in the 1950s, increasing use of cellular vaccines continuously reduced pertussis. Their apparent efficacy was related to the severity of pertussis. When the cri-

teria used were a positive culture, paroxysmal coughing, and whooping followed by vomiting, the apparent efficacy was as high as 90% [37]. When a positive culture was not required and the criterion used was 2 weeks of coughing, the apparent efficacy was <70%.

Cellular vaccines prevent pertussis by several methods. Pertussis is spread only by ill individuals (via coughing); there is no asymptomatic carriage [38]. Vaccination has reduced the incidence and severity of pertussis and colonization by *B. pertussis* [39], resulting in herd immunity [11]. To illustrate, in 1990 the birth rate was ~4 million births/year in the United States, and there were ~24 million 1–6-year-old children in the country. Approximately 85% of children received ≥ 3 DTP injections by 6 years of age, leaving ~3.6 million 1–6-year-old children susceptible to pertussis. The attack rate of pertussis in nonvaccinated children is ~5% annually. There should have been ~180,000 cases of pertussis annually, but there were only ~3500 reported cases, or 2% of the predicted number.

Immunity induced by cellular vaccines or pertussis lasts ~5–20 years [40, 41]. During the past decade, there has been an apparent increase in pertussis in children <1 year of age, older children, and adults, for 3 reasons [42]: (1) inaccurate publicity during the 1980s and early 1990s led to decreased use of cellular vaccines and an increase in pertussis in older children and adults [43]; (2) a greater awareness of pertussis in adults, who do not experience the childhood symptoms of whooping, vomiting, and lymphocytosis; and (3) more laboratories becoming capable of identifying *B. pertussis*.

Pertussis in adults. Pertussis in adults as a source of infection in infants was described in the 1920s but was not considered to be an important cause of coughing in this age group until the 1960s [44, 45]. Only tetanus and diphtheria toxoids were administered to adults, because cellular vaccines were too toxic [46].

PERTUSSIS IN JAPAN

In 1950, it was recommended that DTP be used for routine vaccination of 3-month-old infants in Japan. The incidence of pertussis in Japan rapidly decreased until 1974, when the death of 2 infants after injection of DTP was widely publicized, and pertussis vaccination was temporarily suspended. The age at which initial vaccination with cellular vaccine was performed was increased from 3 months to 2 years. Despite this measure, vaccination decreased to nearly 10%, and the incidence of pertussis increased from 0.4 cases/100,000 population in 1974 to 11.3 cases/100,000 population in 1979. At about the same time, similar events occurred in the United Kingdom.

The first aP vaccine composed of pertussis toxoid and FHA was developed in Japan [47]. Its availability led to an increase in the vaccination rate, to >80%. In 1988, it was recommended that DTaP be used for routine immunization of 3-month-old infants; in 1993, only 130 cases were reported, mostly in incompletely or unvaccinated patients. Components other than FHA and pertussis toxoid were trace components [48, 49]. In light of the evidence for FHA (vide infra), the Japanese aP vaccine can be regarded as a pertussis toxoid vaccine.

ANTIGENS OF *B. PERTUSSIS*

Pittman's hypothesis, that the symptoms of and immunity to pertussis were mediated by a toxin, inspired the study of this and other proteins of *B. pertussis* [49, 50]. A master regulatory gene (*bvg*) for the synthesis of *B. pertussis* virulence factors (PT, FHA, pertactin, and adenylate cyclase) was discovered, and the realization that not all were protective followed [51, 52].

FHA. Although the other *Bordetella* species produce FHA, only immunization with *B. pertussis* confers protection in the ic-challenge potency assay [28, 53]. Neither active nor passive immunization with

polyclonal or monoclonal antibodies to FHA confers protection against ic or pulmonary challenge with *B. pertussis* [35]. In mice, naturally occurring or Tn5 transposon-induced mutants that are deficient in FHA continue to cause death and confer protection [32, 51]. In the first trial of aP vaccines in Sweden, no statistically significant difference was found between the protection conferred by the monovalent pertussis toxoid (JN1H-7) and that conferred by the divalent toxoid plus FHA (JN1H-6) [54]. Seroepidemiological studies showed that only PT antibodies conferred immunity [55–57]. The cellular vaccine produced by Lederle Laboratories was successfully used in the United States and induced 91% protection in a case-controlled study [58]. This vaccine did not contain FHA and did not induce FHA antibodies [59]. The absence of FHA in Lederle Laboratories' cellular vaccine, however, has not been mentioned in analyses of these clinical trials [60–62]. Last, infection with culture-confirmed *B. parapertussis* induced FHA and pertactin antibody levels similar to those that occur after pertussis but did not induce PT antibodies and did not confer immunity against pertussis [63, 64]. In summary, overwhelming evidence indicates that FHA is not a protective antigen for *B. pertussis*.

Pertactin. Neither active nor passive immunization with pertactin confers protection against ic challenge [65]. In a pulmonary challenge model, active immunization with 16 μ g of pertactin induced 94% protection, but 8 μ g of glutaraldehyde-treated PT induced 100% protection [66]. The addition of pertactin did not increase the efficacy of an aP vaccine containing pertussis toxoid and FHA [67]. The age-related appearance of pertactin antibodies without a history of infection with *Bordetella* or vaccination with a pertussis vaccine suggests cross-reacting antigens [68]. The experience in Japan and in patients with parapertussis indicates that pertactin is not essential for immunity to pertussis [49].

Fimbriae. Extensive characterization of the fimbriae (agglutinogens) of *B. pertussis* has been achieved [69]. However, there are no convincing data showing that fimbriae could serve alone or contribute to the efficacy of a pertussis vaccine [70].

PT. PT conforms to the AB model of toxins. The B subunit binds to host surface cells, and the A subunit is an ADP ribosyl transferase [71].

PT causes metabolic effects [72]. Hypoglycemia has been reported in infants convalescent from pertussis and on use of higher-than-recommended doses of pertussis vaccine. The lethal effect that pertussis vaccines have on fetal mice is prevented by administration of glucose. PT induces a paradoxical effect on glucose regulation: administration of adrenaline to mice lowered insulin and increased blood glucose; in mice infected with *B. pertussis* or injected with PT, administration of adrenaline increased blood insulin and lowered glucose. PT-induced hypoglycemia prompted its designation as an islet-activating protein and is probably the mechanism involved in the increased sensitivity to histamine caused by this toxin (histamine sensitizing factor [HSF]) in animals and humans [72, 73]. HSF activity is the basis for a bioassay for residual PT in both cellular and aP vaccines.

It is the inhibitory effect on lymphoid cells that offers the best explanation for the role played by PT in virulence. The striking lymphocytosis is due to the loss of the ability of the lymphocytes to "home" to lymphatic tissue once they have entered the circulation. In vitro, macrophages exposed to PT do not respond to lipopolysaccharide [74]. Antibodies inhibit the action of PT on lymphoid cells, thereby promoting phagocytosis of *B. pertussis*.

Human immunoglobulin, with high levels of anti-PT, is therapeutic, which is consistent with the observation that PT is released from lymphoid tissue and, therefore, is available for neutralization by antitoxin [75, 76]. Rats challenged intrabronchially with *B. pertussis* embedded in

fine agarose beads develop paroxysmal coughing, leukocytosis, and immunologic responses similar to those of humans. Expression of PT by the challenge strain is essential for the coughing [76]. Immunization with pertussis toxoid, but not FHA or pertactin, confers immunity in this model [77, 78].

CLINICAL TRIALS OF aP VACCINES IN EUROPE

First Swedish study. Two vaccines, JN1H-6 (pertussis toxoid and FHA) and JN1H-7 (pertussis toxoid only), and a control (alum) were evaluated in a randomized, controlled, blinded trial [54]. Two injections were administered at 5–11 months of age and then 2 months later. JN1H-6 and JN1H-7 were found to be safer than cellular vaccines, and both conferred statistically significant protection. Initially, it was concluded that the efficacy of JN1H-6 was higher than that of JN1H-7, but the limitations of diagnosis based on serologic testing were soon realized: a calculation with the 181 cases listed in table 1 gives efficacy estimates of –7% for JN1H-7 and 42% for JN1H-6. However, such a crude comparison is based on the fact that the antigens used for diagnosis were also used for immunization [79]. Actuarial analyses from household contacts showed that JN1H-7 was equal to or more protective than JN1H-6 [80]. The apparent lack of correlation between the level of antibodies and protection was vexing, but pertussis occurred at a significantly higher rate in unimmunized control subjects. Conclusions drawn from this study had a profound effect.

WHO criteria. Because of the controversies that followed this study, a consensus for diagnostic criteria of pertussis was reached by a multinational committee [80]. Diagnosis required ≥ 21 days of paroxysmal coughing and a positive culture. Without a culture, diagnosis could be made by the demonstration of a statistically significant increase in either anti-FHA or anti-PT IgG levels. Contact in a

household with a culture-confirmed case was also considered to be diagnostic.

Monocomponent pertussis toxoid vaccine. PT, inactivated with H_2O_2 (National Institute of Child Health and Human Development [NICHD] Ptxd), was found to be safe and immunogenic in adults [81] and in 18–24-month-old children and, in a preliminary trial, was found to be protective in infants [82, 83]. In a randomized, double-blind trial with diphtheria toxoid as the control, 3450 infants were inoculated at 3, 5, and 12 months of age. There were no serious adverse reactions and no uncontrollable screaming or crying. After active surveillance for a median of 17.5 months, the efficacy of NICHD Ptxd was 71% ($P < .001$) [84], and it did not change in the ensuing 6 months. NICHD Ptxd induced the same protective mechanisms as did cellular and multivalent aP vaccines [85]: it (1) reduced the number of positive cultures and the severity of the disease [79] and (2) prevented infection in household contacts (76%) and inhibited transmission of *B. pertussis* to household members [85].

Recipients of NICHD Ptxd or those control subjects who contracted pertussis had a similar increase ($>90\%$) in anti-FHA IgG levels. However, only $\sim 50\%$ of recipients of NICHD Ptxd had an increase in anti-PT IgG levels, whereas $>90\%$ of those control subjects who contracted pertussis had a such an increase [86]. A correlation was observed between the incidence and severity of pertussis 1 month after the third vaccination and anti-PT IgG levels [87]. There was a statistically significant relationship between postvaccination serum IgG against PT and protection against pertussis in recipients of NICHD Ptxd. Children with severe pertussis in the NICHD Ptxd group had significantly lower anti-PT IgG levels than did children with mild or no pertussis ($P < .001$). During 24 months of surveillance, there was an 18-fold decrease in vaccine-induced anti-PT IgG levels without a change in efficacy, indicating that protective levels are considerably lower than

those observed 2–3 months after the third injection. This is similar to the case of diphtheria antitoxin, in which a post-immunization level of 0.1 IU/mL is considered to be predictive of long-term protection, and a postimmunization level of 0.01 IU/mL is considered to be protective.

Mass vaccination with NICHD Ptxd.

From 1979 to 1995, there was no vaccination against pertussis in Sweden. After completion of the trial of NICHD Ptxd, mass vaccination of children born during the 1990s was begun in the Göteborg area (population, 778,597 people) [88]. Control subjects from the clinical trial and children up to 10 years of age were given a full course of NICHD Ptxd. Infants were inoculated at 3, 5, and 12 months of age, in accordance with the Swedish recommendations for DTP. Over the next 3 years, 167,810 doses were administered to 62,129 children, such that ~56% of the children in Göteborg received 3 doses of NICHD Ptxd. Assuming that the attack rate of pertussis in this age group was 5% annually, ~70% of the population up to 10 years of age were supposedly immune to pertussis during the 3-year period of immunization. The number of *B. pertussis* isolates that were identified decreased from 1214 during 1993–1995 to 64 from January 1997 to June 1999 ($P < .0001$). Hospitalizations due to pertussis in individuals of all ages, including adults and unvaccinated young children, decreased from 62 to 5 ($P < .0001$). Herd immunity was observed, as the number of isolates identified in individuals of all ages decreased. This decrease was noted ~1 year before the introduction of multivalent aP vaccines in the remainder of Sweden [89]. A similar effect of herd immunity after mass immunization with an aP vaccine has been reported in Senegal [90].

Clinical trials of multivalent aP vaccines. Several double-blind, randomized, case-controlled trials of aP vaccines [58, 67, 91, 92] have used pertussis toxoid, FHA, and pertactin [57, 90, 91], and one study has also used fimbriae [91]. The apparent greater efficacy of most

multivalent aP vaccines, compared with that of the monovalent NICHD Ptxd vaccine, is the given reason for the inclusion of these *B. pertussis* components [59–61]. However, the lower percentage of positive cultures and the smaller increase in anti-PT and/or anti-FHA IgG levels in vaccine recipients than in control subjects create an artifact in the calculation of the efficacy of multivalent vaccines when WHO criteria are used for diagnosis [79, 84, 86]. To illustrate, if 100 vaccine recipients and 100 control subjects have ≥ 21 days of paroxysmal coughing, 70% of control subjects and 50% of vaccine recipients will have positive cultures. Now there are 30 control subjects and 50 vaccine recipients to be analyzed by an increase in anti-FHA or anti-PT IgG levels. With respect to monovalent pertussis toxoid vaccine, 90% (45/50) of vaccine recipients and 90% (27/30) of control subjects will have an increase in anti-FHA IgG levels. In contrast, 90% (27/30) of control subjects but only 50% (25/50) of vaccine recipients will have an increase in anti-PT IgG levels. Accordingly, 5 vaccine recipients and 3 control subjects will be eliminated from the efficacy calculation. However, this is not the case for recipients of multivalent vaccines. There are still 30 control subjects and 50 vaccine recipients to be analyzed by serologic testing. Increases in anti-FHA or anti-PT IgG levels continue to occur in 90% (45/50) of control subjects but in only 50% (25/50) of vaccine recipients. Accordingly, 25 recipients of DTaP vaccine but only 5 control subjects will be eliminated from the efficacy calculation, resulting in an apparent greater efficacy conferred by multivalent aP vaccines than by monovalent toxoid vaccines. Last, despite the torrent of articles touting *B. pertussis* antigens, no one has suggested that there be an aP vaccine that does not include pertussis toxoid.

Mass vaccination in Sweden with multivalent aP vaccine. In Sweden, the introduction of aP vaccine to the routine immunization of infants began in 1999.

Since then, there has been a continual reduction in the incidence of pertussis [90].

Pertussis in young infants and older children. Cellular pertussis vaccines induced a relatively high rate of local and systemic reactions, including fever and convulsions. Inaccurate and irresponsible publicity reported these vaccines to be ineffective, as well as a cause of central nervous system damage and sudden infant death syndrome [43]. This led to a decrease in their acceptance that began around 1975 and that resulted in a large population of older children and young adults being susceptible to *B. pertussis* [7, 24, 42]. The new pool of susceptible individuals increased the transmission of *B. pertussis* to infants who had not yet completed their vaccination. To protect these susceptible individuals, there has been an increasing call for the immunization of adults with DTaP vaccine rather than with tetanus and diphtheria toxoids [3, 42, 84, 87, 92–94]. Indeed, the immunization of adults with DTaP vaccine is now recommended in several European countries.

ADVERSE REACTIONS TO MULTIVALENT aP VACCINES

The substitution of DTP vaccine with DTaP vaccine has brought attention to an adverse reaction that is considered to be minor. After the fifth injection of DTaP vaccine, local reactions include erythema and swelling >5 cm in $>25\%$ of recipients and fever (mostly mild) in $\sim 30\%$ [1–9]. Approximately 5% of these swelling reactions involve almost the entire limb and last for ~ 1 week. These reactions are only slightly less frequent and severe than those that occur after the fifth injection of DTP vaccine, which causes a higher incidence (60%) and a more severe degree of pain and fever than does DTaP vaccine [7]. A relationship between the level of antibodies elicited by the fifth dose of DTaP vaccine and the size of swelling has been shown [5].

The large local reactions elicited by the fifth dose of DTaP vaccine most likely are Arthus hypersensitivity reactions, which

require high levels of antigens and antibodies. The aP vaccine elicited higher antitoxin levels than did the DTP vaccine. Importantly, the inclusion of FHA, pertactin, and fimbriae in the aP component increased the amount of antigens in the DTaP vaccine. In one study, the PT and FHA were purified by column chromatography [95]. The “crude” and purified PT and FHA were treated with formalin and were combined with diphtheria and tetanus toxoids. The 2 formulations induced similar levels of FHA and PT antibodies, but recipients of the crude PT had larger areas of swelling than did recipients of the purified antigen. Omission of the nontoxoid antigens from the aP vaccine and use of the more immunogenic genetically inactivated diphtheria and PT components is expected to considerably reduce local reactions elicited by the fifth injection of DTaP vaccine [15–18, 96–98]. Given the extensive and heterogeneous changes wrought by treatment with formalin, no adjustments in the requirements are needed, because the mutant proteins are antigenically identical to the wild-type toxins, have only 1 or 2 changes in their amino acid composition, and induce neutralizing antibodies with properties that are indistinguishable from those elicited by formalin-treated toxoids.

Usage of the existing DTaP vaccines by adults may be inhibited by the adverse publicity given to the large local reactions. To account for these local reactions, reduced levels of DTaP components for adolescents and adults (2.5 µg of PT, 5 µg of FHA, 5 µg of fimbriae, 2 limits of flocculation [Lf] of diphtheria toxoid, and 5 Lf of tetanus toxoid; denoted “TdaP”) has been advocated [99]. However, this reduced amount of aP component alone elicited swelling >1 cm in ~10% of recipients, and TdaP vaccine elicited swelling in 14.1% of recipients. Genetically inactivated and purified pertussis and diphtheria toxoids will provide a DTaP vaccine that contains lower amounts of proteins and that induces equal or higher levels of antibodies [17, 98].

Last, the enzymatic activity of tetanus toxin has been identified as a zinc metalloprotease with specificity for the synaptosome [100]. It should be possible to isolate a nontoxic protein that is antigenically identical to tetanus toxin and that could constitute an improved immunogen.

References

- Dagan R, Igbaria K, Piglansky L, van Brusteghem F, Melot V, Kaufhold A. Reactogenicity and immunogenicity of reduced antigen content of diphtheria-tetanus-acellular pertussis vaccines as a booster in 4–7-year-old children primed with diphtheria-tetanus-whole cell pertussis vaccine before 2 years of age. *Vaccine* **1999**; 17:2620–7.
- Centers for Disease Control and Prevention. Use of diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine as a five dose series: supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* **2000**; 49:RR-13.
- Halperin SA, Smith B, Russell M, et al. An adult formulation of a five-component acellular pertussis vaccine combined with diphtheria and tetanus toxoids is safe and immunogenic in adolescents and adults. *Vaccine* **2000**; 18:1312–9.
- Pichichero ME, Edwards KM, Anderson EL. Safety and immunogenicity of six acellular pertussis vaccines and one whole cell pertussis vaccine given as a fifth dose in four- to six-year-old children. *Pediatrics* **2000**; 105:1–8.
- Liese J, Stojanov S, Zink TH, et al. Safety and immunogenicity of Biken acellular pertussis vaccine in combination with diphtheria and tetanus toxoid as a fifth dose at four to six years of age. Munich Study Group. *Pediatr Infect Dis J* **2001**; 20:981–6.
- Kosuwon P, Warachit B, Hutagalung Y, et al. Reactogenicity and immunogenicity of reduced antigen content diphtheria-tetanus-acellular pertussis vaccine (dTPa) administered as a booster to 4–6 year-old children primed with four doses of whole-cell pertussis vaccine. *Vaccine* **2003**; 21:4194–200.
- Halperin SA, Schiefele D, Mills E, et al. Nature, evolution, and appraisal of adverse events and antibody response associated with the fifth consecutive dose of a five-component acellular pertussis-based combination vaccine. *Vaccine* **2003**; 21:2298–306.
- Rennels MB. Extensive swelling reactions occurring after booster doses of diphtheria-tetanus-acellular pertussis vaccines. *Semin Pediatr Infect Dis* **2003**; 14:196–8.
- Robbins JB. Towards a new vaccine for pertussis. In: A. Schlesinger, ed. *Microbiology*. Washington, DC: American Society for Microbiology, **1984**:176–83.
- Robbins JB, Pittman M, Trollfors B, Lagergård TA, Taranger J, Schneerson R. *Primum non nocere*: a pharmacologically inert pertussis toxoid alone should be the next pertussis vaccine. *Pediatr Infect Dis J* **1993**; 12:795–807.
- Schneerson R, Robbins JB, Taranger J, Lagergård T, Trollfors B. A toxoid vaccine for pertussis as well as diphtheria? Lessons to be relearned. *Lancet* **1996**; 348:1289–92.
- Age-specific death rates, 1920–1950. *Vital Statistics Special Report* **1956**:43.
- Ipsen J. Circulating antitoxin at the onset of diphtheria in 425 patients. *J Immunol* **1946**; 54:325–47.
- Pappenheimer AM Jr. Diphtheria. In: Germanier R, ed. *Bacterial vaccines*. New York: Academic Press, **1984**:1–36.
- Uchida T, Pappenheimer AM Jr, Greaney R. Diphtheria toxins and related proteins. I. Isolation and properties of mutant proteins serologically related to diphtheria toxin. *J Biol Chem* **1973**; 248:3838–44.
- Rappuoli R. Isolation and characterization of *Corynebacterium diphtheriae* non-tandem double lysogens hyper-producing CRM197. *Appl Environ Microbiol* **1983**; 46:560–4.
- Giannini G, Rappuoli R, Ratti G. The amino-acid sequence of two non-toxin mutants of diphtheria toxin: CRM45 and CRM197. *Nucleic Acids Res* **1984**; 12:4063–9.
- Podda A, Vescia N, Donati D, et al. Valutazione clinica di fase I di un nuovo vaccino antitetanico/antidifterico per adulti. *Ann Ig* **1991**; 3:79–84.
- Locht C, Keith JM. Pertussis toxin gene: nucleotide sequence and genetic organization. *Science* **1986**; 232:1258–64.
- Nicosia A, Perugini M, Franzini C. Cloning and sequence of the pertussis toxin genes: operon structure and gene duplication. *Proc Natl Acad Sci USA* **1986**; 83:4631–5.
- Arico B, Rappuoli R. *Bordetella parapertussis* and *Bordetella bronchiseptica* contain transcriptionally silent pertussis toxin genes. *J Bacteriol* **1987**; 169:2847–53.
- Marchitto KS, Smith SG, Loch C, Keith JM. Nucleotide sequence homology to pertussis toxin gene in *Bordetella bronchiseptica* and *Bordetella parapertussis*. *Infect Immun* **1987**; 55:497–501.
- Luttinger P. The epidemiology of pertussis. *Am J Dis Child* **1916**; 12:290–315.
- Centers for Disease Control and Prevention. Pertussis—United States, 1997–2000. *MMWR Morb Mortal Wkly Rep* **2002**; 51:73–6.
- Kendrick P, Elderling G. Significance of bacteriological methods in the diagnosis and control of whooping cough. *Am J Public Health* **1935**; 25:147–55.
- Kristensen B. Occurrence of the Bordet Gengou bacillus. *JAMA* **1933**; 101:204–6.
- Medical Research Council. A report of the whooping-cough subcommittee of the antibiotics clinical trials (non-tuberculous conditions) committee of the Medical Research Council: treatment of whooping-cough with antibiotics. *Lancet* **1953**; 1:1109–12.

28. Kendrick PL, G Eldering, MK Dixon. Mouse protection tests in the study of pertussis vaccine: a comparative series using the intracerebral route for challenge. *Am J Public Health* **1947**; 37:803–10.
29. Pittman M. Pertussis and pertussis vaccine control. *J Wash Acad Sci* **1956**; 46:234–43.
30. Kendrick PL, Nadolski EB, Eldering G, Baker J. Antigenic relationships of *Haemophilus pertussis*, the parapertussis bacillus, and *Brucella bronchiseptica* as shown by cross protection tests in mice. *J Bacteriol* **1953**; 66:166–9.
31. Tuomanen EI, Nedelman J, Hendley JO, Hewlitt EL. Species specificity of *Bordetella* adherence to human and animal ciliated respiratory epithelial cells. *Infect Immun* **1983**; 42:692–5.
32. Weiss AA, El Hewlitt, GA Myers, S Falkow. Tn5-induced mutations affecting virulence factors of *Bordetella pertussis*. *Infect Immun* **1983**; 42:33–41.
33. Sato H, Sato Y. *Bordetella pertussis* infection in mice: correlation of specific antibodies against two antigens, pertussis toxin, and filamentous hemagglutinin with mouse protectivity in an intracerebral or aerosol challenge system. *Infect Immun* **1984**; 46:415–21.
34. Sato H, Ito A, Chiba J, Sato Y. Monoclonal antibody against pertussis toxin: effect on toxin activity and pertussis infections. *Infect Immun* **1984**; 46:422–8.
35. Eldering G, Kendrick PL. Incidence of parapertussis in the Grand Rapids area as indicated by 16 years' experience with diagnostic cultures. *Am J Public Health* **1952**; 42: 27–31.
36. Linneman CC, Perry EB. *Bordetella parapertussis*: recent experience and a review of the literature. *Am J Dis Child* **1977**; 131:560–3.
37. Onorato IM, Wassilak SG, Meade B. Efficacy of whole-cell pertussis vaccine in preschool children in the United States. *JAMA* **1992**; 267:2745–849.
38. Linneman CC Jr, Bass JW, Smith MHD. The carrier state in pertussis. *Am J Epidemiol* **1968**; 88:422–7.
39. Grob PR, Crowder MJ, Robbins JF. Effect of vaccination on severity and dissemination of whooping cough. *Brit Med J (Clin Res Ed)* **1981**; 282:1925–8.
40. Lambert HJ. Epidemiology of a small pertussis outbreak in Kent County, Michigan. *Public Health Rep* **1965**; 80:365–9.
41. Cherry JD, Beer T, Chartrand SA, et al. Comparison of values of antibody to *Bordetella pertussis* antigens in young German and American men. *Clin Infect Dis* **1995**; 20:1271–4.
42. Robbins JB, Schneerson R, Trollfors B. Pertussis in developed countries. *Lancet* **2002**; 360:657–8.
43. Gangarosa EJ, Galazka AM, Wolfe CR, et al. Impact of anti-vaccine movements on pertussis control: the untold story. *Lancet* **1998**; 351:356–61.
44. Mannerstedt G. Pertussis in adults. *J Pediatr* **1934**; 5:596–600.
45. Nelson JD. The changing epidemiology of pertussis in young infants: the role of adults as reservoirs of infection. *Am J Dis Child* **1978**; 132:371–3.
46. Linnenman CC, Ramundo N, Perlstein PH, et al. Use of pertussis vaccine in an epidemic involving hospital staff. *Lancet* **1975**; 2:540–3.
47. Sato Y, Kimura M, Fukumi H. Development of a pertussis component vaccine in Japan. *Lancet* **1984**; 1:122–6.
48. Sato Y, Sato H. Further characterization of Japanese acellular pertussis vaccine prepared in 1988 by 6 Japanese manufacturers. *Tokai J Exp Clin Med* **1988**; 13:79–88.
49. Kuno-Sakai H, Kimura M, Watanabe H. Verification of components of acellular pertussis vaccines that have been distributed solely, been in routine use for the last two decades and contributed greatly to control of pertussis in Japan. *Biologicals* **2004**; 32:29–35.
50. Pittman M. Pertussis toxin: the cause of the harmful effects and prolonged immunity of whooping cough: a hypothesis. *Rev Infect Dis* **1979**; 1:401–12.
51. Weiss AA, Hewlitt EL. Virulence factors of *Bordetella pertussis*. *Annu Rev Microbiol* **1986**; 40:661–86.
52. Robbins JB, Schneerson R, Bryla DA, Trollfors B, Taranger J, Lagergård T. Immunity to pertussis: not all virulence factors are protective antigens. In: Gupta S, Sher A, Ahmed R, eds. *Mechanisms of lymphocyte activation and immune regulation VII*. New York: Plenum Press, **1998**:207–18.
53. Watanabe M, Nakase Y. Mutant of *Bordetella pertussis* which lacks ability to produce filamentous hemagglutinin. *Infect Immun* **1982**; 35:1018–23.
54. Ad Hoc Group for the Study of Pertussis Vaccines. Placebo-controlled trial of two acellular pertussis vaccines in Sweden: protective efficacy and adverse events. *Lancet* **1988**; 1: 955–60.
55. Zackrisson G, Taranger J, Trollfors B. History of whooping cough in nonvaccinated Swedish children, related to serum antibodies to pertussis toxin and filamentous hemagglutinin. *J Pediatr* **1990**; 116:190–4.
56. Granström M, Granström G. Serological correlates in whooping cough. *Vaccine* **1993**; 11: 445–8.
57. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Low levels of antipertussis antibodies plus lack of history of pertussis correlate with susceptibility after household exposure to *Bordetella pertussis*. *Vaccine* **2003**; 21:3542–9.
58. Heininger U, Cherry JD, Christenson PD, et al. Comparative study of Lederle/Takeda acellular and Lederle whole-cell pertussis-component diphtheria-tetanus-pertussis vaccines in infants in Germany. *Vaccine* **1994**; 12: 81–6.
59. Steinhoff MC, Reef GE, Decker MD, et al. A randomized comparison of reactogenicity and immunogenicity of two whole-cell pertussis vaccines. *Pediatrics* **1995**; 96:567–70.
60. Plotkin SA, Cadoz M. The acellular pertussis vaccine trials: an interpretation. *Pediatr Infect Dis J* **1997**; 16:508–17.
61. Olin P. Commentary: the best acellular pertussis vaccines are multicomponent. *Pediatr Infect Dis J* **1997**; 16:517–9.
62. Cherry JD. Comparative efficacy of acellular pertussis vaccines: an analysis of recent trials. *Pediatr Infect Dis J* **1997**; 16(Suppl 4):S90–6.
63. Taranger J, Trollfors B, Lagergård T, Zackrisson G. Parapertussis infection followed by pertussis infection [letter]. *Lancet* **1994**; 344: 1703.
64. Bergfors E, Trollfors B, Taranger J, Lagergård T, Sundh V, Zackrisson G. Parapertussis and pertussis: differences and similarities in incidence, clinical course, and antibody responses. *Int J Infect Dis* **1999**; 3:140–6.
65. Romanos MA, Clare JJ, Beesley KM, et al. Recombinant *Bordetella pertussis* pertactin (P69) from the yeast *Pichia pastoris*: high-level production and immunologic properties. *Vaccine* **1991**; 9:901–6.
66. Shahin RD, Brennan MJ, Li AM, Meade BD, Manclark CR. Characterization of the protective capacity and immunogenicity of the 69-kD membrane protein of *Bordetella pertussis*. *J Exp Med* **1990**; 171:63–73.
67. Liese JG, Meschievitz CK, Harzer E, et al. Efficacy of a two-component acellular pertussis vaccine in infants. *Pediatr Infect Dis J* **1997**; 16:1038–44.
68. Isacson J, Trollfors B, J Taranger, T Lagergård. Acquisition of IgG serum antibodies against two *Bordetella* antigens (filamentous hemagglutinin and pertactin) in children with no symptoms of pertussis. *Pediatr Infect Dis J* **1995**; 14:517–21.
69. Miller E, Ashworth LAE, Robinson A, Waight PA, Irons LI. Phase II trial of whole-cell pertussis vaccine vs. an acellular vaccine containing agglutinogens. *Lancet* **1991**; 337:70–3.
70. Jones DH, McBride BW, Jeffery H, O'Hagan DT, Robinson A, Farrar GH. Protection of mice from *Bordetella pertussis* respiratory infection using microencapsulated pertussis fimbriae. *Vaccine* **1995**; 13:675–80.
71. Tamura M, Nogimori K, Murais S, et al. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. *Biochemistry* **1982**; 21:5516–22.
72. Pittman M. The concept of pertussis as a toxin-mediated disease. *Pediatr Infect Dis* **1984**; 3:467–86.
73. Stronk MG, Pittman M. The influence of pertussis vaccine on histamine sensitivity of rabbits and guinea pigs and on the blood sugar in rabbits and mice. *J Infect Dis* **1955**; 96:152–61.
74. Meade BD, Kind PD, Ewell JB, et al. In vitro inhibition of murine macrophage migration by *Bordetella pertussis* lymphocytosis-promoting factor. *Infect Immun* **1984**; 45:718–25.
75. Granström M, Olinder-Nielsen AM, Holmblad P, Mark A, Hanngren K. Specific immunoglobulin for treatment of whooping cough. *Lancet* **1991**; 338:1230–3.
76. Adler A, Morse SI. Interaction of lymphoid

- and nonlymphoid cells with the lymphocytosis-promoting factor of *Bordetella pertussis*. *Infect Immun* **1973**; 7:461–7.
77. Woods DE, Franklin R, Cryz SJ Jr, Ganss M, Peppler M, Ewanowich C. Development of a rat model for respiratory infection with *Bordetella pertussis*. *Infect Immun* **1989**; 57: 1018–24.
 78. Hall E, Parton R, Wardlaw AC. Responses to acellular pertussis vaccines and component antigens in a coughing-rat model of pertussis. *Vaccine* **1998**; 16:1595–603.
 79. Storsaeter J, Hallander HO, Farrington CP, Olin P, Mollby R, Miller E. Secondary analyses of the efficacy of two acellular pertussis vaccines evaluated in a Swedish phase II trial. *Vaccine* **1990**; 8:457–61.
 80. Storsaeter J, Blackwelder WC, Hallander HO. Pertussis antibodies, protection and vaccine efficacy after household exposure. *Am J Dis Child* **1992**; 146:167–72.
 81. World Health Organization (WHO). WHO meeting on case definitions of pertussis [MIM/Epi/PERT/91.1:10–11]. Geneva: WHO, **1991**.
 82. Sekura RD, Zhang Y-L, Roberson R, et al. Clinical, metabolic and antibody responses of adult volunteers to an investigational vaccine composed of pertussis toxin inactivated by hydrogen peroxide. *J Pediatr* **1988**; 113: 806–13.
 83. Krantz I, Sekura R, Trollfors B, Taranger, et al. Immunogenicity and safety of a pertussis vaccine composed of pertussis toxin inactivated by hydrogen peroxide in 18- to 23-month-old children. *J Pediatr* **1990**; 116:539–43.
 84. Trollfors B, Taranger J, Lagergard T, et al. placebo-controlled trial of a pertussis toxoid vaccine. *N Engl J Med* **1995**; 333:1045–50.
 85. Trollfors B, Taranger J, Lagergard T, et al. Immunization of children with pertussis toxoid decreases spread of pertussis within the family. *Pediatr Infect Dis J* **1998**; 17:196–9.
 86. Taranger J, Trollfors B, Bergfors E, et al. Immunologic and epidemiologic experience of vaccination with a monocomponent pertussis toxoid vaccine. *Pediatrics* **2001**; 108:E115.
 87. Taranger J, Trollfors B, Lagergard T, et al. Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. *J Infect Dis* **2000**; 181:1010–3.
 88. Taranger J, Trollfors B, Bergfors E, et al. Mass vaccination of children with pertussis toxoid: decreased incidence in both vaccinated and nonvaccinated persons. *Clin Infect Dis* **2001**; 33:1004–9.
 89. Olin P, Gustafsson L, Barreto L, et al. Declining pertussis incidence in Sweden following the introduction of acellular pertussis vaccine. *Vaccine* **2003**; 21:2015–21.
 90. Preziosi MP, Yam A, Wassilak AG, et al. Epidemiology of pertussis in a West African community before and after introduction of a widespread vaccination program. *Am J Epidemiol* **2002**; 155:891–6.
 91. Gustafsson L, Hallander HO, Olin P, Reizenstein E, Storsaeter J. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. *N Engl J Med* **1996**; 334:349–55.
 92. Greco D, Salmaso S, Mastrantonio P, et al. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. *N Engl J Med* **1996**; 334:341–8.
 93. Campins-Marti M, Cheng HK, Fordyth K, et al. Recommendations are needed for adolescent and adult pertussis immunization: rationale and strategies for consideration. *Vaccine* **2001**; 20:641–6.
 94. Cherry JD, Baraff LJ, Hewlett E. The past, present, and future of pertussis: the role of adults in epidemiology and future control. *West J Med* **1989**; 150:319–28.
 95. Kimura M, Kuno-Sakai H, Kamiya H, et al. Immunogenicity and reactogenicity of the component acellular pertussis vaccine produced by a combination of column purified pertussis toxin and filamentous hemagglutinin. *Acta Paediatr Jpn* **1995**; 37:562–74.
 96. Burnette WN, Cieplak W, Mar VL, Kaljot KT, Sato H, Keith JM. Pertussis toxin S1 mutant with reduced enzyme activity and a conserved protective epitope. *Science* **1988**; 242: 72–4.
 97. Pizza M, Covacci A, Bartoloni A, et al. Mutants of pertussis toxin suitable for vaccine development. *Science* **1989**; 246:497–500.
 98. Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 acellular pertussis vaccines: overview and serologic response. *Pediatrics* **1995**; 96:548–57.
 99. Minh NNT, He Q, Ramalho A, et al. Acellular vaccines containing reduced quantities of pertussis antigens as a booster in adolescents. *Pediatrics* **1999**; 104:1–6.
 100. Montecucco C, Schiavo G. Mechanism of action of tetanus and botulinum neurotoxins. *Mol Microbiol* **1994**; 13:1–8.