PERSPECTIVE

Toward a new vaccine for pertussis

John B. Robbins[1,2], Rachel Schneerson[2], Joanna Kubler-Kielb[a], Jerry M. Keith[2], Birger Trollfors[b], Evgeny Vinogradov[c], and Joseph Shiloach[d]

[a]*National Institute of Child Health and Human Development and* [d]*National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892;* [b]*Department of Pediatrics, Sahlgrenska Hospital, 413 45 Gothenburg, Sweden; and* [c]*National Research Council, Ottawa, ON, Canada K1A 0R6*

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To overcome the limitations of the current pertussis vaccines, those of limited duration of action and failure to induce direct killing of *Bordetella pertussis*, a synthetic scheme was devised for preparing a conjugate vaccine composed of the *Bordetella bronchiseptica* core oligosaccharide with one terminal trisaccharide to aminooxylated BSA via their terminal ketodeoxyoctanate residues. Conjugate-induced antibodies, by a fraction of an estimated human dose injected into young outbred mice as a saline solution, were bactericidal against *B. pertussis*, and their titers correlated with their ELISA values. The carrier protein is planned to be genetically altered pertussis toxoid. Such conjugates are easy to prepare, stable, and should add both to the level and duration of immunity induced by current vaccine-induced pertussis antibodies and reduce the circulation of *B. pertussis*.

new pertussis vaccine | lipooligosaccharide conjugate

Pertussis, a highly contagious respiratory disease, has been the subject of research worldwide since its cause, *Bordetella pertussis*, was discovered in 1906 (1). However, despite a century of study in the laboratory and clinic and widespread use of the acellular vaccine for infants and young adults (2, 3), there is yet no agreement about a definition of immunity to pertussis, an entirely satisfactory vaccine, or an explanation for outbreaks throughout the world (4–7). Implicated as causes of these outbreaks are an increased awareness of the disease, better diagnostic tools, improved surveillance, waning vaccine-induced immunity, mutations in *B. pertussis* antigens, and low immunization rates, some due to exemptions from vaccination. Is there an increased incidence of pertussis in the United States in addition to the renewed recognition of pertussis in older children and adults? Many have not considered the low vaccination coverage and booster immunization of unregistered immigrants and their children, especially in large cities, as a cause of these outbreaks (8). However, deficiencies of the current acellular vaccine is in our opinion the most important factor in causing these outbreaks.

To provide an improved vaccine, we review the development of pertussis vaccines (9). Pertussis as a distinct, highly contagious, and serious disease has been known for centuries. In the 1930s, it was the most common cause of death in children in the United States (10). Soon after the discovery of *B. pertussis* as the causative organism, scientists evaluated the efficacy of vaccines composed of *B. pertussis* suspended in rabbit blood made by T. Madsen, Serum Institute of Denmark, during an epidemic in 1929 in the Faroe Islands (11). The vaccine was administered i.m. to children and young adults. Surveillance was maintained for 2 y, and the results showed 6 deaths due to pertussis among 3,020 vaccinees and 26 among 1,027 nonvaccinees. Similar rates were obtained for mild and moderate cases. This study showed it was possible to prevent pertussis by vaccination. Soon thereafter, a similar study was conducted by L. W. Sauer at Northwestern University (Chicago, IL) (12). Eight strains of *B. pertussis* were grown on Bordet media made with human blood and then inactivated with phenol. The vaccines contained 6–7 $\times$ 10^7 organisms/mL, and 1 mL was injected s.c. three to eight times into more than 300 nonimmune children. Temperatures up to 102 °F, lasting 2 d, occurred in most recipients. Clinical data, collected over 3 y, suggested efficacy of the vaccination.

During the late 1930s, several firms in the United States and Europe prepared pertussis vaccines, but there was no information about their standardization or clinical data. Pioneers in this field, Kendrick and Eldering at the Michigan State Board of Health, described an assay using intracerebral challenge with *B. pertussis* of mice immunized by i.p. injection of a test vaccine (13). Standardization of this procedure by Pittman and colleagues at the National Institutes of Health revolutionized the field, because the immunogenicity of these vaccines could now be expressed in units (14, 15). Soon, manufacturers combined these cellular pertussis vaccines with diphtheria (DTx) and tetanus tox-oids (TTx) adsorbed onto alum (DTP*ads*). DTP*ads* was recommended for routine immunization of infants and 6 y olds. These cellular vaccines significantly reduced the incidence of pertussis in young children throughout the United States and other developed countries (16). Its use, however, was associated with local reactions, fever, and seizures and was considered too toxic for adults (17) Some accused DTP*ads* of causing CNS injury, but this was disproved (18, 19). However, the ensuing publicity resulted in the decreased use of pertussis-containing vaccines that persists to date. In addition, many criticized the intracerebral (i.c.) challenge assay as not being related to vaccine-induced immunity to pertussis. However, i.c. challenge of mice with viable *B. pertussis* mirrors the events in human pertussis: first, the organisms do not cause a blood or purulent infection during infection but adhere to the cilia of the bronchi during disease and to the cilia of cerebral ventricles in the assay (both respiratory and CNS cilia have a common ectodermal origin) (20). Second, only pertussis toxin (PT) antibodies, whether actively induced or passively administered, conferred protection against lethal infection in mice including the Food and Drug Administration assay (21, 22). We wonder why

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[1]To whom correspondence should be addressed. E-mail: johnbennettrobbins@gmail.com.

[2]Retired.

the pertussis vaccine is not controlled by measurement of serum neutralizing antibodies (antitoxin) as is done for DT (23). Unfortunately, there are no published regulations for the current acellular pertussis vaccine by the US Food and Drug Administration.

It was a contribution, by the same Margaret Pittman, that expanded our understanding of pertussis on a molecular level and that inspired scientists to study this disease (24). She reasoned that pertussis was a toxin-mediated disease and that the toxin was the cause of the many biologic effects ascribed to *B. pertussis* such as histamine sensitization factor, lymphocytosis-promoting factor, islet cell activation factor, and protective antigen. Scientists soon isolated the PT, elucidated its amino acid structure and DNA regulation, and developed mutations that preserved its immunogenicity while eliminating its toxicity (25–28). An acellular bicomponent vaccine containing PT and filamentous hemagglutinin (FHA), was shown to be safe and effective in Japan (29). Purification of other *B. pertussis* components led to several acellular licensed vaccines composed of one to five components (30, 31). The combination of purified proteins, including diphtheria, tetanus, and pertussis proteins and *Haemophilus influenzae* type b conjugate in one vaccine induced Arthus reactions in recipients of more than three injections of the combination vaccines (32, 33). Accordingly, the levels of DTx in acellular pertussis vaccine for individuals >12 y of age was lowered (Tdap), which resulted in the disappearance of the Arthus reactions (33). In individuals who did not have pertussis or were not vaccinated with pertussis-containing vaccines, one injection of Tdap failed to induce protective levels of IgG anti-PT (100 IU), and its overall effectiveness in >13 y olds was estimated to be only 53% (34). The wide resurgence of pertussis initiated calls for an improved vaccine (4–7, 35). However, there is no agreement about how to improve acellular vaccines that may contain one to five protein components. There is, however, agreement that pertussis toxoid (PTx) is an essential component of cellular and acellular vaccines (36). The evidence for this is as follows. (*i*) Low levels of anti-PT were related to susceptibility to infection with this pathogen (37, 38). A monocomponent PTx showed efficacy in a field trial in Sweden (39). This same PTx has been used in Denmark as the only pertussis vaccine for 15 y (40, 41) and its efficacy is similar to that of the multivalent vaccines, and there has been no pertussis epidemic in Denmark since 2002 in contrast to neighboring countries, where epidemics have occurred. (*ii*) A convalescent level of ≥100 EU (ELISA units) to PT is the only reliable method for serologic diagnosis of pertussis (42). No valid data assign protective activities to the other vaccine proteins: (*i*) FHA did not confer immunity against intracerebral or pulmonary challenge with *B. pertussis* in mice by active or passive immunization with monoclonal or polyclonal antibodies (21). (*ii*) FHA and pertactin antibodies, induced by infection with *Bordetella parapertussis,* do not confer immunity to pertussis (there were no PT antibodies) (43, 44). (*iii*) A cellular pertussis vaccine without FHA (Lederle Laboratories), once used for routine immunization of infants in the United States, had similar protective activity as vaccines containing FHA (45, 46). (*iv*) Inclusion of FHA did not confer additional protection to acellular pertussis vaccines or additional therapeutic effect to passively administered anti-PT (47, 48). (*v*) Neither active nor passive immunity to pertactin conferred protection to mice challenged i.c. (49). In one study, a relatively high dose of pertactin (16 μg) provided only incomplete immunity to mice challenged in the upper respiratory tract, whereas PTx conferred 100% protection at half that dose (8 μg) (50). In humans, seroepidemiologic studies showed that pertactin and FHA antibodies arose independently of pertussis infection and did not prevent pertussis (51). (*vi*) Japanese regulatory agencies have requirements only for FHA and PTx (52). This experience in Japan, where pertussis has almost been eradicated, indicates that pertactin is not essential for induced immunity to pertussis. (*vii*) Strains of *B. pertussis* isolated from patients with pertussis that do not express pertactin have been identified in the United States, Europe, and Japan (53–55). (*viii*) Not commonly appreciated is that convalescence from pertussis, at any age, does not confer lifelong immunity to pertussis (56, 57). In addition, indirect data showed that addition of fimbriae to the four-component vaccine adds only a small increment of efficacy (not significant) (30). Lastly, some have proposed to add adenylate cyclase or outer membrane vesicles to this mixture, but no efficacy for these investigative vaccines has been published (58, 59).

PT antibodies induced by pertussis vaccines invoke two main actions. A primary protective action, which is indirect, inhibits the action of PT on phagocytic cells, thus allowing them to opsonize the *B. pertussis* (60). Similar to the effect induced by widespread immunization with DTx, this indirect effect of anti-PT accounts for the incomplete immunity on an individual basis (~80% for both toxoids) (61, 62). Another effect of anti-PT is to reduce coughing that, in turn, decreases transmission of *B. pertussis* in the susceptible population (21). Because *B. pertussis* is an inhabitant of and a pathogen for humans only, this reduced transmission results in "herd" immunity.

From the above discussion, it follows that improvement of the current pertussis vaccine can begin with two steps: (*i*) removal of the nonessential vaccine components; and (*ii*) improving the essential component PTx by using a nondenatured, genetically detoxified mutant, one of which has been shown to be a better immunogen than the chemically modified PTx at a smaller (1/5) dose (63, 64). A third step will be discussed below.

Consistent with the observations on antiprotein antibodies, the duration of vaccine- and disease-induced IgG antiprotein wanes, so that maximal level declines about 10-fold in 2–5 y (65–68). Older children and adults were not immunized with cellular pertussis vaccines because of adverse reactions, leaving many nonimmune individuals (17). We quote Alison Weiss: "booster immunization of adults with acellular pertussis vaccine was not found to increase bactericidal activity over preimmunization levels. Identifying ways to promote bactericidal immune responses might improve the efficacy of pertussis vaccines" (69). Because vaccine-induced IgG antibodies to the surface polysaccharides of Gram-negative pathogens induce a bactericidal effect and immunity, we studied the lipooligosaccharide (LOS) of *B. pertussis* as a potential vaccine component (70–72).

A Pertussis Vaccine Designed to Induce Bactericidal Antibody

Protein conjugates of polysaccharides induce antibodies whose protective levels may last for decades even for the life of the recipient (73). The reason is that polysaccharides are composed of repeat small saccharides that are common in nature and may stimulate cross-reacting antibodies (74). Proteins, in contrast, have a complex specific structure, with very few cross-reactive structures, i.e., DT antibodies are induced only by vaccination or disease and that is the reason why most proteins, such as PTx, elicit a comparatively short duration of protective levels (3–5 y) (56, 57, 65–68).

LPS is both an important virulence factor and a protective antigen of Gram-negative bacteria. Protection induced by our *Shigella sonnei* conjugates was related to the levels of serum IgG anti–O-SP (71, 72). *B. pertussis* lacks an O-specific polysaccharide domain of its LPS, and is termed lipo-oligosaccharide (LOS), differing from most enterobacteriacae such as *Escherichia coli* and *Salmonella,* which have an LPS (75). The *B. pertussis* LOS is

expressed in two forms denoted as band A and band B, distinguished by a terminal trisaccharide of band A at its nonreducing end (76, 77). Alone, *B. pertussis* LOS is a poor immunogen due to its low molecular weight (~2,500 K_d) (75). Fever, usually assumed to be mediated by the LPS, is not a common finding in pertussis patients of all ages. Serum LOS antibodies are found in children who recovered from pertussis, indicating that this saccharide is expressed during disease (78). Neither cellular nor acellular vaccines regularly elicit bactericidal antibody (79–83). Only some investigative vaccines induce bactericidal antibodies and some "naturally occurring" anti-LOSs elicit a cidal reaction with *B. pertussis* (80, 83).

With the goal of regularly inducing antibodies that have a direct bactericidal effect on *B. pertussis,* we prepared conjugates of its LOS core. We used *Bordetella bronchiseptica*, reported to share the *B. pertussis* core structure, for ease of cultivation, and bound its reducing end to BSA by oxime chemistry (84, 85). Several mutants were investigated; only conjugates of the core saccharide with a single trisaccharide at the nonreducing end, with about eight chains per protein carrier, induced high levels of bactericidal antibodies (86). Because of its availability, we used BSA but we plan to use the genetically altered PTx modified by Arg-9 to Lys and Glu-129 to Ala of the S1 peptide, as the carrier protein (23, 25) to provide both anti-PT and bactericidal antibodies. This approach is feasible because we showed that PT can serve as a carrier for pneumococcus type 14 polysaccharide (87). We expect the bivalent conjugate-induced antibodies to be fully protective and long-lived. Because *B. pertussis* is an inhabitant of and a pathogen for humans only, the addition of an immunogen that induces bactericidal antibodies opens the opportunity to combine high rates of immunization in infants with eradication of *B. pertussis*. Such a conjugate will be simple and less costly to prepare and should confer a high degree of immunity for a longer period than our current pertussis vaccines.

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