

Pertussis vaccine research*

CHARLES R. MANCLARK¹

The mechanisms of infection and immunity in pertussis are not well understood, and as a result, the development of a new, improved vaccine is difficult. This paper describes the limitations of currently available vaccines, and outlines the problems associated with the introduction of new prophylactics, such as defining the bases of toxicity and efficacy and organizing meaningful clinical trials. Until these problems are resolved, efforts are needed to improve currently available whole-cell vaccines. The possible role of passive immunity in the control of the disease is also discussed.

Pertussis has been controlled in developed countries with current pertussis vaccines. Modern supportive facilities and medications are available for the child who contracts pertussis and although the disease is still distressing and costly, and may produce permanent sequelae, mortality has been reduced to very low levels. In the countries where pertussis has been controlled, current interest centres on problems associated with the administration of the vaccine. Opponents of pertussis immunization suggest that the attendant risks now outweigh the advantages and have called for the discontinuation of pertussis vaccination programmes. Experience has shown, however, that when the number of pertussis vaccinations declines there is an unacceptable increase in the incidence of the disease.

In the developing countries, the control of pertussis is impeded by the lack of vaccine delivery systems that can achieve adequate coverage of susceptible populations, with a potent vaccine that meets WHO requirements for safety. Pertussis is common and may have serious sequelae in infants who contract the disease and who do not have access to appropriate supportive health care facilities.

PERTUSSIS VACCINE PROPHYLAXIS

Bordetella pertussis infects only man and infection is by the respiratory route, usually from patients with early or catarrhal infections or possibly from carriers. The bacterium survives only for very brief periods outside the human body.

The proper use of vaccine is the most effective way to control pertussis.^a Chemotherapeutic agents are of limited value during the prodromal stages of the disease although they have been useful in the control of secondary bacterial infections. The merit of passive immunization has yet to be established.

Present pertussis vaccines have been judged to be safe and effective, although most would agree that there is room for improvement. Current whole-cell vaccines are a compromise in which a certain level of toxicity is accepted in order to achieve a sufficient level of potency. The boundary between vaccine toxicity and potency is not well defined and

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¹ Research Microbiologist, Bureau of Biologics, 8800 Rockville Pike, Bethesda, MD 20205, USA.

^a MANCLARK, C. R. & HILL, J. C., ed. *International symposium on pertussis*. DHEW (NIH) 79-1830, Washington, DC, US Government Printing Office, 1979, 387 pp. This publication contains scientific reports, reviews, and a bibliography of most of the significant pertussis research done prior to 1979. Complimentary copies may be obtained from the author.

the situation is further complicated by the technical limitations of laboratory methods of defining these parameters. Although there are always problems inherent in animal assays, the main difficulty is that the bases for vaccine potency and toxicity are not known. Accordingly, current laboratory control tests should be regarded rather as useful correlative indices than as absolute measures.

Most pertussis vaccines are whole-cell preparations that were developed before many of the recent advances in immunology, biochemistry, and genetics, and the development of an improved vaccine is a desirable goal that should be pursued. The laboratory effort required, while considerable in terms of time, talent, and facilities, will be modest compared with the effort that would be necessary to establish the efficacy and safety of a completely new vaccine. Any vaccine that is significantly different from existing whole-cell vaccines will require extensive clinical trials. In view of our incomplete knowledge of the immunology of *B. pertussis* and of the protective immune responses in humans, proof of protection from disease will be the only acceptable measure of vaccine efficacy.

At the present time there is enough disease in the world to permit the evaluation of a new vaccine. However, if disease prevalence is eventually reduced by a better but only partially effective vaccine, it may not then be possible to carry out meaningful clinical trials.

Clinical trials of new pertussis vaccines

Clinical trials of a new vaccine will be complicated by serious ethical, legal, and logistic problems, since it would be difficult to justify injecting children with a new vaccine when safe, effective vaccines are already available. One of the most difficult problems will be to find meaningful control groups for a clinical study. The validity of the trial and of the resultant decisions concerning the vaccines under test will depend on the satisfactory constitution of the control group, and yet it will be difficult to justify withholding vaccine from a susceptible population.^b

Clinical trials to demonstrate the safety of new vaccines could be carried out in many different parts of the world. A sample of 50 000–100 000 children, observed for one month, would be required to exclude rare serious side-effects.

Clinical trials of efficacy should be carried out in two parts. Preliminary studies to evaluate vaccine efficacy (protection from disease) should be done in those parts of the world where pertussis is a common disease, and could be expected to be completed within 5 years. If the vaccine is to be used in a developed country, definitive studies of efficacy should be done in those parts of the world where pertussis is not a common disease because of the differences in antigenic experience in the test populations. Because we must rely on measures of efficacy based on protection from disease, evaluation in a developed country may require many years to complete and is likely to be the most difficult obstacle to the development of a new vaccine.

HOST-PARASITE INTERACTIONS IN PERTUSSIS

Since clinical studies will be complicated and time-consuming, it is important to have good expectations of success before undertaking them. The developmental work on a new vaccine requires a study of the host-parasite interactions in pertussis and it is necessary to understand the mechanisms of infection and disease and the basic mechanisms of immunity at all levels.

^b KABAT, E. A. Ethics and the wrong answer. *Science*, **189**: 505 (1975).

At present, the host-parasite interactions in pertussis are not well understood, but in the following discussion the disease process has been divided into three steps, which are considered separately.

Attachment

The first step in infection is the attachment of *B. pertussis* to target cells in the respiratory tract. Filamentous haemagglutinin (FHA) may be involved in attachment but recent studies implicate it more as a proximity factor rather than a factor responsible for the specificity of attachment normally associated with pertussis.^c The mechanism of immunity is not known although it has been proposed that IgA and possibly cell-mediated immunity have a role at this level.

Local disease

B. pertussis produces a local infection; the organism is not invasive. Symptoms of disease develop at the site of infection but the pathogenic mechanisms and components of *B. pertussis* responsible are not known. Toxins, such as dermonecrotic toxin (DNT) and lymphocytosis promoting factor (LPF) are produced, but their roles are poorly understood. Dermonecrotic toxin has been administered via the intranasal route to experimental animals, but the preparations were rather crude and the results difficult to interpret. Goldman & Baseman (unpublished data, 1980) have described a peptide called tracheal cytotoxin (TCT) from *B. pertussis* that causes ciliostasis and pathogenic changes in epithelial cells. Although the results fit the projected model for pathogenesis, additional studies are necessary to establish the role of the peptide in the disease. A material described as polymorphonuclear leukocyte-inhibitory factor (PIF) has been isolated and partially purified and may be an important virulence factor in *B. pertussis*.^{d,e} There is speculation that an immune response directed at PIF or TCT may be significant in the disease process. However, at the present time, no reasonable model exists to explain the mechanisms of immunity to local disease in pertussis.

Systemic disease

Although the organism produces a local infection, it is apparent that metabolites of *B. pertussis* move away from the site of infection and produce systemic effects. The lymphocytosis associated with infection is one example, but it is not known how LPF and other mediators of systemic effects contribute to the disease process. The bases for immunity to the systemic effects of disease are not understood but it is likely that immunoglobulins, particularly IgG, are important.

IMMUNOCHEMISTRY OF *B. PERTUSSIS*

An examination of host-parasite interactions should be coupled with a definitive immunochemical study of *B. pertussis*, and much current interest centres on the isolation,

^c JONES, G. B. The attachment of bacteria to the surfaces of animal cells. In: Reissig, J. L., ed., *Receptors and recognition. Microbial interactions, Series B, Vol. 3*. London, Chapman and Hall, 1977, pp. 139-176.

^d UTSUMI, S. ET AL. Polymorphonuclear leukocyte-inhibitory factor of *Bordetella pertussis*. I. Extraction and partial purification of phagocytosis- and chemotaxis-inhibitory activities. *Biken's journal*, **21**: 121-135 (1978).

^e IMAGAWA, T. ET AL. Polymorphonuclear leukocyte-inhibitory factor of *Bordetella pertussis*. II. Localization in the outer membrane. *Biken's journal*, **22**: 1-10 (1979).

purification, and characterization of the biologically active components of the cell. The ultimate goal is to assemble the necessary antigens in a vaccine which, ideally, will be specific and more potent, require fewer injections, and confer a longer-lasting immunity than present whole-cell vaccines. Toxic antigens would be modified before use.

Substantial progress has been made in the isolation and characterization of potentially pathogenic components of *B. pertussis*. In addition to TCT and PIF already mentioned, much recent interest has centred around FHA and LPF because of their possible roles in pathogenesis and because they may be important immunogens. LPF has been the subject of intensive study for many years, because, in addition to its ability to produce lymphocytosis following injection of vaccine or infection with *B. pertussis*, it is a potent T-cell mitogen and adjuvant. Histamine sensitizing factor (HSF) can make rats and mice unusually sensitive to the lethal effects of histamine and may be involved in the enhanced production of reaginic and haemagglutinating antibodies. Islet-activating protein (IAP) causes fasting hyperinsulinaemia and/or enhanced insulin secretion in response to insulin secretagogues. Several recent studies suggest that lymphocytosis promoting, histamine sensitizing, and insulin secreting activities are the properties of a single protein.

Japanese scientists have developed a new component vaccine which is to be licensed by the Japanese Government and will be widely used in Japan. The vaccine contains FHA, but the method of manufacture probably permits small amounts of LPF and other cellular components to be present. The presence of LPF is significant since it appears to enhance the immunogenicity of FHA and may be an immunogen for man. The FHA vaccine meets the WHO requirements for pertussis vaccine, manifests low rates of adverse reaction, and produces satisfactory agglutinin responses in clinical studies. Although the vaccine has not been shown to protect humans from pertussis, its protective effect will be monitored in the clinical trials.

If studies of the host-parasite interrelationships in pertussis are productive, it may be possible to develop laboratory methods to predict vaccine efficacy. Such methods would greatly simplify the evaluation of candidate vaccines and could be used in conjunction with monitoring of clinical protection from disease.

Ethical considerations are an important problem in the clinical testing of experimental vaccines. If, however, the basic studies on a candidate vaccine are done well and demonstrate that the new vaccine has a good chance of success, then it becomes less ethical not to do the clinical studies.

CLASSICAL WHOLE-CELL PERTUSSIS VACCINES

For modified vaccines that still retain the salient characteristics of a whole-cell vaccine, the criteria of potency established in the British Medical Research Council trials in the 1950s are still appropriate.^{f,g,h} Potency as measured in the mouse protection test and demonstration of appropriate agglutinins in clinical subjects is sufficient evidence of efficacy for these vaccines. However, vaccines that no longer fulfil the definition of a whole-cell vaccine would require extensive clinical trials to demonstrate that they give protection from disease. Modest but significant changes in a vaccine are easily accomplished in the laboratory but it will be difficult to demonstrate the clinical efficacy of such vaccines:

^f MEDICAL RESEARCH COUNCIL. The prevention of whooping-cough by vaccination. *British medical journal*, 1: 1463-1471 (1951).

^g MEDICAL RESEARCH COUNCIL. Vaccination against whooping-cough: relation between protection in children and results of laboratory tests. *British medical journal*, 2: 454-462 (1956).

^h MEDICAL RESEARCH COUNCIL. Vaccination against whooping-cough: final report. *British medical journal*, 1: 994-1000 (1959).

In view of these problems, certain types of research on whole-cell vaccines may be counterproductive and should be deferred until more information is available. For example, the role of agglutinogens in immunity may never be resolved with existing knowledge and it may be more productive to concentrate on other problems. To cover the possibility that agglutinogens do have a role in immunity and vaccine efficacy, a standard spectrum of serotypes could be included in all vaccines. Similarly, it may be unwise to attempt to find less toxic strains for vaccine production, since the factors producing toxicity, e.g., LPF, may be the most potent immunogens.

The development and testing of a new vaccine will require considerable time and it is thus apparent that current whole-cell pertussis vaccines will be needed in the interim; and much can be done to improve them. Research is needed to understand and correct the inconsistencies in vaccine production, testing, and use, and the information obtained would benefit both whole-cell vaccines and the purified vaccines of the future.

When, as in the case of pertussis vaccine, the bases for efficacy are not known, the best expedient is to determine empirically the most reliable method of manufacture and then always make the vaccine by that method. WHO has developed a protocolⁱ for standardization of vaccine production, and a simplified flow chart for pertussis vaccine production based on the WHO manual is shown in Fig. 1. A few instances where research studies might be productive are mentioned below.

Seed culture

The selection of production strains of *B. pertussis* is complex. Production strains are usually selected on the basis of the performance of vaccine concentrates in the mouse potency test and the freedom from toxicity test, and the ability of the strains to grow in the manufacturer's production medium. Production stock cultures should be stored and used in a true seed lot system to minimize genetic drift in production cultures. Very little is known about the basic genetics of *B. pertussis*. The characteristics of the prototroph are not known and no useful genetic markers are available to monitor changes in the prototroph or production cultures. Basic genetic studies and research on the physiology of *B. pertussis* are needed.

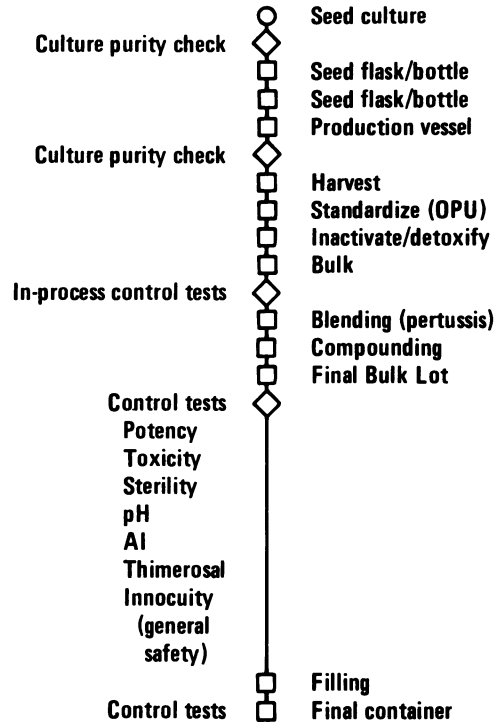


Fig. 1. A simplified flow chart for the production of pertussis vaccine (the International Nonproprietary Name (INN) for thimerosal is thiomersal).

ⁱ Manual for the production and control of vaccines. Pertussis vaccine. WHO unpublished document, BLG/UNDP/77.3 Rev. 1, 1977.

Production culture

An understanding of the physiology and metabolism of *B. pertussis* would permit the development of production media for the growth of typical cells with predictable characteristics. Diagnostic culture media would also benefit from physiological and metabolic studies, as they provide important laboratory tools for the evaluation of vaccine efficacy in epidemiological studies.

Control tests

A control test must be reproducible and must correlate with clinical experience or it is meaningless and these tests should be direct, specific, and require no extrapolation. For example, if the detoxification of a vaccine concentrate is assessed by the loss of dermo-necrotic toxin, the best test to use is the suckling mouse test for DNT since it is a direct and very sensitive measure. It could be misleading to infer the absence of DNT from survival rates or proper weight gains in the mouse weight-gain test. Similarly, if it is necessary to know the LPF content of a vaccine, specific assays for LPF, HSF, or IAP should be done. There is a need to re-examine the control tests for pertussis vaccine, especially those that are included in national and international regulations. Most important of all, however, there is a need for simple, specific, sensitive, *in vitro* tests to replace the animal assays used now.

Vaccine usage

The differences that exist in pertussis immunization practices throughout the world underline the confusion about the best way to protect against the disease. A compilation of the bases for the different immunization schedules and methods would be useful. The reasons for choosing different routes, injection sites, age of initial immunization, and number and timing of subsequent immunizations would be of interest and could provide valuable information for improving vaccine usage.

PASSIVE IMMUNITY IN PERTUSSIS

Artificial passive immunity

The use of hyperimmune globulin or antibody in pertussis prophylaxis has wide acceptance but, so far, there is no evidence of efficacy in well-controlled trials. Based on currently available information, it is likely that pertussis immune globulin (human) will lose its United States licence unless it can be shown to be effective. Clinical trials to establish the efficacy of pertussis immune globulin or whole serum antibody should therefore be done.

Natural passive immunity

Natural passive immunity in pertussis has been thought to be unimportant because of the long period of time between clinical disease or immunization and pregnancy. The transmission of immunity through the human placenta or milk has not been demonstrated. Recent observations, however, suggest that natural passive immunity may play a role in the epidemiology of pertussis, but more research is needed to confirm this.

Pertussis morbidity and mortality is highest in infants. Protective herd immunity as a

result of vaccination programmes in the very young is probably a consequence of the high levels (>80%) of immunization in cohorts. In many parts of the world it may not be possible to achieve the high level of vaccination necessary to control pertussis or to reach infants soon enough to protect them from disease. In such places vaccine-induced natural passive immunity may be a useful way of avoiding the problems of immunological unresponsiveness and the risks associated with early immunization and may simplify the delivery of effective prophylactic immunity to newborn infants.

There are many unknown factors and any discussion of vaccine-induced natural passive immunity in pertussis can only be speculative. If it can be shown that pregnant women or women of childbearing age can be immunized with an effective, purified, non-toxic pertussis vaccine, and if it can be demonstrated that protective immunity can be transmitted through the human placenta or milk to protect the newborn infant, we may have an important tool for the control and eventual eradication of pertussis. This is especially true for developing countries where the prompt delivery of adequate health care to newborn infants is difficult. At the very least, the concept of passive immunity in pertussis deserves consideration, especially if we are successful in developing purified immunogens.

CONCLUSIONS

The immediate research priorities for current whole-cell pertussis vaccines should concern the production and delivery of safe, potent, reliable, and uniform products in sufficient quantity to meet the demands of immunization programmes.

In addition to research on whole-cell vaccines, emphasis should be placed on the development of a purified, definitive immunogen for pertussis, but this will require basic studies of host-parasite interactions. The concept of a single protective antigen is probably not valid and the ultimate vaccine will probably contain several purified antigens. It is possible that development of a definitive immunogen could lead to the control and eventual eradication of pertussis in the world.

Opportunities for clinical trials are limited and they should be used only for the evaluation of the new pertussis vaccines that have the best prospects for success. Vaccines that have evolved from a thoughtful analysis of studies on the immunochemistry of *B. pertussis*, the host-parasite interactions, and ethical and legal considerations associated with the use of an experimental vaccine are most likely to be successful in the clinic. This is the proper time to develop a definitive pertussis vaccine, since the technology and interest are available and the disease is sufficiently prevalent in the world to permit clinical evaluation.
