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 315

AD HOC WORKING GROUP ON DEVELOPMENTS
 IN PERTUSSIS VACCINE
 (Geneva, 25-27 October 1983)

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The WHO Ad Hoc Working Group on Developments in Pertussis Vaccine met in Geneva, from 25-27 October 1983. Dr F. Assaad, Director, Division of Communicable Diseases of the World Health Organization, opened the meeting on behalf of the Director-General.

1. INTRODUCTION

Whooping cough is a disease of world-wide distribution. It causes serious problems in both the developed and developing countries. Despite ample literature on pertussis, little is really known about the microbiology, epidemiology, prevention and treatment of the disease. Recent research has resulted in the isolation and characterization of cellular components from Bordetella pertussis, but their role, individually and collectively, in pathogenesis or as protective antigens, has not been elucidated. This lack of basic knowledge of the disease, its diagnosis, the etiologic agent and the significant protective immune response has complicated the development of an improved vaccine.

2. PRESENT SITUATION

Whole-cell pertussis vaccines in use throughout the world are produced from a variety of different strains of B. pertussis cultivated in stationary or submerged liquid culture or on solid media. The organisms are killed and detoxified using thimerosal, formalin, long-term storage at 2-8°C, at various time-temperature relationships, or some combination of these methods. Most pertussis vaccines are combined with diphtheria and tetanus toxoids and adsorbed on an aluminium or calcium carrier. The time at which immunization starts, the interval between injections, the immunization route (intramuscular or subcutaneous), the preferred site of injection and the number of doses which constitute a total immunizing dose, also vary between countries. Vaccine potency is measured by the intracerebral challenge mouse potency assay and freedom from excessive toxicity is usually determined by the mouse weight gain test.

Diphtheria and tetanus toxins are usually toxoided before purification and then compounded with pertussis vaccine; an aluminium adsorbent is used. Sometimes, however, the diphtheria and tetanus toxins are purified as toxins, toxoided and then compounded with pertussis vaccine; a calcium carrier is also occasionally used. Vaccines may be adsorbed to the mineral adjuvant in situ or the adsorbent can be prepared separately and then added to the vaccine. There is a limit for the amount of adsorbent that can be contained in a vaccine, but there is little or no control of the physical characteristics of the adsorbent or the degree of adsorption of the final vaccine on the aluminium or calcium carrier.

Most whole-cell pertussis vaccines contain biologically active lymphocytosis promoting factor (LPF) and endotoxin. The endotoxin content of whole-cell vaccines is best reduced by decreasing the number of bacteria necessary to achieve acceptable potency.

The group summarized the reports submitted by the participants concerning the vaccine in use in individual countries and came to the following conclusions:

1. Little is known of the stability of some vaccines in use. The potency of some of the vaccines might deteriorate rather rapidly.

2. The duration of vaccine induced immunity is unknown and poorly studied. Immunization schemes have also varied considerably in different countries.
3. The rate of minor complications and side-effects of vaccination is high but some of the side-effects are serious and cause public concern.
4. There has been increased public concern that the risks associated with the use of pertussis vaccine outweigh the risks of disease, and in some countries vaccine acceptance has decreased and consequently there has been an attendant increase in disease. The difficulties that have arisen over vaccination programmes led to the conclusion that there is a need for a less reactogenic, more effective pertussis vaccine and this goal should be pursued.
5. Although the mouse intracerebral potency assay may be an useful index of potency for whole cell pertussis vaccines, no laboratory test has been developed and validated which measures the potency of the acellular vaccines and relates to their clinical efficacy. Recently developed serological methods can measure the antigenic content of vaccines or the antibody response to infection or vaccination, but the antigens and/or antibodies measured by these procedures have not been correlated to clinical immunity from disease..
6. The clinical diagnosis can be confused with other upper respiratory infections. This has caused difficulties in the interpretation of epidemiological data as well as data collected in field trials of vaccines. There is a great need for sensitive and specific quick and simple laboratory tests for confirming the diagnosis of whooping cough.

Recent advances in the understanding of the host parasite interactions in pertussis, the immunochemistry of B. pertussis itself and the development of studies of acellular (component) pertussis vaccines in different parts of the world, have led to the prospect of less reactogenic, more potent vaccines to replace the whole-cell vaccines presently in use. The development of laboratory methods to characterize acellular pertussis vaccines and to evaluate the clinical safety and efficacy of candidate vaccines are a prerequisite to their routine clinical use.

The purpose of the present meeting was to determine what collaborative studies could be coordinated by the World Health Organization in order to implement a programme to evaluate candidate vaccines through the standardization of laboratory procedures, reagents and protocols for clinical trials.

3. RECENT RESEARCH ON PERTUSSIS VACCINE

Recent research on the host-parasite interactions in pertussis and the immunochemistry of B. pertussis has identified prospective immunogens for inclusion in an acellular (component) vaccine. There is nearly universal agreement that LPF is an important protective antigen and that filamentous haemagglutinin (FHA) should also be included in a vaccine. Studies of antigens at or near the surface of the B. pertussis cell including, but not limited to, fimbriae/agglutinogens, and outer membrane proteins, would indicate that these may be important protective antigens. Other potentially

important protective antigens which require further study are: heat labile toxin (detoxified), adenylate cyclase, tracheal cytotoxin and possibly outer membrane proteins which are produced in vivo or are produced in vitro under conditions which approximate the in vivo environment.

Interest in an improved pertussis vaccine to replace the current whole-cell vaccine centers, in part, on the acellular (component) vaccines presently in use for routine vaccination programmes in Japan. However, the protective activity of these vaccines is still under study.

The Japanese acellular (component) pertussis vaccine, which contains LPF-HA and F-HA, is prepared from the culture supernatant of B. pertussis, phase I; this is subjected to ammonium sulfate fractionation, extraction with a high concentration of sodium chloride, and sucrose density gradient centrifugation to obtain an HA preparation practically free of endotoxin. The HA preparation is treated with formalin to destroy the activities which cause leukocytosis promotion and histamine sensitization. Finally, aluminium hydroxide is added to the preparation as an adjuvant.

According to the Japanese investigators, the new vaccine has a sufficient level of potency, as measured in their modified mouse test, and also contains less than one-tenth of the specific toxicity (leukocytosis promotion, histamine sensitization and endotoxicity) found in the whole-cell vaccine. Comparison of the incidence of side-effects caused by whole-cell and acellular (component) vaccines based on about 5000 injections of each vaccine showed that the incidence of fever, redness and hardening was respectively 29.7%, 68.0% and 26.0% for the whole-cell vaccine and 2.7%, 15.4% and 7.3% for the component vaccine. No severe side-effects such as fever-related convulsion were reported. This vaccine, called Precipitated Purified Pertussis Vaccine, has been used in mass immunization in Japan since the autumn of 1981. Other investigators however, are more cautious and believe that there is a need for further study of efficacy of the vaccine. At the meeting the observations based on laboratory investigations of the vaccine were reported. In one study, a purified pertussis component (DPT 21) vaccine, produced by a manufacturer in Japan, and whole-cell vaccines were compared in various routine tests and experimental models. The potency of the vaccines were examined in the mouse protection test as described in the WHO Technical Report No 638. In addition, the stability of the vaccines was tested at 37°C. After incubation of the vaccines at 37°C, a significant decrease of potency was observed for both vaccines. The purified vaccine did not induce significant leukocytosis nor histamine hypersensitivity. LPS activity could not be demonstrated. The purified vaccine did not interfere with the glucose metabolism five hours after vaccination, due to the low level of LPS. However, four days after vaccination both purified and whole-cell vaccine induced a hypoglycemia and hyperinsulinemia. In contrast to the whole-cell vaccine, the purified vaccine did not induce an impairment of the autonomic responsiveness within the cardiovascular system in the rat. In another report it was stated that the (component) vaccine contains protein of which about 90% is FHA and LPF. The remaining 10% has not been characterized, but is probably a mixture which contains agglutinogens. Toxicity has been inactivated with formalin and there is little or no LPF biological activity. The endotoxin content has been reduced to at least one-twentieth of the starting material. Some acellular vaccine lots have as little as 1/100 000 of the endotoxin contained in a whole-cell vaccine.

The name "Precipitated Purified Pertussis Vaccine" infers that a single vaccine has been studied and used in the Japanese clinical trials, but there are apparently some important differences in the vaccines which were used. Although the six licensed manufacturers in Japan use a prescribed manufacturing procedure, there are some minor differences between the vaccines produced and there are, furthermore, variations from lot to lot which are inherent in the production method. Changes have also been introduced in the formulation of the vaccine. For example, the present formulation contains FHA and LPF, but no detectable agglutinogens, whereas previous formulations contained appreciable amounts of agglutinogens and other antigens in addition to FHA and LPF. These changes in formulations during the clinical trials clearly complicate the evaluation of vaccine efficacy. Only a relatively small number of individuals have been evaluated in matched case contact studies and apparently no record has been made of the formulation used for any or all of the vaccinations received by each subject.

Since the Japanese acellular (component) pertussis vaccines have remarkably less endotoxin, have been rigorously toxoided with formalin and contain less bacterial mass per dose they would be expected to be less reactogenic when injected into children. Comparisons of whole-cell and acellular (component) pertussis vaccines showed that the acellular (component) vaccines do indeed induce fewer febrile reactions and less erythema and induration than do whole-cell vaccines, but it was also mentioned that the acellular (component) vaccines were less potent than whole-cell vaccines when assayed by the standard mouse potency test.

4. CONTROL TESTS SUGGESTED FOR PERTUSSIS VACCINES

One of the topics discussed at the meeting of the Ad Hoc Working Group on Developments in Pertussis Vaccine was the quality control of the vaccine. The participants agreed that the intracerebral (i.c.) mouse protection test and the mouse weight gain test may not be adequate and should be replaced by more relevant tests as soon as possible. For this reason, a more precise description of the antigen content, safety and immunogenicity of new vaccines is needed.

A proposal for a collaborative study on acellular (component) DPT vaccines was made by the group with the aim to characterize the vaccines for antigen content, safety and potency. It was agreed that all tests should be performed in at least two laboratories. The protocol of the study was prepared and the collaborating laboratories were identified by the group (see annex).

The group agreed that the efficacy and safety of a potential vaccine will, ultimately, have to be evaluated in controlled field trials. There are, however, a number of problems associated with the introduction and study of new pertussis vaccines. Amongst these is the current concern for public confidence. If a trial with a new vaccine is carried out in a country which already has a current vaccination programme, then the efficacy of new and old vaccines should be compared.

Manufacturers of vaccines are requested to pay attention to the following suggestions and eventually to introduce one of the tests mentioned below and to report their experience to WHO:

(a) Antigen content

LPF, FHA, agglutinogens, in relation to total protein content.

(b) Toxicity (in place of MWG test).

LPF in a biological test such as leukocytosis, HSF or CHO* test.

LPS in a limulus lysate test also include quantity with the aid of ELISA.

HLT in suckling mice.

IAP test.

Reversion to toxicity by bioassay.

(c) Potency

Considering that the i.c. challenge test is only of limited value in assessing the potency of acellular pertussis vaccine, the following specific tests are suggested as replacement.

Detection of antitoxic immunity either by challenge of immunized mice with LPF or by estimation of the neutralizing activities of the serum in a CHO test.

Determination of the immune response to FHA and agglutinogens 1, 2 and 3.

(d) Stability

The stability of the vaccine should be studied after incubation at 37°C for various periods of time. Based on the results, appropriate conditions will be defined.

5. CLINICAL TESTING OF CANDIDATE VACCINES

Ultimately, the efficacy and safety of a potential vaccine will have to be evaluated in controlled field trials. Such trials should also establish the duration of immunity.

Since it is likely that different groups will produce slightly different vaccines, perhaps with only shades of differences between them, it will be important to establish international standards for the assessment of antigen content and antibody responses.

A study to establish the incidence of minor reactions to a vaccine should be relatively straightforward and could be carried out with only a small number of adult volunteers and then subsequently in infants. The assessment of the incidence of serious rare reactions, however, will be difficult and is likely to require a large number of vaccinees and a prolonged surveillance programme. The fact that pertussis vaccine is normally given early in the first year of life at the stage when a range of unexplained neurological diseases tend to be present is a major complicating factor and a knowledge of

* Chinese hamster cell line (CHO cells).

the background level of such events in unvaccinated children will be essential. The clinical investigation of the safety of a new vaccine should include full immunological and viral investigations of infants with claimed serious reactions together with carefully matched controls. An investigation of certain genetic markers, such as human leukocyte antigen (HLA), may also be useful.

The justification for a clinical trial would require considerable laboratory evidence of probable efficacy. Also, the candidate vaccine should be as fully characterized as possible with regard to antigenic content and its composition should remain unaltered throughout the trial.

For trials to be epidemiologically satisfactory for measuring efficacy and reactogenicity they should be carried out in a country where there is a sufficiently high incidence of the disease and where appropriate surveillance systems are in place. They should also be ethically acceptable.

6. STANDARDIZATION AND CONTROL OF NEW VACCINES

New standards and control measures for the new vaccines will have to be established since it is very unlikely that current requirements will be appropriate. These measures, the development of which will need collaborative studies, should as far as possible be firmly based upon a sound understanding of protective mechanisms involved in immunity.

7. RESEARCH NEEDS

7.1 Immune mechanisms and protective antigens

7.1.1 Identification of protective antigens

There is a need for a better definition of bacterial structures and extracellular products that are important in the pathogenesis and in the establishment of immunity. It is particularly important to know which antigens are responsible for the protective immunizing effect in man. This knowledge is essential for a more rational selection of candidate strains for the development of new vaccines and for the purification and detoxification of potential vaccine components.

7.1.2 Genetics and Molecular Biology of *B. pertussis*

Genetic and biochemical analysis of *B. pertussis* is urgently required to provide a detailed understanding of the factors associated with virulence and protection and of their molecular organization. Application of this knowledge should facilitate the rational identification of antigens that should be included in new vaccines. Such information would also be invaluable in controlling strains selected for vaccine production in order to produce vaccines with proper and predictable characteristics. Furthermore the molecular cloning of *B. pertussis* genes in another host microorganisms could permit more efficient large scale production of appropriate antigens and biologically active macromolecules. The detailed examination of potential vaccine components for possible antigenic variation is also particularly important if new vaccines are to contain only a few purified component proteins.

7.1.3 Measurement of Antibody Responses

Tests should be developed for examining the antibody response to the structures or products of B. pertussis which have been shown to play an important role in pathogenesis and/or establishment of immunity. Newer sensitive and specific techniques such as immunoblotting should be employed. Simplified assays for measuring antibody levels in serum and other body fluids are also required. Antibody response following natural disease in man should be compared with that produced by vaccination.

7.2 Mucosal immunity

7.2.1 Methods to measure mucosal immunity

Studies are needed to define practical ways of measuring mucosal immunity in the respiratory tract of man. Such studies should include examination of saliva and breastmilk. Studies of breastmilk antibody should also include an investigation of its ability to provide passive immunity to the baby, a property that might be reinforced by planned immunization of the mother. Pertussis morbidity and mortality is most serious in very young infants and vaccine-induced "natural" passive immunity may be a useful way of avoiding the problems of immunological unresponsiveness in infants and the risks associated with early immunization. Immunization of mothers may also simplify the creation of effective immunity in newborn infants.

7.2.2 Methods of stimulating mucosal immunity

There should be a search for methods to elicit a mucosal immune response through evaluation of various antigen forms, routes of administration and use of adjuvants. Mucosal and serum antibody responses should be compared and their relative contributions to protection assessed. Whether the human secretory immune system, once stimulated, can provide long lasting protection against B. pertussis infection, and the extent to which such protection occurs at mucosal sites distant from the site of antigen exposure, is not known. There should be studies on the mucosal immune response in man and especially in different population groups. Differences in the immune response to B. pertussis in different population groups should be investigated. The possible influence of age, nutritional status, genetic factors and co-existing microbial and parasitic infections should also be taken into account.

7.3 Development of animal models

The lack of a suitable animal model has long impeded the study of host-parasite interaction in pertussis. Models which more closely mimic the human disease than the mouse intracerebral infection are clearly required. In view of the empirical nature of both the currently used intra-cerebral mouse protection test for vaccine potency, and the mouse weight gain test for vaccine toxicity, better characterized and scientifically more acceptable assays are needed.

The model employing respiratory infections in mice may be more appropriate than intracerebral infections. Respiratory and intracerebral challenge should, however, be compared to evaluate the similarities and differences there may be between the two routes; the results obtained when the two methods are used to rank the effective protective potencies of various potential vaccine components should also be compared. New model systems should not only further our understanding of host-parasite interactions but also help to evaluate in vitro assays which might eventually replace animal tests in the routine control of vaccine potency and safety.

ANNEX

Proposal for a collaborative study with Japanese acellular DPT vaccine.

1. Collaborating Laboratories:

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Professor B. F. Semenov, MIVS, Moscow, USSR
Dr M. Tiru, NBL, Stockholm, Sweden

2. Aim of the study:

Characterization of the Japanese acellular DPT vaccine for antigen content, safety and potency. All tests will be performed in at least two laboratories.

3. Preparations to be tested:

Japanese component vaccine lot J NIH-2
Reference preparations FHA 4, LPF-HA 7
Pertussis antigen P.B.P. 1217-Tx and P.B.P. 1217-Td (see annex)

4. Antigen content:

Samples A and B will be analysed for:

LPF in ELISA, cell-culture (Chinese Hamster Ovary Cells) and mice.
FHA in ELISA

LPS in ELISA and limulus lysate test agglutinogens in ELISA and agglutinin-absorption test.

Peptide spectrum in SDS PAGE only for sample A.

All tests will be done in comparison with the respective reference preparations 4 and 7.

Vaccine J NIH-2 will be assayed after dissolution of the adsorbents with Na citrate.

The D and T content will be estimated by ELISA or radial immunodiffusion. The vaccine will also be analysed for the pertussis components mentioned above.

5. Safety

a) Specific toxicity

The samples A, B and Vaccine J NIH-2 will be examined for residual amounts of LPF in functional tests, such as:

(Hapto globulin) HP-ELISA, cell culture (lymphocytes, rat glioma cells, CHO cells, murine encephalopathy test, leukocytosis and histamine sensitivity.

b) The samples A, B and vaccine J NIH-2 will be tested for dornecrotic toxin suckling mice.

c) LPS see paragraph 4.

6. Immunogenicity

The vaccine J NIH-2 will be tested for immunogenicity as to:

a) agglutinin response

b) Anti LPF in ELISA and CHO tests and anti FHA response (compare with sample before vaccination)

c) Mouse i.c. challenge test 2 and 3 weeks after vaccination paying special attention to parallelism in comparison with the international reference preparation.

Some investigators may like to carry out additional tests such as:

a) Behaviour studies in vaccinated mice (Professor Semenov)

b) Non-specific immunomodulation induced by the vaccine in mice (Professor Semenov)

c) Protective activity on the basis of intranasal challenge test (Dr Robinson)

d) Anti LPS response in mice (Dr Nagel)

e) Pharmacological activities

It is advisable to include a standard preparation in these studies.

The participants intend to finish the study at the end of January 1984. All results will be sent to Dr J. G. Kreeftenberg for collation. Copies of all data will be sent to DCD and BDD. The final report will be made after evaluation of the results by all participants.

Continuation and extension of the study will be discussed possibly in a meeting in Geneva.

ANNEX TO COLLABORATIVE STUDY WITH JAPANESE
ACELLULAR DPT VACCINE

Reference Antigen

F-HA Reference (4) 200 µg Protein/ml in 0.05M phosphate buffer, pH 7.0, containing 0.5 M NaCl and 50% Glycerol. HA titer; 6,400 (fixed CRBC) anti-F-HA ELISA; 5,670 U/ml.

LPF-HA Reference (7) 200 µg Protein/ml in 0.05M phosphate buffer, pH 7.0, containing 0.5M NaCl and 50% Glycerol. 9,630 LPF U/ml (in vivo) 12,000 Hp-ELISA U/ml.

Vaccine J NIH-2 Dried precipitated purified-pertussis, diphtheria, tetanus combined vaccine 300 vials. Sterile diluent J NIH-2 0.7 ml
Pertussis antigen 15 µg PN/ml (F-HA 7ug PN/ml)
(LPF-HA 8ug PN/ml)
Diphtheria toxoid 13 ug PN/ml (32 Lf/ml)
Tetanus toxoid 2 µg PN/ml (8 Lf/ml)
Stabilizer; (Haemacell (as polygeline) 0.5%
(Sucrose 3%
(Lactose 3%
(Glucose 3%
0.7 ml/vial and freeze-dried.
Moisture content: < 1%

Test results of the J NIH-2

- o Protein nitrogen content; 30 µg PN/ml
- o Sterility test; Pass
- o Freedom from HLT; Pass
- o Freedom from abnormal toxicity (GP. mouse); Pass
- o Mouse body weight decreasing toxicity (ET); 28 BWDU/ml
- o Mouse leukocytosis - increasing toxicity (LPF); 0.3 LPU/ml
- o Mouse histamine-sensitizing toxicity (HSF); 0.015 HSU/ml
- o Pyrogen test; Pass
- o Detoxification of diphtheria toxoid; Pass
- o Detoxification of tetanus toxoid; Pass
- o Aluminium content; 0.23 mg/ml
- o Thimerosal content; 0.0095 (W/V)%
- o Potency of diphtheria; 74 IU/ml
- o Potency of tetanus; 36 IU/ml
- o Potency of pertussis; 28.5 IPU/ml
(25,28,30,24,35,29)
- o Stability test for pertussis potency; stable 80°C, 30 min. (in lyophilized condition).

o Vaccine sample (A)

Pertussis antigen (No. P.B.P. 1217-TX)
before detoxification
94 µg PN/ml

Yellow cap.
1.5ml/tube x 20
in 0.05M phosphate buffer pH 7.0,
containing 0.5M NaCl and 6.5% sucrose
23,300 LPU/ml (in vivo)
13,400 Hp-ELISA U/ml
13,000 α-LPF.ELISA U/ml. Free from pyrogen
Two major clear bands were seen in acid
pH PAGE

o Vaccine sample (B)

Pertussis antigen (No. P.B.P. 1217-Td)
after detoxification
50 µg PN/ml

White cap.
1.5ml/tube x 20
in 0.05M phosphate buffer pH 7.0,
containing 0.5M NaCl.
LP, HS, ET activities; non-detectable.

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