The rise in pertussis cases urges replacement of chemically-inactivated with genetically-inactivated toxoid for DTP

John B. Robbins a, *, Rachel Schneerson a, Jerry M. Keith a, Joseph Shiloach b, Mark Miller c, Birger Trollorsd a

a National Institute of Child Health and Human Development, National Institutes of Health, 31 Center Drive, Bethesda, MD 20892-2423, USA
b National Institute of Digestive Diseases and Diabetes, National Institutes of Health, 31 Center Drive, Bethesda, MD 20892-2423, USA
c Fogarty International Center, National Institutes of Health, 31 Center Drive, Bethesda, MD 20892-2423, USA
d Department of Pediatrics, University of Goteborg, VRegion.SE, S-41685 Goteborg, Sweden

Received 11 December 2006; accepted 12 December 2006
Available online 21 December 2006

Abstract

The number of pertussis cases reported to the CDC increased from 5158 in 1995 to 21,503 in 2005. Most of the increase was in individuals greater than 10 years of age. This increase occurred also in other developed nations despite high coverage of infants and young children with the acellular pertussis vaccine. In Goteborg Sweden, virtual elimination of pertussis occurred following immunization of 70% of the children less than 10 years old with monocomponent pertussis toxoid (PTx). Immunity following disease or vaccination with either the cellular or acellular pertussis vaccine wanes gradually so that older children and adults may again become susceptible. Currently, PTx is made from chemically-inactivated pertussis toxin (PT). The most immunogenic PTx is made from genetically-inactivated mutant PT that induces higher levels of IgG anti-PT at all ages. Because of its greater immunogenicity, the genetically-inactivated PTx can be expected to be more protective on an individual and on a community basis for a longer duration than the current product. Manufacturers have declined to produce the genetically-inactivated PTx because of the expense required to change to the improved vaccine and not because of scientific issues.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Pertussis toxoid; Genetically inactivated; DTP

1. Introduction

This article was prompted by reports that the number of pertussis cases reported to the CDC in 2005 was 21,503 (CDC, unpublished data) up from 3589 in 1985 and from 5158 in 1995 [1]. This is an unacceptable number of cases for a vaccine-preventable disease. The largest proportion of this increase is in individuals >10 years of age now accounting for 67% of reported cases. There has been no appreciable change in the number of reported cases in individuals <10 years of age (these incidence numbers are an estimate based upon passive surveillance). Similar data have been reported from other nations with a comprehensive vaccine program [2].

Immunity to pertussis remains controversial. Insights into the pathogenesis of and immunity to infectious diseases may be gained by examining the similarities between Corynebacterium diphtheriae and the pathogen in question Bordetella pertussis [3]. Both organisms are inhabitants of humans only, both are confined to the surface of the respiratory epithelium, both excrete a toxin that is an ADP ribosyl transferase, and both are preventable and treatable by serum IgG antitoxin. Because of its high morbidity and mortality, there has never been a randomized double-blinded controlled study of diphtheria toxoid vaccine. Diphtheria has been virtually eliminated in the U.S. and in other countries that have a high level of routine vaccination of infants and children. The efficacy of diphtheria toxoid is only 75%, diphtheria may occur in
patients with “protective” levels of anti-toxin (>0.01 IU/mL), and at least one half of the population in the U.S. does not have such levels [4]. But when at least half of the population has anti-toxin levels ≥0.01 IU/mL, transmission of the pathogen is interrupted and tox+ C. diphtheriae is rarely detected. This is the basis for “herd” immunity or protection of the non-immune and should be the objective of a pertussis immunization program. In fact, virtual elimination of B. pertussis has been achieved in a small study in Goteborg, Sweden [5]. Because of its greater immunogenicity, the genetically-inactivated mutant pertussis toxoid would increase both the efficacy and probably the duration of DTP-induced immunity at all ages.

2. Background information

B. pertussis, isolated in 1906 by Bordet and Gengou, is a slow growing, non-fermentative Gram-negative bacillus that causes a highly contagious respiratory disease [6]. In a 1914 survey of about 17,000 cases in the lower East Side of Manhattan, Luttinger reported that the highest age incidence of pertussis was in 3–5 years old but that most of the deaths were in <1 years old [7]. Our schedule of DTP vaccination at 2, 4, and 6 months of age was designed to prevent the high death rate in infants. Luttinger also noted that pertussis in adults had slightly different clinical symptoms than in infants: whooping and lymphocytosis are not common in the older age group. At that time, the term “Grandmother’s pertussis” was used as these “babysitters” were an important cause of pertussis in infants. Spreaders of B. pertussis are patients who are coughing [8]. An important feature of pertussis was identified by Kendrick and Eldering [9]. Positive cultures of B. pertussis were common in the 1st week of the disease, a time of only non-specific upper respiratory symptoms. When the disease becomes “full blown”, both the number of organisms and the frequency of positive cultures decline i.e. pertussis occurred in the absence of B. pertussis, the sine qua non of a toxin-mediated disease.

In 1933, Madsen in Denmark and Sauer in the U.S. demonstrated efficacy against pertussis in children with vaccines composed of inactivated B. pertussis [10,11]. But neither the protective antigen/s nor the host immune factors were known and these first vaccines were not standardized. Standardization was done by Dr. Pearl Kendrick’s mouse intracerebral challenge (IC) as quantitated by Dr. Margaret Pittman, who assigned a unitage for the potency of cellular pertussis vaccines [12–14]. The IC challenge potency assay is mediated by IgG anti-pertussis toxin (PT) whether actively induced or passively administered [15]. Mice do not cough and there is no spread of B. pertussis within litters. But the IC challenge model mirrors the events on the pulmonary epithelium because B. pertussis adheres to the cilia of the cerebral ventricles and there are no positive blood cultures or direct invasion of the brain by the pathogen [16]. Only B. pertussis within the genus Bordetella causes death of mice when administered at clinically relevant doses; PT-deficient mutants are not lethal and along with Bordetella parapertussis and Bordetella bronchiseptica do not confer protection in the IC challenge assay [17,18]. This assay was successful in controlling the potency of new lots of pertussis vaccines. Important information gained by immunization with cellular pertussis vaccines may be summarized:

(1) The efficacy of cellular pertussis vaccines was about 70–80% and was inversely related to the severity of disease [19,20].
(2) Vaccine-induced immunity waned in about 4–6 years [21,22].
(3) Immunization reduced the number of positive cultures in vaccinated patients with pertussis [23–25].
(4) Cellular vaccines induced “herd” immunity by reducing transmission of the organism [26].
(5) The frequency and severity of adverse reactions, such as fever and convulsions, induced by cellular pertussis vaccines were considered unacceptable for adults and, therefore, only diphtheria and tetanus toxoids (DT) were offered to this age group [27]. Accordingly, older children and adults in the U.S. have not been vaccinated with pertussis vaccine.

All these properties of cellular vaccine were mimicked by the use of pertussis toxoid vaccine.

3. Development of acellular pertussis vaccines

In 1979, Margaret Pittman’s classic article, describing pertussis as a toxin-mediated disease, provided the stimulus for research of pertussis components on a molecular level [28]. We emphasize two important contributions:

(1) The diverse biologic activities of B. pertussis, including “lymphocytosis-promoting factor”, “islet-cell activating factor”, “histamine-sensitizing factor”, “protective antigen”, are mediated by a single protein, pertussis toxin. Although the genome for this protein is present in other Bordetella, it is expressed by B. pertussis only [29,30].
(2) A genetic locus designated as Bordetella virulence gene (Bvg) regulate the synthesis of at least five proteins that participate in the virulence of B. pertussis: pertussis toxin (PT), filamentous hemagglutinin (FHA), 59 Kd outer membrane protein or pertactin, pili or fimbriae (FIM) and adenylate cyclase [31]. However, not all virulence factors are protective antigens [32]. With the exception of adenylate cyclase, all of these proteins act at different combinations and have been added to a pertussis vaccine. The FDA has licensed 1, 2, 3, and 5 valent vaccines—all contain a pertussis toxoid.

PT is a physiologic toxin and not a cytotoxin. It is proposed that PT exerts its action by inactivation of macrophages and of neutrophils on the epithelial surfaces of the upper respiratory tract thus permitting survival of B. pertussis [33,34]. IgG anti-
PT would neutralize the toxin and permit opsonization of the pathogen.

4. Clinical trials

The first major trial of acellular pertussis vaccine was conducted in the Stockholm area of Sweden. Two investigational vaccines, JNIH-6 composed of pertussis toxoid (PTx) and FHA, JNIH-7 composed of Ptx only, and a control composed of the aluminium adsorbent, were administered at 5 and 7 months of age [35]. The results were controversial because the criteria for diagnosis of pertussis had not been agreed upon. Using actuarial analysis, Blackwelder et al. showed that addition of FHA did not contribute to immunity induced by PTx [36]. An international committee was convened to develop criteria for the diagnosis of pertussis [37]. They proposed that a cough of ≥ 3 weeks duration and a culture of B. pertussis were sufficient for a diagnosis (later, the PCR assay was considered as evidence of the presence of B. pertussis). If there was cough of ≥ 3 weeks but no culture or PCR, there must be serologic evidence of B. pertussis infection characterized by a significant rise of serum IgG antibodies to FHA or PT, or a household contact with a PCR or culture-confirmed case. The situation remains confusing because this proposal was not accepted universally: two groups did not require a cough [38,39], and the CDC requires only ≥ 14 days of coughing [40]. In addition, there is evidence that antibodies to FHA and B. pertussis antigens other than PT may be induced by organisms other than B. pertussis [41]. Although there is no licensed, validated, standardized assay available in the U.S, at least five studies over the span of 10 years have demonstrated that assay of IgG anti-PT provides the best serologic method to diagnose pertussis [42–47]. Serum IgG anti-PT will also be induced by vaccination. Two randomized placebo-controlled trials showed that a monocompontent PTx confers immunity on an individual basis [35,48]. The apparent higher efficacy of multicomponent acellular pertussis vaccines is due to an artifact caused when an antigen in the vaccine is also used for serological evaluation of disease [49]. The study with a monocompontent PTx in Goteborg provided the following data:

(1) There was a statistically significant correlation between the level of IgG anti-PT antibodies and protection against disease [50]. Correlary is that the higher the level of IgG anti-PT (immunogenicity of the vaccine), the higher will be the rate of individual protection.

(2) Vaccination reduced transmission of B. pertussis in family contacts [5,51]. This is the basis for the “herd” immunity. After immunization of about 60% of children up to 10 years of age in Goteborg pertussis was virtually eliminated at all ages including those too young to be immunized and adults [5].

These findings have been confirmed recently with a 5-year experience with a monocompontent PTx vaccine in Denmark [52].

5. Development of genetically-inactivated pertussis toxoids

The S1 polypeptide or A subunit of pertussis toxin, the enzymatic and the dominant immunoprotective region, does not contain lysine and therefore, cannot be effectively inactivated at low doses by formaldehyde [15,53,54]. To achieve chemical inactivation, the PT must be treated extensively with formaldehyde or with added glutaraldehyde. To-date, the PTx of all licensed multivalent pertussis vaccines are chemically-inactivated.

Genetically-inactivated PTx mutants were developed simultaneously at the National Institute of Allergy and Infectious Diseases (NIAID) and at SCLA VO (Chiron) [55,56]. Manufacturers have shied away from producing the mutant PTx because of patent issues and not because of scientific data. The 20-year patent shelter is complete this year but manufacturers have declined to change to the mutant PTx because of the expensive extensive testing required by regulatory agencies for licensing a “new vaccine”. The recombinant mutant has two substitutions in the S1 peptide including the change of arginine 9 to lysine and has trace or no detectable in vitro enzymatic activity or in vivo such as lymphocytes-promoting activity. The insertion of lysine also renders the mutant PT reactive with formaldehyde at a fraction of that used to inactivate the native toxin [57]. Studies conducted by NIAID in 2–3, 15–20 months old and adults showed the greater immunogenicity of the mutant at all ages (5–10 μg of the mutant PTx elicited similar or higher levels of IgG anti-PT as 25 μg of the chemically-inactivated PTxs) [58,59]. This greater immunogenicity was related to a slightly higher efficacy of the mutant toxoid vaccine in young children [60]. The mutant PTx was about 10 times as immunogenic as the newly-licensed reduced protein dT vaccine for older children and adults [61]. The difference in the level of chemically-activated PTx-induced IgG-anti-PT of older children and adults, not previously immunized with a pertussis vaccine, is even wider [62]. In summary, the genetically-inactivated mutant PTx is more immunogenic at all ages compared to the chemically-inactivated PTxs and, therefore, can be expected to be more protective for a longer duration on both an individual and community basis than the current PTxs.

6. Why is there an increase in the number of pertussis cases?

There are several plausible reasons to explain this increase. First, disease- and vaccine-induced serum IgG antibody to proteins is of limited duration (ranging from 5 to 10 years) [24–26]. Second, older children and adults are the most important source of B. pertussis because this age group was not immunized with the cellular pertussis vaccine [63,64]. Third, the anti-vaccine movement, especially that directed towards cellular pertussis vaccines during the 1980s, was responsible for many parents insisting that their
children receive DT, rather than DTP [65,66]. Personal exemptions that increase the risk of contracting pertussis, based upon religious or other reasons, persist [67]. Fourth, there is a greater awareness among physicians about this disease and increased laboratory facilities for isolation of the pathogen and for serologic diagnosis. Although the criteria for diagnosis are inconsistent, it appears that ∼20% of persistent cough illness in teenagers and adults are caused by B. pertussis [68,69]. Fifth, there were shortages of many vaccines in the U.S., including DTP, in rural and depressed areas that relied upon supply by Federal programs [70]. A major manufacturer eliminated their DTP program prompted by several changes of the FDA vaccine requirements that resulted in replacement of the appropriate vaccine manufacturing guidelines of CBER with the rules of the Bureau of Drugs. These new regulations were promulgated under a directive that allowed the FDA to propose potential hazards (so-called Cautionary Principle) for manufacturing of drugs (not vaccines). As a result we do not have a U.S.-based manufacturer of DTP. Lastly, the lesser serum IgG anti-PT antibody responses of premature infants, especially those treated with dexamethasone for respiratory distress, could have contributed to a rise of pertussis in infants and young children [71]. We suggest that data should be sought about prematurity in patients with pertussis because of the increase in prematurity observed in recent years.

7. Lower polysaccharide antibody response in combination vaccines with chemically-inactivated PTxs

Unexpectedly, combination vaccines prepared with chemically-inactivated Ptxs showed a depression of polysaccharide antibody responses elicited by Haemophilus influenzae type b and pneumococcal polysaccharide conjugate vaccines [72]. The use of aluminum phosphate, rather than aluminum hydroxide, might reduce this depressive effect [73]. We suggest a mechanism for this depressive effect of chemically-inactivated PTxs: both formaldehyde and glutaraldehyde react with lysine residues which are absent in the immunodominant S1 or the A subunit of PT. Inactivation of PT by these reagents, therefore, results in extensive denaturation of the B subunit. In spite of this, it is likely that a low level ADP ribosylation activity of the A subunit remains and exerts a depressive affect upon the surrounding lymphoid tissue. This is likely the reason why 25 μg of the chemically-treated PTx are required to induce levels of IgG anti-PT comparable to 5–10 μg of the mutant toxin. The mutant PTx, in contrast, has a lysine substituted for the arginine 9 residue and its trace level of enzymatic activity is completely inactivated by the lower level of formalin [55]. Experiments to test this hypothesis are planned.

8. Summary

In our opinion, vaccine-induced immunity to diphtheria is similar to the immunity produced by cellular and acellular pertussis vaccines. The mechanism of protection for both is indirect, incomplete and of limited duration. Preliminary data suggest that a high rate of immunity in the entire population will reduce transmission of and therefore eliminate B. pertussis [74]. Change of the chemically-inactivated pertussis PTxs to the more immunogenic genetically-inactivated mutant will increase the individual efficacy of the acellular pertussis vaccine at all ages, reduce the incidence and severity of adverse reactions, avoid the suppressive effect of DTP upon the serum antibody response to the polysaccharide component of conjugate vaccines and hasten the onset of “herd” immunity to this pathogen. This change of the PTxs to the mutant PTx with its increased immunogenicity should be facilitated by the governmental and non-governmental organizations involved in vaccine usage.

Acknowledgement

The comments and suggestions about this manuscript by Trudy Murphy, CDC, are gratefully acknowledged.

References


