ADVANCES IN

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BERNARD J. ABBOTT

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The Current Status of Pertussis Vaccine: An Overview

CHARLES R. MANCLARK
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Whooping cough has been a major cause of infant morbidity and mortality in the United States. With the introduction of an improved and standardized pertussis vaccine in the 1940s, there followed a remarkable decline in pertussis in the United States, most of the Western world, and Australia, New Zealand, and Japan. Clinicians and public health authorities agree that the use of pertussis vaccine has been and remains the most effective method of controlling whooping cough. Antimicrobials are of limited value during the prodromal stages of the disease; they may be useful in the control of bacterial infections which are secondary to *Bordetella pertussis* infection, but they have had a negligible effect on the control of whooping cough. In addition to vaccines, an observed decrease in the amount and severity of pertussis and the use of modern medical supportive procedures have contributed to the apparent control of pertussis.

The early days of pertussis research were occupied with determining the etiologic agent of the disease and methods for its culture and maintenance. Eventually production culture methods evolved and a vaccine was developed, standardized, and shown to be effective in the British clinical trials conducted during the 1940s and 1950s. Much of pertussis vaccine research and development occurred prior to the advent of modern biochemistry, physiology and microbial genetics. Because the vaccine appears to be effective, few attempts have been made to change the early formulations. With the exception of one extracted vaccine, all pertussis vaccines produced in the United States are whole cell products. As much as these vaccines resemble each other, the variability in the manufacture of vaccines from manufacturer to manufacturer and the variation from lot to lot from the same manufacturer is usually not appreciated.

Pertussis vaccine is one of the more troublesome products to produce and assay. As an example of this, pertussis vaccine has one of the highest failure rates of all products submitted to the Bureau of Biologics for testing and release. Approximately 15–20% of all lots which pass the manufacturers' tests fail to pass the Bureau's tests. Many of the problems associated with the production of pertussis vaccine result from the fact that little is known about the physiology and genetics of *B. pertussis*. The usual pure culture techniques are not employed when transferring

[1] This introductory statement and the four articles that follow are from a seminar entitled "Pertussis Vaccine: Current Status" held during the 75th Annual Meeting of the American Society for Microbiology, New York City, April 28, 1975.

or propagating cultures, nor are there any simple markers of production cultures to insure that they are typical of the prototroph or that cultures of high protective potency and low reactogenicity are selected for use in the production of vaccine. Recent studies in our laboratory have shown that *B. pertussis* cultures and vaccines contain high levels of adenylate cyclase and that this enzyme is related to a number of biological activities. Assays for adenylate cyclase are easily done and may provide a useful marker for vaccine development and for definitive genetic and physiologic studies.

Since a genetic study has not been done and prototrophic *B. pertussis* cannot be properly characterized, it follows that a definitive study of the physiology of *B. pertussis* has not been done. Consequently, there is evidence that current culture methods select against prototrophic cells in favor of nutritionally and antigenically deficient mutants.

Even though whooping cough has been controlled in those countries which have employed pertussis vaccine prophylaxis, many unanswered questions concerning the host–parasite relationship remain. A panel of experts has been convened at the Bureau of Biologics to study the efficacy and safety of all biologics, including those with a pertussis vaccine component. Much current interest by the panel and other public health researchers centers around the following topics.

Does pertussis vaccine protect against whooping cough and/or prevent *B. pertussis* infection? Very little is known about the pathogenesis of *B. pertussis*, and even less is known about the mechanism and/or duration of vaccine- or disease-induced immunity. Immunity is probably not mediated by serum antibody, but secretory antibody *may* play a role. In the absence of evidence to the contrary, by default, immunity is usually considered to be cellular. What is the reservoir or infection between epidemics? What is the prevalence of asymptomatic carriage?

Pertussis disease and infection are underreported. One of the difficulties in evaluating the efficacy or failure of pertussis vaccine is caused by problems in the laboratory and in the clinical diagnosis of pertussis. In the absence of the whoop pertussis may be undiagnosed. Some viral infections may mimic whooping cough. A definitive diagnosis requires the isolation and identification of the etiologic agent. Unfortunately, diagnosis is complicated by deficiencies in existing laboratory procedures. Also, most laboratories are not properly equipped or trained to use the methods that do exist. The result is that pertussis is either not diagnosed or is misdiagnosed.

We know that pertussis vaccines passing the required toxicity and safety tests can cause adverse reactions in children. Local reactions are relatively common and include edema, erythema, induration, pain, and sometimes ulceration at the injection site. Systemic reactions are less common, but include fever, collapse, seizures, persistent screaming, and,

rarely, paralysis and death. Adverse reaction rates are not accurately reported, but more adverse reactions are probably experienced with the use of pertussis vaccine than with other biologicals.

With control of the disease, we may be approaching a time in which more vaccine-related problems than those due to the disease will be experienced. The higher rate of vaccine reactions than disease has been responsible for decisions in several countries making pertussis immunization an optional procedure. It is the opinion of public health authorities in the United States that the benefits of vaccination outweigh the risks of disease. Unfortunately, the reduced use of pertussis vaccine in those countries where it has been made an option may provide the ultimate proof of the efficacy of pertussis vaccine.

Much work with improved pertussis vaccines is based on the results of the aforementioned British clinical vaccine trials done in the 1940s and 1950s. These trials showed, among other things, that the clinical efficacy of pertussis vaccine correlated with the mouse potency test. The premise that evolved was that the protective antigen measured in the mouse potency test was *the* antigen that conferred immunity in infants. Since then other biological entities in pertussis vaccines have been identified. A partial list would include: protective antigen (or mouse protective antigen), histamine sensitizing factor (HSF), lymphocytosis promoting factor (LPF), hemagglutinin, hemolysin, endotoxin, dermonecrotic toxin (heat labile toxin), late-appearing toxin (late weight loss factor), adjuvant (especially for IgE-like antibody), active and passive anaphylaxis-promoting activity factor, shock-promoting activity factor, and lethal toxin.

As a result of studies done since the British trials, it is known that although there is not an absolute correlation between the mouse protective antigen and HSF or LPF, vaccines can be ranked with regard to these components. For example, vaccines containing high levels of mouse protective antigen generally would contain high levels of HSF, LPF, and so on. It is likely, therefore, that the vaccines used in the British trials that tested high for mouse protective antigen probably would have contained high levels of HSF, LPF, and so on, if those tests had been done.

Pertussis is a localized *infection* without invasiveness. Septicemia is not produced. Only the ciliated epithelium of the respiratory tract is involved. There is evidence that pathologic changes, probably due to soluble substances released by the organism or to diffuse physiologic changes initiated by the disease, occur at sites distant from the site of infection. One approach to understanding the host–parasite relationship would be to determine the mechanisms responsible for the organism's predilection for and attachment in the upper respiratory tract and to study those substances (toxins, sensitogens, metabolites, and so on) that mediate the symptoms of disease production at sites distant from the site of infection.

Some investigators consider the mouse protective antigen to be a distinct entity that can be separated from the other biological activities of the cell. What little is known about mouse protective antigen would indicate that it is a rather innocuous substance and it would be difficult to understand its role in the *disease* process. If one subscribes to the thought that protective antigen is the same as HSF, LPF, or other substances in the cell, then it is apparent that the roles of these metabolites would have to be determined in human disease and/or immunity to disease.

The various factors and cellular components of *B. pertussis* have not been directly related to human disease or reactions to vaccine. These relations require that the various components be isolated and characterized.

One area that has received little direct consideration is the mechanism by which *infection* is established and a determination of how it may be prevented. One approach to such a problem is to determine the characteristics of *B. pertussis* cell surfaces that confer specificity and are related to attachment and localization in the target tissues of the respiratory tract.

Although pili have not been satisfactorily demonstrated for *B. pertussis*, it may be productive to consider the possibility that pili may exist and that they play a role in the attachment process. Piliated gram-negative bacteria have been associated with a number of infectious processes of similar surfaces. In many instances the presence of pili has been associated with a number of infectious processes of similar surfaces. In many instances the presence of pili has been associated with hemagglutinin. *Bordetella pertussis* has a hemagglutinin. Extracted mouse protective antigens have been shown to have hemagglutinating activity and electron micrographs demonstrate a fine fiberlike structure. If it can be shown that *B. pertussis* is piliated, then the role of such structures in pertussis infection or immunity should be determined.

The ultimate goal of any investigations of *B. pertussis* and the host–parasite relationship in pertussis should be the development of an improved vaccine. Because of the ethical and logistic problems in carrying out field trials on an improved pertussis vaccine, every attempt should be made to include all possible approaches to vaccine immunity in a single trial. One of the several dilemmas facing the manufacturer or developer of a new vaccine is justifying the development and use of a new vaccine if present vaccines are judged to be safe and effective. In addition, pertussis vaccine is a relatively low-profit item and the cost of developing and field testing it would be very high.

The clinical testing of a new product poses the most difficult problems. It may be possible to field test a vaccine in one of the underdeveloped countries where whooping cough is more common than in the United States, but would such trials be ethical? Would the testing of a vaccine in an underdeveloped country be a proper evaluation of a product that is to be used in the United States? Would an underdeveloped country have

the laboratory facilities and technical competence necessary to evaluate the efficacy of the vaccine? If a meaningful clinical trial could be conducted in the United States or elsewhere, since the vaccine recipient is an infant, what are the problems associated with informed consent? Most important, are present laboratory procedures sufficient to assure that a new vaccine is safe?

The costs of developing and testing an improved vaccine could be borne by the manufacturer and passed on to the consumer. The government could pay for the development of a vaccine through the mechanism of grants or contracts, and the costs could be spread to the taxpayer. There are advantages and disadvantages of each approach, but if the government supported the research, the newly developed process probably would become public property.

It may be that the problems associated with the proper and ethical clinical testing of an improved pertussis vaccine are so great as to be considered insurmountable, and some other approach to make pertussis vaccine more effective and less reactogenic may be necessary. Some may conclude that any clinical trial involving infants is immoral and unethical. However, it is possible that it may turn out to be more immoral and unethical to abstain from such clinical studies if it is demonstrated that the probability of risk is low and of success, high.

On April 16, 1973, the Department of Health, Education, and Welfare announced the formation of a Panel on Review of Bacterial Vaccines and Toxoids with Standards of Potency. The panel was composed of physicians and scientists who were among the leaders in the fields of microbiology, immunology, preventive medicine, and public health, with a wide range of experience in clinical medicine and research.[2] These experts have

[2] Panel on Review of Bacterial Vaccines and Toxoids with Standards of Potency:

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been charged with the responsibility of reviewing, among other things, the safety and efficacy of vaccines containing a pertussis component. After many months of study and deliberation, a Provisional Generic Statement on Pertussis Vaccine was released on November 21, 1975. The following recommendations have been abstracted from the panel's statement. These recommendations represent a concensus of present thought and provide reasonable goals and guidelines for research to improve pertussis vaccine, as well as for the clarification of our understanding of the host–parasite relationship in pertussis.

A. The panel strongly recommended that adequate public support be provided for studies of the pathogenesis of pertussis and the biology of the organism, particularly as related to the immunology of pertussis, the complications of the disease, and the untoward reactions to immunization. Without such basic studies a more effective and safer pertussis vaccine cannot be developed.

B. Surveillance of pertussis in well-defined populations should be undertaken. Such surveillance would have three purposes: first, to determine the incidence in the United States, including distribution by age and vaccine status; second, to evaluate the possibility that a change in serotypes of *B. pertussis* in a community causes outbreaks of pertussis in individuals previously immunized with serotypes formerly present; and third, to determine whether the current infrequency of the disease in the United States may ultimately result in a population of older children and adults whose immunity has waned because of a lack of repeated exposure to the organism.

Further, the panel is convinced that currently employed surveillance systems to identify adverse reactions to pertussis are inadequate and recommends that definitive steps be taken by the appropriate subdivisions of the Public Health Service to improve them. Several alternatives are available. Perhaps the same channels as those proposed for reporting of adverse drug reactions can be utilized. Special field stations with sufficient populations under surveillance may have to be established and funded.

C. Specific recommendations of the panel regarding the production, use, and evaluation of pertussis vaccines include the following:

The weight-gain test in mice used to determine toxicity of pertussis vaccine needs revision to include specifications regarding mouse strain(s) to be used. Studies should be undertaken to develop other assays predictive of human reactivity. Obviously, better definition of the organism's biological characteristics would facilitate prediction and prevention of reactogenicity in man.

The agglutination test used to determine vaccine response in humans should be standardized. It is recommended that a reference serum be

used for comparison. A reference laboratory should be available at the Bureau of Biologics. The interval between immunization and obtaining serum for testing of the serologic response must be specified. An acceptable titer obtained by a standardized method should be defined; fold titer rises or geometric mean titers are not adequate indicators of induced immunity.

The regulation for manufacturers regarding the human dose should be updated to reflect current recommendations and practices. To achieve this, a requirement that pertussis vaccine have a potency of "4 units per single human dose" could be substituted.

The vaccine label should warn that if shock, encephalopathic symptoms, convulsions, or thrombocytopenia follow a vaccine injection, no additional injections with pertussis antigens should be given (immunizations can be continued with DT). The label should also include a cautionary statement about fever, excessive screaming, and somnolence.

Any fractionated vaccine that differs from the original whole cell vaccine should be field tested if possible until better laboratory methods for evaluating immunogenicity in man are developed. Field testing should include agglutination testing and, if possible, evaluation of clinical efficacy in man.

D. Pertussis vaccine is one of the immunizing agents for which it is strongly urged that legislation be enacted to provide reasonable federal compensation to the few individuals injured and disabled by meritorious public health programs. Such legislation would protect manufacturers and physicians against liability in situations in which the injury was not a consequence of defective or inappropriate manufacture or administration of the vaccine.

The scientific community and public health authorities are in general agreement that pertussis vaccine is the most effective measure employed in the control of pertussis disease. Recent concern has been expressed that vaccines currently in use have not benefited from modern genetic, physiologic, and immunologic knowledge and are less immunogenic and more reactogenic than is desirable or necessary. These and other concerns are reflected in the recommendations of the panel and are discussed in greater detail by Doctors Morse, Parker, Anderson, and Cameron in the following presentations.

Biologically Active Components and Properties of *Bordetella pertussis*[1]

STEPHEN I. MORSE

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I. Introduction

A variety of biological effects may be observed in experimental animals following injection of phase I *Bordetella pertussis* cells or products. A partial list includes: sensitization to the lethal effects of the pharmacological agents histamine and serotonin as well as to nonspecific stresses such as cold and peptone shock; unresponsiveness to the induction of hyperglycemia by epinephrine, which has been attributed to the causation of a β-adrenergic blockade; leukocytosis with predominating lymphocytosis; adjuvanticity with respect to both antibody production—including reaginic antibody (IgE)—and cell-mediated immunity; and direct, acute toxic effects which are in general related to either a distinct heat-labile toxin or to heat-stable lipopolysaccharide endotoxin.

In addition, components of *B. pertussis* can be shown to induce striking effects in *in vitro* systems. Some of these *in vitro* effects are clearly relevant to *in vivo* events, e.g., adjuvant activity in spleen cell cultures and depression of cyclic adenosine 3′,5′-monophosphate (cAMP) formation. Others, such as hemagglutination, hemolysis, and mitogenic stimulation of murine, as well as human lymphocytes, have yet to be shown to be related to *in vivo* occurrences.

Paradoxically, while clinical pertussis has become of decreasing importance where effective vaccines are routinely used, there has been an increased interest in the biological activities of *B. pertussis*. This interest is

[1] Supported in part by research grant AI 09683 from the National Institutes of Health.

especially manifest in the numerous studies designed to isolate and characterize the responsible bacterial factors and to determine the underlying mechanisms involved in the observed reactions. Although there is still a long way to go in both cases, a sense is emerging that what apparently appear to be quite disparate biological activities in some instances may be caused by a single component inducing a single basic specific biochemical effect which, in turn, is manifested by superficially unrelated events (see Section X). Heretofore, each event has been attributed to the activity of a distinct bacterial component with a distinct mechanism of action.

Interest has also been furthered by the interrelated facts that we comprehend neither the pathogenesis of whooping cough nor the component(s) of pertussis vaccine which engenders protection. It is likely that identification of the bacterial determinant of pathogenicity and virulence and the corollary definition of the protective antigen will ultimately lead to the development of an effective, well-defined immunizing preparation. Moreover, it is possible that such a preparation would be freer of adverse reactions than current products. Admittedly, although the true incidences of serious reactions to pertussis vaccine in infants is not known with precision, they are generally conceded to be extremely rare. However, it is now realized that protection is not lifelong and that previously immunized older children and adults are as susceptible as those not previously immunized. The disease is often milder, but it may be serious; morbidity is apparent in terms of lost time from work, school, and so on; and the patient is a source of infection to others. It is in this population that local and systemic complications of vaccination are more frequent and severe (Linnemann *et al.*, 1975).

The purpose of this article is to review some of the information on the biologically active components of phase I *B. pertussis* and their effects. A certain amount of selectivity of topics and references is necessary because of the vast number of reports in the field, and some of the selectivity may reflect the writer's interests, and perhaps his biases.

For background information, the reader is referred to excellent review articles by Kind (1958), Munoz and Bergman (1968), Pittman (1970), and Munoz (1971).

II. Sensitization to Histamine

Parfentjev and his co-workers (1947a,b,c) found that in mice previously injected with pertussis vaccine, the subsequent injection of pertussis vaccine or pertussis extracts was followed by lethal shock. Subsequently, Parfentjev and Goodline (1948) demonstrated that pertussis-vaccinated mice became highly sensitive to the lethal effects of histamine. The trivial term for the factor or factors of *B. pertussis* which causes sensitization is histamine-sensitizing factor or HSF.