Does whole-cell pertussis vaccine protect black South African infants?

Assessment of post-vaccination events and antibody responses to pertussis toxin, filamentous haemagglutinin and agglutinogens 2 and 3

A. RAMKISSOON, H. M. COOVADIA, W. E. K. LOENING, M. NDLOVANA

Summary

The whole-cell pertussis vaccine currently used in South Africa has not been adequately evaluated for post-vaccination events and immunogenicity. A trial of this vaccine combined with diphtheria and tetanus toxoids (DTP) was undertaken in 115 black babies who received primary vaccination at 2, 4 and 6 months of age. Serological IgG responses to the major antigens of *Bordetella pertussis*, filamentous haemagglutinin (FHA), pertussis toxin (PT) and fimbriae (agglutinogens 2 and 3 (AGG 2 + 3), were evaluated by enzyme-linked immunosorbent assay in sera obtained at birth, and before vaccination at 2, 4 and 6 months and at 9 months. Surprisingly, after 3 doses of DTP, responses to PT and FHA were found merely to restore levels of IgG to PT and FHA to those found in cord blood. In contrast with the positive increases in these antibodies found in other series of whole-cell vaccination, the anti-PT seroconversion rate was only 19% and the anti-FHA rate only 24%. High levels of anti-AGG 2 + 3 were produced with 67,2% seroconversion.

The frequency and nature of post-vaccination events were recorded. Incidences of all reactions to the vaccine were low (7,6%). Fever (3,2%) and excessive crying (2,4%) were the most frequently occurring minor events. The rate of neurological post-vaccination events (without sequelae) during the brief follow-up period was 2 hypotonic-hyporesponsive episodes (8,03/1 000 doses) and 1 convulsion (4,02/1 000 doses).

Significant pertussis antibody levels were found in maternal and cord sera with levels in the latter frequently being higher. Three cases of pertussis occurred during the study period. Only 1 of the subjects had completed primary vaccination. In view of these findings, the need for a proper efficacy and safety study of the currently used DTP vaccine is urgently indicated in South Africa.

S Afr Med J 1991; **79:** 645-649.

Department of Paediatrics and Child Health, University of Natal, Durban
A. RAMKISSOON, B.SC., M.SC. (MED. SCI.)
H. M. COOVADIA, F.C.P. (S.A.), M.D.
W. E. K. LOENING, M.B. CH.B., F.C.P. (S.A.)
M. NDLOVANA

Accepted 21 Aug 1990.

Pertussis (whooping cough) remains a major cause of morbidity and mortality in the very young. According to World Health Organisation estimates, 60 000 deaths due to pertussis occur yearly in Africa, virtually all in unvaccinated infants.[1]

Conventional whole-cell pertussis vaccines at present used in most parts of the world consist of inactivated cells of *Bordetella pertussis* and are therefore a mixture of antigens, some of which, while essential for protection, also produce adverse reactions. These are considered the most reactive of childhood vaccines used in routine vaccination schedules, although the possible contribution of the diphtheria and tetanus components of triple vaccines is frequently ignored. Whole-cell vaccines are associated with a wide range of side-effects, which include local and systemic reactions and some temporally associated neurological disorders.[2] Use of the vaccine was discontinued, for example, in Japan after the sudden death of recently vaccinated infants and in Sweden where protection after vaccination with unabsorbed vaccine was poor and side-effects unacceptable.

The safety and efficacy of whole-cell pertussis vaccines have been the subject of discussion in medical publications for the past decade. Efficacy rates ranging from 20% to 95% have been

reported throughout the world.[3] The best evidence that they are indeed effective in reducing morbidity and mortality in pertussis is the increased incidence of the disease and epidemics that follow withdrawal of vaccine usage.[4-6] It is widely recognised that the benefits of effective vaccination outweigh the risks.

Pertussis vaccines vary from country to country, as do vaccination schedules and recommendations, and clinical monitoring techniques. Vaccine efficacy appears to be good in some areas of South Africa, but for other areas there is no information and recent outbreaks of the disease have occurred in Cape Town and Durban.[7] The disease is not notifiable in South Africa and consequently no hard data on efficacy have been reported. There has also been no assessment of responses to the South African whole-cell pertussis vaccine. We therefore undertook to assess post-vaccination events and to measure serum IgG antibody responses to three major antigens, pertussis toxin (PT), filamentous haemagglutinin (FHA), and fimbrial agglutinogens 2 and 3 (AGG 2 + 3) in recipients of this vaccine. Maternal antibodies were also measured, since little is known about maternofetal transfer of pertussis antibodies, especially in African countries where inhibition of placental transfer might occur for a variety of reasons.

Subjects and methods

In the 3-month period March - May 1988, 115 healthy full-term newborn infants from KwaMashu, a suburb of Durban inhabited exclusively by blacks, were enrolled in the study after parental informed consent was obtained.

The subjects formed part of a larger study of the antibody responses and symptoms to the acellular Japanese J-NIH-6 whooping cough vaccine.[8] The subjects received BCG and trivalent oral polio vaccine (TOPV) at birth and were asked to return at 2, 4, 6 and 9 months of age. Whole-cell DTP and TOPV were administered at the time of the 2-, 4- and 6-month visits in accordance with current WHO recommendations. Measles vaccine was administered at 9 months of age. Blood samples were taken before vaccination at each visit. Three deaths, which were not vaccine-related, occurred before the subjects were 2 months old. All had been assigned to the whole-cell vaccine group and had therefore received only BCG and TOPV at birth. Of the 112 remaining subjects, 91 (81%) returned at 2 months of age, 83 (74%) at 4 months, 75 (67%) at 6 months and 60 (54%) at 9 months of age.

This was an open uncontrolled study. All healthy, full-term babies born at the Polyclinic in KwaMashu, during the period of enrolment were recruited into the study providing written parental consent was obtained and that they resided in an area suitable for follow-up. Babies were assigned to one of three vaccination groups in sequence. Each group comprised 115 children. Two groups received acellular pertussis vaccine and one group received whole-cell pertussis vaccine as detailed above. Mothers and nursing staff were unaware of which vaccine was given.

Each child was weighed and examined by a paediatrician (H.M.C. and W.E.K.L.) at every visit before vaccination. Children with acute illnesses were vaccinated after the illness subsided. The children were also carefully monitored for illnesses up to 9 months of age.

Nutritional status of subjects was assessed at each clinic visit by the anthropometric indices of length and weight for age, and clinical features of protein-energy malnutrition, and vitamin or trace element deficiencies. The US National Center for Health Statistics Reference Population was used as a standard. Children with a weight-for-age less than the third centile were considered underweight and children with a length-for-age less than 90% of the fiftieth centile were considered stunted.[9]

An illustrated questionnaire was given to all parents to record post-vaccination events for a period of at least 14 days after each vaccination. Detailed instructions pertaining to interpretation of the questionnaire were conveyed in Zulu by one of us (M.N.). Eight symptoms and signs were registered, viz. fever; loss of appetite; excessive crying; fretfulness; convulsions; hypotonic hyporesponsive episodes; swelling or induration at injection site (irrespective of size); or other symptoms regarded as possibly vaccine-associated. Fever was measured qualitatively (mothers' impression) because of the limited educational background of the population. Convulsions and hypotonic-hyporesponsive episodes were explained in some detail by M.N. Before the second and third doses of DTP the parents were questioned about any side-effects that had occurred after the previous injection. The parents were instructed to inform the paediatricians in charge of the trial immediately in case of serious reactions (usually through M.N.).

A sample of the mother's blood and the cord blood was obtained for each infant. Whole blood samples were also taken at 2, 4 and 6 months of age, immediately before vaccination. A fifth sample was taken at 9 months of age. Sera obtained from these samples were coded and frozen at −20°C until antibody assays could be performed. All sera from one individual were tested in the same assays on the same day. In some cases the sample was not sufficient to carry out all the tests required and therefore the numbers of samples giving rise to the data shown in the tables are not uniform.

IgG antibodies to FHA, PT and AGG 2 + 3 were assayed by enzyme-linked immunosorbent assay (ELISA) at the Centre for Applied Microbiology and Research, Public Health Laboratory Service, Porton, UK, by one of us (A.R.). The ELISA procedure used was essentially as described by Rutter *et al.*[10] Concentrations of antigens and conjugate used were determined by chequerboard titrations.

Materials

The adsorbed DTP vaccine used was manufactured by the South African Institute for Medical Research, Johannesburg. The vaccine was made up of 50 LF/ml diphtheria toxoid, 12 LF/ml tetanus toxoid, 20 000 million *B. pertussis*/ml; 2,5 mg/ml aluminium phosphate (adjuvant) and 0,01% thiomerosol (preservative). The vaccine was given to the infants in 0,5 ml doses by intramuscular injection in the left thigh at 2, 4 and 6 months of age. A single lot (No. A595) was used. The date of manufacture was 1 January 1988 and date of expiry 1 January 1990.

FHA and PT used as antigens in the ELISA were purchased from the Research Foundation for Microbial Diseases of Osaka University, Japan. Co-purified AGG 2 + 3 was provided by Dr A. Robinson, Centre for Applied Microbiology and Research, UK.

The Japanese reference pertussis antiserum was a gift from the Research Foundation for Microbial Diseases of Osaska University, Japan. It was supplied from a single lot and contained 250 ELISA units of PT-IgG antibody and 400 ELISA units of FHA IgG antibody to pertussis/ml. The reference serum was assigned a value of 400 ELISA units/ml of anti-AGG 2 + 3. The unitage of the test serum relative to the reference serum was calculated by means of parallel-line assays.

Results

Clinical record

Three cases of suspected whooping cough occurred in female infants. The first case occurred in a subject 2 months after the

first dose of vaccine was administered, i.e. at 4 months of age. The infant presented with subconjunctival haemorrhage and cough of 1 week's duration. The second case occurred in an unvaccinated infant who was 2 months of age. The third case occurred in an 8-month-old infant who had been fully vaccinated (3 doses of vaccine). No clinical signs of pertussis occurred in the latter two babies. Diagnosis was made retrospectively and based on the rapid rise in levels of all three antibodies. All infants had marked increases followed by rapid decline in levels of all three antibodies at the time of suspected pertussis infection.

The highest incidence of common childhood infections occurred between 6 and 9 months of age (68,3% of cases); infections of the upper respiratory tract were the most common (15,5% of infections), followed by skin infections (6,8%) and infections of the gastro-intestinal tract (3,2%) (Table I).

Nutritional status

A total of 6 children were found to be malnourished at various times during the study. Two children were underweight at birth but recovered before the first vaccination at 2 months of age. One child was underweight and stunted at 2 months of age, 2 children were underweight at 6 and 9 months of age. Their nutritional status had all reverted to normal by the next clinic visit. One child developed protein-energy malnutrition at 6 months of age and was still malnourished at 9 months of age.

Post-vaccination events

Parental evaluation of post-vaccination events occurring in the 14 days after vaccination was available for 249 doses of whole-cell pertussis vaccination. The number of post-vaccination events was very low (Table II). These subjects appeared normal on subsequent clinical examination. Fever (3,2%) and excessive crying (2,4%) were the most frequent systemic reactions. No infant had a local symptom (swelling or induration) at the injection site, which is most unusual. Other than local symptoms, post-vaccination events were transient and all responded to simple treatment at home without any sequelae.

Three neurologically associated post-vaccination events occurred. One male infant experienced febrile convulsions as well as a cough with wheeze, within 7 days after the third dose of vaccine. He was admitted to hospital 30 days later with measles and bronchopneumonia. No neurological sequelae resulted. Two hypotonic-hyporesponsive episodes (collapse, shock) occurred within 7 days after the first dose of vaccine. It was not possible to ascertain the exact time after vaccination at which these three events occurred.

Immune response

Transplacental transfer of pertussis antibodies

Antibodies to all 3 antigens tested were present in relatively high quantities in maternal sera and often detected in higher quantities in cord blood (hence active transfer across the placenta occurred) (Table III). All mothers had relatively high levels of IgG antibody to FHA and AGG 2 + 3. Only 7% of mothers had very low levels of IgG anti-PT, i.e. < 5 U.

Filamentous haemagglutinin and pertussis toxin

Geometric mean titres (GMT) (U) of IgG anti-FHA and IgG anti-PT in cord blood and at 2, 4, 6 and 9 months of age are shown in Tables III and IV.

TABLE I. INCIDENCE OF INFECTION IN BLACK INFANTS FROM BIRTH TO 9 MONTHS OF AGE

	0 ⩽ 2 mo. (N = 91)		> 2 ⩽ 4 mo. (N = 83)		> 4 ⩽ 6 mo. (N = 75)		> 6 ⩽ 9 mo. (N = 60)		Total	
Type of infection	No.	%	No.	%	No.	%	No.	%	No.	%
Upper respiratory tract	4	4,4	11	13,2	12	16,0	21	35,0	48	15,5
Skin	2	2,2	5	6,0	7	9,3	7	11,7	21	6,8
Gastro-intestinal tract	0	—	2	2,4	4	5,3	4	6,7	10	3,2
Lower respiratory tract	0	—	4	4,8	0	—	5	8,3	9	2,9
Eye	1	11,0	0	—	2	2,7	0	—	3	1,0
Measles	0	—	0	—	0	—	2	3,3	2	0,7
Viral meningitis	0	—	0	—	0	—	1	1,7	1	0,3
Pyrexia of unknown origin	0	—	0	—	1	1,3	0	—	1	0,3
Chicken pox	0	—	0	—	0	—	1	1,7	1	0,3
Total	7	7,7	22	26,5	26	34,7	41	68,3	96	31,1

TABLE II. POST-VACCINATION EVENTS AFTER WHOLE-CELL PERTUSSIS VACCINATION

	Dose 1 (N = 91)		Dose 2 (N = 83)		Dose 3 (N = 75)		Total (N = 249)	
Post-vaccination event	No.	%	No.	%	No.	%	No.	%
Fever	4	4,4	3	3,6	1	1,3	8	3,2
Excessive crying	5	5,5	1	1,2	0	—	6	2,4
Hypotonic-hyporesponsive episodes	2	2,2	0	—	0	—	2	0,8
Loss of appetite	0	—	0	—	1	1,3	2	0,8
Irritability	1	1,1	0	—	0	—	1	0,4
Convulsions	0	—	0	—	1	1,3	1	0,4
Swelling at injection site	0	—	0	—	0	—	0	—
Tenderness at injection site	0	—	0	—	0	—	0	—
Total	12	13,2	4	4,8	3	4,0	19	7,6

TABLE III. MATERNAL AND CORD BLOOD LEVELS (GMT ± SEM)* OF PERTUSSIS ANTIBODIES (RANGE)

Antibody	Mother's blood	Cord blood
Anti-PT - IgG	**41,6 ± 6,5 (2,3 - 211,4) (*N* = 55)**	**48,2 ± 9,8 (1,4 - 466,6) (*N* = 56)**
Anti-FHA - IgG	**72,2 ± 12,1 (12,3 - 590,9) (*N* = 56)**	**78,3 ± 15,1 (5,3 - 595,4) (*N* = 55)**
Anti-AGG 2 + 3 - IgG	**418,37 ± 210,1 (16,2 - 11 603,1) (*N* = 55)**	**539,9 ± 300,2 (0,6 - 14 156,2) (*N* = 54)**

* Geometric mean titre ± standard error of the mean.

The anti-FHA GMT was relatively high at birth and then declined steadily throughout the period of vaccination. After three doses of the vaccine, i.e. at 9 months of age, a ⩾ 4-fold rise in GMT occurred in 24,1% of subjects but a decrease occurred in 44,9% of subjects, and 31,0% of subjects had antibody levels which remained the same or increased only slightly.

The anti-PT GMT fell to a nadir at 4 months of age, rose at 6 months and then fell again at 9 months (i.e. 3 months after the third dose). There was no mean increase in antibody levels after 3 doses of vaccine (Table IV) — 19,0% of subjects experienced a 4-fold rise in level, 63,8% a fall, and 17,2% had levels that remained the same or increased only slightly after 3 doses of DTP (at 9 months of age).

The three children with suspected whooping cough had a rapid rise in levels of all three antibodies at the time of infection (data not included). Cord blood levels of anti-PT and anti-FHA were lower than the mean level in two cases and higher than the mean in the third case. A response to PT and to FHA did occur in the malnourished children, however the levels of antibody produced were usually lower than the mean of the normally nourished group (data not included).

Agglutinogens 2 + 3

There was an increase in GMT to AGG 2 + 3. IgG anti-AGG 2 + 3 levels fell from birth to 2 months and then rose with each dose of vaccine (Table IV). The seroconversion rate (a ⩾ 4-fold rise from pre-vaccination levels) was 67,2%. Although the group as a whole had increased antibodies to AGG 2 + 3, the response of individuals was absent or poor in some cases. A decrease in GMT occurred in 16,4% of recipients; and 16,4% had levels that remained the same or increased only slightly after 3 doses of vaccine (at 9 months of age). A rapid rise in anti-AGG 2 + 3 level occurred at the time of infection in the 3 suspected cases of whooping cough (data not provided). The anti-AGG 2 + 3 levels in all malnourished children were lower than the mean level (data not provided).

Discussion

The South African whooping cough vaccine has been reported to be very effective in the Cape.[7] In the present study this triple vaccine with a whole-cell pertussis component appeared to be fairly safe. The children were carefully monitored for complications and the rates of minor local and systemic post-vaccination events were low. Only 7,6% of subjects had a local or systemic symptom whereas rates of 50% and 72% have been reported in some studies in developed countries.[11,12] Fever, the most frequently found post-vaccination event, occurred in only 3,2% of vaccinees compared with 29,7% and 25,4%, respectively in Japanese and Swedish studies of whole-cell vaccine.[13,14] Local symptoms, absent in the present study, occurred in 16,7%[15] - 72,2%[12] of vaccinees in other studies. The South African DTP was associated temporally with major post-vaccination events (convulsions and hypotonic-hyporesponsive episodes), which did not appear to produce detectable sequelae. The rate of convulsions (1/249 doses, i.e. 4,02/1 000 doses) was high compared with other DTP vaccine studies in the UK (0,4/1 000 doses)[16] and the USA (0,57/1 000 doses).[2] In our studies of acellular pertussis[8] and measles vaccines among black children in South Africa (unpublished observations) rates of convulsions were 1,7/1 000 and 0/136 doses, respectively. Therefore this post-vaccination event appears to be related to vaccination with the South African DTP.

A hypotonic-hyporesponsive episode occurred in 2 children within 7 days after vaccination (8,03/1 000 doses). In a study in the USA[2] this type of reaction occurred at a rate of 0,57/1 000 doses, while in our study of an acellular pertussis vaccine (to be reported separately) this problem did not occur.

There are no data on the incidence of these events in unvaccinated children, since it was not ethically feasible to include such children in the study. The risk from these major post-vaccination events cannot be fully quantified in a study of this size and duration, particularly as the study population had a high incidence of infectious diseases. The possibility of these

TABLE IV. SEROLOGICAL RESPONSES TO WHOLE-CELL PERTUSSIS VACCINATION IN INFANTS (GMT ± SEM)* (RANGE)

		Pertussis IgG antibody (U)		
Age (mo.)	Sampling time	Anti-FHA	Anti-PT	Anti-AGG 2 + 3
2	**Before vaccination**	**82,8 ± 46,0 (1,6 - 135,1) (*N* = 50)**	**31,7 ± 10,3 (1,0 - 483,9) (*N* = 49)**	**212,5 ± 98,0 (6,35 - 4 246,4) (*N* = 48)**
4	**8 wks after dose 1**	**68,3 ± 20,0 (0,9 - 325,9) (*N* = 54)**	**20,5 ± 6,2 (0,9 - 310,8) (*N* = 54)**	**359,1 ± 148,5 (0,6 - 6 556,4) (*N* = 50)**
6	**8 wks after dose 2**	**62,5 ± 14,9 (0,9 - 255,6) (*N* = 60)**	**23,4 ± 6,9 (0,6 - 4 453,9) (*N* = 57)**	**503,2 ± 114,5 (0,9 - 290,0) (*N* = 54)**
9	**12 wks after dose 3**	**60,8 ± 12,5 (0,9 - 183,6) (*N* = 50)**	**18,4 ± 4,2 (1,2 - 186,2) (*N* = 45)**	**738,1 ± 165,3 (0,6 - 5 978,7) (*N* = 44)**

* Geometric mean titre ± standard error of the mean.

reactions being due to other intercurrent phenomena cannot be excluded.

In view of the reported efficacy, it was both surprising and interesting that serum IgG antibody responses to FHA and PT, two major antigens of *B. pertussis*, occurred infrequently and that no increase in GMT of either antibody was found after 3 doses of vaccine. Overall, a response to AGG 2 + 3 was observed but the rate of seroconversion was only 67,2%. Serum antibody responses to all these antigens have been reported in immunogenicity studies of whole-cell vaccines elsewhere.[16–18] Also, recent trials of acellular vaccines have shown that these confer some level of protection[19,20] and have shown that increases in serum IgG antibodies to the vaccine antigens are a feature of the response.[14,17] However, for humans, no serological correlates of protection against pertussis infection have yet been discovered, although evidence for protection by anti-PT and anti-FHA antibodies has been obtained in mice[21–23] and serospecific protection correlated with serum agglutinin type has been reported[18] for this species. No one class or type of pertussis agglutinin antibody has been demonstrated to be protective in humans and it is unknown whether antibodies to agglutinogens alone are sufficient to confer immunity.[11]

Because of the lack of responses to FHA and PT in the vaccinees, the high levels of all 3 antibodies found in maternal and cord sera suggest a high prevalence of infection among women of child-bearing age as well as active transplacental transfer of antibody. All the maternal sera had substantial levels of antibodies to FHA and to AGG 2 + 3 and many also had similar levels of anti-PT (93% had anti-PT > 5 U). However, there was considerable variation and this was reflected in the cord blood antibody levels.

For 1 infant with suspected whooping cough, cord blood levels of all 3 antibodies were higher than the mean cord blood levels, whereas for the other 2 suspected cases they were lower. The malnourished children overall responded no less well to vaccination than did other vaccinees. Nevertheless, the immune response was delayed in some cases and further study of pertussis vaccination of malnourished children is needed.

This study raises serious questions about the South African whooping cough vaccine, which need to be resolved. Most importantly a wider study of vaccine efficacy coupled with serological monitoring is required to determine whether the results presented here are typical or if they arise from batch-to-batch variability of the vaccine. The poor immunogenicity of the DTP used in this study, at least with regard to FHA and PT, signifies either (*i*) this batch would not provide protection, contrary to expectations based on reported efficacy; or (*ii*) any protection was likely to be due to some vaccine constituent(s) other than PT or FHA.

This research was supported in part by grants from the South African Medical Research Council, the British Council, and the Anglo-American and De Beers Chairman's Fund.

We would like to thank the KwaZulu Health Authorities for permitting this trial to be undertaken at clinics under their jurisdiction. We would also like to thank Dr Elizabeth Miller, P.H.L.S., of the UK for her erudite criticism of this manuscript; Dr L. A. E. Ashworth, P.H.L.S. CAMR, Porton, UK, for his support, use of his laboratory and assistance with ELISA measurements; and the Institute for Biostatistics of the South African Medical Research Council for analysis of data.

REFERENCES

1. Saito T, Kobayashi N, eds. *Current Status of the World's Children 1988*. Oxford: Oxford University Press for UNICEF, 1988; **15:** 6.
2. Cody CL, Baraff LJ, Cherry JD, Marcy SM, Manclark CR. Nature and rates of adverse reactions associated with DTP and DT immunizations in infants and children. *Pediatrics* 1981; **68:** 650-660.
3. Fine PEM, Clarkson JA. Reflections on the efficacy of pertussis vaccines. *Rev Infect Dis* 1987; **9:** 866-883.
4. Centers for Disease Control. Pertussis surveillance, 1979 to 1981. *MMWR* 1982; **31:** 333-336.
5. Centers for Disease Control. Pertussis: England and Wales. *MMWR* 1982; **31:** 629-632.
6. Kanai K. Japan's experience in pertussis epidemiology and vaccination in the past thirty years. *Jpn J Med Sci Biol* 1980; **33:** 107-143.
7. Metcalf CA, Prisman LD, Smith D. Whooping cough — a neglected disease in southern Africa (Correspondence). *S Afr Med J* 1989; **76:** 634.
8. Ramkissoon A, Coovadia HM, Loening WEK. A Phase II study of the acellular pertussis vaccine: assessment of antibody response and side-effects in African babies. Paper presented at the 19th International Congress of Paediatrics, 23 - 28 July 1989; Paris, France. (Abstracts Book p 384.)
9. Waterlow JC, Buzina R, Keller W, Lane JM, Nichaman MZ, Tanner JM. The presentation and use of height and weight data for comparing the nutritional status of groups of children under the age of 10 years. *Bull WHO* 1977; 55(4): 489-498.
10. Rutter DA, Ashworth LAE, Day A, Funnell S, Lovell F, Robinson A. Trial of a new acellular pertussis vaccine in healthy adult volunteers. *Vaccine* 1988; **6:** 29-32.
11. Pichichero ME, Badgett JM, Rodgers GC jun, McLinn S, Trevino-Scatterday B, Nelson JD. Acellular pertussis vaccine: immunogenicity and safety of an acellular pertussis vs. a whole cell pertussis vaccine combined with diphtheria and tetanus toxoids as a booster in 18 - 24-month-old children. *Pediatr Infect Dis J* 1987; **6:** 352-363.
12. Barkin RM, Pichichero ME. Diphtheria-pertussis-tetanus vaccine: reactogenicity of commercial products. *Pediatrics* 1979; **63:** 256-260.
13. Sato Y, Kimura M, Fukumi H. Development of a pertussis component vaccine in Japan. *Lancet* 1984; **1:** 122-126.
14. Blennow M, Granstrom M, Olin P *et al*. Preliminary data from a clinical trial (Phase 2) of an Acellular Pertussis vaccine, J-NIH-6. *Dev Biol Stand* 1986; **65:** 185-190.
15. Baraff LJ, Leake RD, Burstyn DG *et al*. Immunologic response to early and routine DTP immunisation in infants. *Pediatrics* 1984; **73:** 37-42.
16. Thomas MG, Ashworth LAE, Miller E, Lambert HP. Serum IgG, IgA, and IgM responses to pertussis toxin, filamentous hemagglutinin, and agglutinogens 2 and 3 after infection with *Bordetella pertussis* and immunisation with whole-cell pertussis vaccine. *J Infect Dis* 1985; **151:** 646-649.
17. Edwards KM, Lawrence E, Wright PF. Diphtheria, tetanus and pertussis vaccine: a comparison of the immune response and adverse reactions to conventional and acellular pertussis components. *Am J Dis Child* 1986; **140:** 867-871.
18. Ashworth LAE, Robinson A, Irons LI, Morgan CP, Isaacs D. Antigens in whooping cough vaccine and antibody levels induced by vaccination of children. *Lancet* 1983; **2:** 878-881.
19. Aoyama T, Murase Y, Gonde T, Iwata T. Type-specific efficacy of acellular pertussis vaccine. *Am J Dis Child* 1988; **142:** 40-42.
20. Ad Hoc Group for the Study of Pertussis Vaccines. Placebo-controlled trial of two acellular pertussis vaccines in Sweden — protective efficacy and adverse events. *Lancet* 1988; **1:** 955-960.
21. Sato H, Ito A, Chiba J, Sato Y. Monoclonal antibody against pertussis toxin: effect on toxin activity and pertussis infections. *Infect Immun* 1984; **46:** 422-428.
22. Oda M, Cowell JL, Burstyn DG, Manclark CR. Protective activities of the filamentous hemagglutinin and the lymphocytosis-promoting factor of *Bordetella pertussis* in mice. *J Infect Dis* 1984; **150:** 823-833.
23. Sato H, Sato Y. *Bordetella pertussis* infection in mice: correlation of specific antibodies against two antigens, pertussis toxin and filamentous hemagglutinin with mouse protectivity in an intracerebral or aerosol challenge system. *Infect Immun* 1984; **46:** 415-421.