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Poor Immune Responses to a Birth Dose of Diphtheria, Tetanus, and Acellular Pertussis Vaccine

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Abstract

Objectives—To evaluate the safety and immunogenicity of an additional birth dose of diphtheria, tetanus, and acellular pertussis vaccine (DTaP).

Study design—Fifty infants between 2 to 14 days of age were randomly assigned to receive either DTaP and hepatitis B vaccines (experimental) or hepatitis B alone (control) at birth. At 2, 4, 6, and 17 months of age, DTaP and routine vaccines were administered to both groups. Safety data were collected after each dose, and sera were obtained at birth, 6, 7, 17, and 18 months. Immune responses to pertussis toxin, filamentous hemagglutinin, pertactin, and fimbriae were measured by enzyme-linked immunosorbent assay; responses to other vaccines were assessed.

Results—No differences were seen between the 2 groups in either local or systemic reactions; all vaccines were well tolerated. Compared with the control group, infants in the experimental group demonstrated significantly lower geometric mean antibody concentrations for pertussis toxin and pertactin 6, 7, and 18 months, for fimbriae at 6, 7, 17, and 18 months, and for FHA at 18 months, and lower geometric mean antibody concentrations for diphtheria at 7 months. Immune responses to all other vaccine antigens were comparable.

Conclusion—Administration of an additional dose of DTaP at birth was safe but was associated with a significantly lower response to diphtheria and 3 of 4 pertussis antigens compared with controls.

Pertussis (whooping cough) is a respiratory tract infection caused by *Bordetella pertussis*, a gram-negative bacillus. The disease is most severe in infants and young children, with hospitalization and complication rates highest in this age group.¹ Most deaths associated with confirmed pertussis occur in infants <6 months of age, too young to have received the primary series of diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP).² Additional reports of increasing rates of infant pertussis in Canada and Europe also have appeared.^{3–7} Given these observations, we hypothesized that an additional dose of acellular pertussis vaccine might enhance protection. Therefore we evaluated the safety and immunogenicity of administering an additional dose of DTaP at birth and compared it with the standard DTaP vaccination schedule alone.

METHODS

Study Design

This was a prospective, randomized, controlled pilot study conducted in healthy full-term infants between 2 and 14 days of age who were available for the entire study period and whose parents or guardians provided written informed consent. Subjects were randomly assigned to either the experimental group that concomitantly received DTaP in the left thigh and hepatitis B vaccine in the right thigh or to the control group that received hepatitis B vaccine in the right thigh alone. Subjects in both groups received DTaP at 2, 4, 6, and 17 months of age concomitantly with the other vaccines recommended routinely by the American Academy of Pediatrics (Table I). The study was approved by the Vanderbilt University Institutional Review Board and conducted at the Vanderbilt University Hospital and Vanderbilt Children's Hospital.

Study Objectives

The 2 main objectives of the trial were to assess the safety and immunogenicity of an additional birth dose of DTaP given with the standard DTaP schedule at 2, 4, 6, and 17 months of age compared with the standard schedule alone.

Vaccines

All of the vaccines were administered according to the schedule outlined in Table I. A single lot of Daptacel DTaP (sanofi pasteur, Swiftwater, Pennsylvania) was provided by the manufacturer and used throughout the study. Each standard 0.5-mL dose of Daptacel contained diphtheria toxoid 15 Lf, tetanus toxoid 5 Lf, pertussis toxoid (PT) 10 μ g, filamentous hemagglutinin (FHA) 5 μ g, pertactin (PRN) 3 μ g, and fimbriae 2 and 3 (FIM) 5 μ g. The other vaccines included the following: hepatitis B vaccine (HepB) (Recombivax HB, Merck, Whitehouse Station, New Jersey), inactivated trivalent poliovirus vaccine (IPV) (IPOL, sanofi pasteur), *Haemophilus influenzae* type b conjugate vaccine (Hib) (ActHIB, sanofi pasteur); pneumococcal conjugate vaccine 7-valent (PCV7) (Prevnar, Wyeth, Madison, New Jersey), varicella virus vaccine live (V) (Varivax, Merck); and measles mumps rubella (MMR), (M-M-R, Merck). The components of each of these licensed vaccines are provided in the Appendix (available at www.jpeds.com).

Safety Evaluation

After each injection with DTaP, the infant was observed for 30 minutes for any immediate reactions. Parents/guardians, unaware of which vaccines were injected into which thigh, were given a digital thermometer, instructed in its use, and taught how to record local site reactions (pain, erythema, or thigh swelling by measuring thigh diameter), fever (rectal temperatures), systemic adverse events (crying for >3 hours, irritability/fussiness, drowsiness, and loss of appetite), and antipyretic use in a diary for 7 days after the birth vaccinations and for 3 days with the subsequent immunizations.

Phone calls were made by the study nurse on days 1 to 3 and day 8 after vaccination to review the diary cards. Illnesses or events that required physician intervention or concomitant medication use within 30 days of immunization were recorded. Serious adverse events were assessed for the entire 18-month study duration.

Immunogenicity Evaluation

Venous blood samples were obtained just before administration of the birth doses at 2 to 14 days of life, and again at 6, 7, 17, and 18 months of age. In 1 subject the parents requested that a cord blood sample be substituted for the prevaccination birth sample. Immunoglobulin

G (IgG) antibodies to PT, FHA, PRN, and fimbriae 2/3 (FIM) were assayed using standardized enzyme-linked immunosorbent assay (ELISA) as previously described^{8–12} and were performed in the Pediatric Research Laboratories at Vanderbilt University.

ELISA units were assigned based on the U.S. Food and Drug Administration (FDA) human reference pertussis antisera (Lots 3 and 4). The minimum level of detection for each IgG antibody was established at 2 ELISA Units (EU)/mL. The limit of quantification was 2 EU/mL, 3 EU/mL, and 5 EU/mL for IgG PT, IgG FHA, and IgG FIM, respectively. Sequential serum samples from each subject were run simultaneously in the same assay.

To evaluate the possibility of interference with the other standard approved vaccines, the FDA required that serum specimens collected at 7 and 18 months also be analyzed for responses to polyribosylribitol phosphate (PRP) Hib capsule antigen, pneumococcal serotypes 6B, 14, and 23F capsule antigen, and diphtheria and tetanus toxoids by ELISA. Microneutralization assays for poliovirus types 1, 2, and 3 were also performed on sera obtained at 7 and 18 months and hepatitis B surface (HBS) antibody concentrations were assessed by EIA on the 7 month sera only. All of these serologic assays were performed at MEP Laboratories in Rochester, New York.

The protective levels for vaccine antigens were defined by the FDA as diphtheria antitoxin and tetanus antitoxin 0.01 IU/mL at 7 months and 0.10 IU/mL at 18 months; PRP antibody 0.15 μ g/mL at 7 months and 1.00 μ g/mL at 18 months; neutralizing antibody levels for poliovirus types 1, 2 and 3 1:8, pneumococcal polysaccharides (Pn) (6B, 14, and 23F) antibody 0.15 μ g/mL at 7 months and 0.5 μ g/mL at 18 months, and anti-HBS antibody 10 mIU/mL.¹³ Because serologic correlates of immunity do not exist for pertussis antigens, the GMC and the number with a 4-fold rise in antibody concentration from baseline were determined for each pertussis antigen (Tables II and III).^{13,14}

Statistical Analysis

To assess the comparability of the experimental and control groups, a number of demographic and physical characteristics were compared by use of the 2-sample *t* test if the variables were continuous and Fisher exact test if the variables were categorical. Local and systemic reactions after vaccinations were compared by use of the Fisher exact test (2-sided). The geometric mean concentration (GMC) of anti-PT, anti-FHA, anti-PRN, and anti-FIM were calculated using the log-transformation of concentrations and taking the anti-log of the mean of these transformed values. The mean GMC levels were compared using repeated measures analysis of variance. The repeated measures analysis of variance models used an unstructured covariance structure to incorporate correlation between subjects. Both overall differences and time point comparisons for the treatment and control groups used the *t* test statistic (using the corrected standard error and degrees of freedom on the basis of the repeated measures design). The frequency of seroresponses, defined as a 4-fold increase from the prevaccination antibody level, was compared by the χ^2 test statistic.

RESULTS

Study Populations

To enroll the targeted 50 infants, a total of 270 parents/guardians were approached. The demographic and physical characteristics of the infants enrolled in the 2 groups did not differ. Overall, the mean age of the infants at enrollment was 3.2 days (range 2 to 14 days). Thirty infants were male, 20 were white, 26 were black, 1 was Asian, and 3 were Hispanic. A total of 8 infants withdrew during the entire 18-month study period (3 in the experimental group and 5 in the control group). Reasons for withdrawal were as follow: 5 subjects moved, 2 were unable to be reached by phone/certified letter, and 1 withdrew consent for additional

studies. A total of 47 subjects remained enrolled at the 2-month visit, 46 subjects at the 4-month visit, 45 infants at the 6- and 7-month visits, and 42 infants at the 17- and 18-month visits. The first subject was enrolled on February 10, 2004, the last subject was enrolled on August 1, 2004, and the last serum sample was obtained on June 26, 2006.

Safety Assessment

The birth doses of vaccines were well tolerated with no infant developing fever $\geq 100.5^{\circ}\text{F}$ within 7 days after either the DTaP and hepatitis B vaccines in the experimental group or the hepatitis B alone in the control group. No statistically significant differences were noted in local or systemic reactions, fever, use of concomitant medications, or serious adverse events between the experimental and control groups after any vaccine doses.

Immunogenicity

GMCs for each of the pertussis antigens at birth, and at 6, 7, 17, and 18 months of age in the experimental and control groups are shown in Table II. The control group demonstrated significantly higher titers for PT and PRN at 6, 7, and 18 months, for FIM at 6, 7, 17, and 18 months, and for FHA at 18 months compared to the experimental group. When the percentage of infants with a 4-fold rise after vaccination in the experimental and control groups are compared at 7 months, more infants in the control group had a 4-fold rise in antibody levels to PRN than in the experimental groups (82% vs 52%, $P = .032$), and at 18 months, more infants in the control group had a 4-fold rise in antibody levels to PT and FIM than in the experimental groups (40% vs 9%, $P = .0296$ and 85% vs 55%, $P = .047$), respectively. We investigated the impact of maternally-derived, preexisting antibody concentrations on infant responses to pertussis antigens and found no significant impact on immune responses to DTaP in either group on the basis of preexisting antibody, although prevaccination pertussis antibody levels were generally low in both groups.

GMCs were compared between the 2 groups for the other vaccine antigens (Table IV). The only significant differences at 7 months were higher GMCs of antibody for diphtheria and pneumococcal serotype 14 in the control group and at 18 months higher microneutralization titers for poliovirus 1 and 3 in the experimental group.

If infants did not acquire protective antibody titers after vaccination, the FDA required additional doses of vaccine be administered. All infants at 7 and 18 months attained antibody levels associated with protection for diphtheria, tetanus, and poliovirus, and no additional doses of these vaccines were given. However, 2 subjects in the experimental group and 1 in the control group received an additional dose of Hib and 2 in the control group received an additional dose of hepatitis B at 7 months of age because they had not achieved antibody responses associated with protection. At 18 months of age, 2 in the experimental group and 2 in the control group received an additional dose of Hib for the same reason.

DISCUSSION

Our study investigated the possibility of accelerating DTaP vaccination by administering a birth dose to infants. Because morbidity and mortality of pertussis are more severe in young infants, too young to have completed their primary DTaP series,² we sought to determine whether neonatal vaccination increased the serologic response to the pertussis antigens. In contrast with what we anticipated, an additional birth dose of DTaP resulted in lower GMCs to 3 of the 4 pertussis antigens when measured at 7 months and all four of the antigens at 18 months. Because the immune correlates of protection against pertussis are not well established, it is not known whether the antibody concentrations achieved by the infants in

the experimental group were protective. However, the antibody concentration that correlates with protection for diphtheria is known, and despite the lower diphtheria GMC in the experimental group at 7 months of age, all infants in the experimental group achieved these protective levels.

Although the birth doses of DTaP were not more immunogenic, they were well tolerated, with neither the number nor extent of local nor systemic adverse events differing between the experimental and control groups. These data confirm earlier reports of the safety of neonatal pertussis vaccination.^{15–25} Importantly, none of the infants in our trial experienced fever associated with the DTaP birth dose, avoiding the need for extensive sepsis evaluation. Finally, although reports of increased thigh swelling after the fourth and fifth doses of DTaP in older children had previously appeared,²⁶ we did not observe this event in the current trial.

The exact mechanism that underlies the lower response in the experimental group compared with the control group remains unexplained. In an earlier study by Baraff et al.,¹⁵ administering a birth dose of whole-cell pertussis vaccine combined with diphtheria and tetanus toxoids (DTP) also resulted in significantly lower titers to PT at 9 months of age compared with control infants. Similarly, several other studies of neonatal vaccination with DTP demonstrated lower antibody responses compared with routine DTP vaccination.^{22–24} In contrast, 2 other studies administering only aP at birth, without diphtheria or tetanus toxoids, followed by the DTaP series at either 3, 5, and 11 months or 2, 4, and 6 months, resulted in enhanced immune responses.^{16,27} These studies suggest that the combination of D and T with the aP at birth may have resulted in interference with antigen presentation or B-lymphocyte priming, explaining the lower antibody levels in the experimental group. Because we did not perform B- or T-lymphocyte studies in this trial, we cannot confirm or refute these possibilities. However, the challenges and opportunities presented by neonatal vaccination have been reviewed by others.^{28–30}

Although elevated levels of maternally-derived antibodies have been reported to interfere with infant immune responses to DTP in previous clinical trials,^{15,31–33} this was not found to be the case in our study. This may have been because the vaccine used in our study was DTaP, where preexisting antibody levels in earlier DTaP trials were not found to impact on subsequent immune responses.^{32,33} In addition the levels of maternally-derived antibodies in our study were very low.

Our study has several limitations. This was a pilot study, and therefore the sample size was small and not powered to detect rare adverse events associated with administering a birth dose of DTaP or to measure the efficacy of the vaccine. We did not measure antibody levels at 2 or 4 months and therefore do not know whether infants in the experimental group generated higher titers than the control group at those time points. Also, we did not measure memory B- and T-lymphocyte responses. Differences in GMCs may exist between the experimental and control groups to other antigens, but our study may have been underpowered to detect the differences. Finally, safety evaluation of administering DTaP at birth is not complete until a natural pertussis challenge has been observed. This was not observed in our study.

Further studies to assess the safety and immunogenicity of neonatal vaccination should be conducted. However, in the meantime, parents and caregivers of young infants should be encouraged to receive booster immunizations with acellular vaccines to minimize pertussis exposure to this very vulnerable group of infants.³⁴

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Glossary

DTaP	Diphtheria and tetanus toxoids and acellular pertussis vaccine
DTP	Diphtheria and tetanus toxoids
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FHA	Filamentous hemagglutinin
FIM	Fimbriae 2 and 3
GMC	Geometric mean concentration
HBS	Hepatitis B surface
HepB	Hepatitis B vaccine
Hib	<i>Haemophilus influenzae</i> type b conjugate vaccine
IgG	Immunoglobulin G
IPV	Inactivated trivalent poliovirus vaccine
MMR	Measles mumps rubella
PCV7	Pneumococcal conjugate vaccine 7-valent
PRN	Pertactin
PRP	Polyribosylribitol phosphate
PT	Pertussis toxoid
V	Varicella virus vaccine live

References

1. Pertussis—United States, 1997–2000. MMWR Morb Mortal Wkly Rep. 2002; 51:73–6. [PubMed: 11837909]
2. Pertussis deaths—United States, 2000. MMWR Morb Mortal Wkly Rep. 2002; 51:616–8. [PubMed: 12236302]
3. Celentano LP, Massari M, Paramatti D, Salmaso S, Tozzi AE. Resurgence of pertussis in Europe. *Pediatr Infect Dis J*. 2005; 24:761–5. [PubMed: 16148840]
4. Nteyayabo B, De Serres G, Duval B. Pertussis resurgence in Canada largely caused by a cohort effect. *Pediatr Infect Dis J*. 2003; 22:22–7. [PubMed: 12544404]
5. Crowcroft NS, Andrews N, Rooney C, Brisson M, Miller E. Deaths from pertussis are underestimated in England. *Arch Dis Child*. 2002; 86:336–8. [PubMed: 11970924]
6. Smith C, Vyas H. Early infantile pertussis; increasingly prevalent and potentially fatal. *Eur J Pediatr*. 2000; 159:898–900. [PubMed: 11131347]

7. Heininger U, Stehr K, Cherry JD. Serious pertussis overlooked in infants. *Eur J Pediatr.* 1992; 151:342–3. [PubMed: 1396888]
8. Cherry JD, Heininger U, Christenson PD, Eckhardt T, Laussucq S, Hackell JG, et al. Surrogate serologic tests for the prediction of pertussis vaccine efficacy. *Ann N Y Acad Sci.* 1995; 754:359–63. [PubMed: 7625674]
9. Deen JL, Mink CA, Cherry JD, Christenson PD, Pineda EF, Lewis K, et al. Household contact study of *Bordetella pertussis* infections. *Clin Infect Dis.* 1995; 21:1211–9. [PubMed: 8589145]
10. Hodder SL, Cherry JD, Mortimer EA Jr, Ford AB, Gornbein J, Papp K. Antibody responses to *Bordetella pertussis* antigens and clinical correlations in elderly community residents. *Clin Infect Dis.* 2000; 31:7–14. [PubMed: 10913389]
11. Mink CA, Sirota NM, Nugent S. Outbreak of pertussis in a fully immunized adolescent and adult population. *Arch Pediatr Adolesc Med.* 1994; 148:153–7. [PubMed: 8118532]
12. Meyer CU, Zepp F, Decker M, Lee M, Chang SJ, Ward J, et al. Cellular immunity in adolescents and adults following acellular pertussis vaccine administration. *Clin Vaccine Immunol.* 2007; 14:288–92. [PubMed: 17267589]
13. Plotkin SA. Immunologic correlates of protection induced by vaccination. *Pediatr Infect Dis J.* 2001; 20:63–75. [PubMed: 11176570]
14. Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. *Vaccine.* 1998; 16:1901–6. [PubMed: 9796041]
15. Baraff LJ, Leake RD, Burstyn DG, Payne T, Cody CL, Manclark CR, et al. Immunologic response to early and routine DTP immunization in infants. *Pediatrics.* 1984; 73:37–42. [PubMed: 6318185]
16. Belloni C, De Silvestri A, Tinelli C, Avanzini MA, Marconi M, Strano F, et al. Immunogenicity of a three-component acellular pertussis vaccine administered at birth. *Pediatrics.* 2003; 111(Pt 1): 1042–5. [PubMed: 12728086]
17. Provenzano RW, Wetterlow LH, Sullivan CL. Immunization and antibody response in the newborn infant. I. Pertussis inoculation within twenty-four hours of birth. *N Engl J Med.* 1965; 273:959–65. [PubMed: 5831734]
18. Sako W, Treuting WL, Witt DB, Nichamin SJ. Early immunization against pertussis with alum-precipitated vaccine. *J Am Med Assoc.* 1945; 127:379–84.
19. Abayomi I, Woodland M, Morley D. Whooping cough vaccine at birth. *J Trop Pediatr Environ Child Health.* 1973; 19:3–4. [PubMed: 4490499]
20. Di Sant Agnese PA. Combined immunization against diphtheria, tetanus, and pertussis in newborn infants. I. Production of antibodies in early infancy. *Pediatrics.* 1949; 3:20–33. [PubMed: 18107037]
21. Di Sant Agnese PA. Combined immunization with diphtheria, tetanus, and pertussis in newborn infants. II. Duration of antibody levels. Antibody titers after booster dose. Effect of passive immunity to diphtheria on active immunization with diphtheria toxoid. *Pediatrics.* 1949; 3:181–94. [PubMed: 18111425]
22. Di Sant Agnese PA. Combined immunization with diphtheria, tetanus, and pertussis in newborn infants. Relationship to age of antibody production. *Pediatrics.* 1949; 3:333–44. [PubMed: 18113114]
23. Di Sant Agnese PA. Simultaneous Immunization of Newborn Infants against Diphtheria, Tetanus, and Pertussis; production of antibodies and duration of antibody levels in an eastern metropolitan area. *Am J Public Health Nations Health.* 1950; 40:674–80. [PubMed: 15413708]
24. Du Pan RM. The vaccination of the newborn infant against pertussis. *J Pediatr.* 1958; 53:180–6. [PubMed: 13564382]
25. Waddell WW, L'Engle CS. Immune response to early administration of pertussis vaccine. *J Pediatr.* 1946; 29:487–92. [PubMed: 21002164]
26. Rennels MB, Deloria MA, Pichichero ME, Losonsky GA, Englund JA, Meade BD, et al. Extensive swelling after booster doses of acellular pertussis-tetanus-diphtheria vaccines. *Pediatrics.* 2000; 105:e12. [PubMed: 10617749]
27. Knuf M, Schmitt H-J, Wolter J, Schuerman L, Jacquet J-M, Kieninger, et al. Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. *J Pediatr.* 2008; 152:655–60. [PubMed: 18410769]

28. Kovarik J, Siegrist CA. Immunity in early life. *Immunol Today*. 1998; 19:150–2. [PubMed: 9577089]
29. Fischer GW, Ottolini MG, Mond JJ. Prospects for vaccines during pregnancy and in the newborn period. *Clin Perinatol*. 1997; 24:231–49. [PubMed: 9099512]
30. Marchant A, Newport M. Prevention of infectious diseases by neonatal and early infantile immunization: prospects for the new millennium. *Curr Opin Infect Dis*. 2000; 13:241–6. [PubMed: 11964793]
31. Van Rie A, Wendelboe AM, Englund JA. Role of maternal pertussis antibodies in infants. *Pediatr Infect Dis J*. 2005; 24(Suppl):S62–5. [PubMed: 15876928]
32. Van Savage J, Decker MD, Edwards KM, Sell SH, Karzon DT. Natural history of pertussis antibody in the infant and effect on vaccine response. *J Infect Dis*. 1990; 161:487–92. [PubMed: 2313127]
33. Englund JA, Anderson EL, Reed GF, Decker MD, Edwards KM, Pichichero ME, et al. The effect of maternal antibody on the serologic response and the incidence of adverse reactions after primary immunization with acellular and whole-cell pertussis vaccines combined with diphtheria and tetanus toxoids. *Pediatrics*. 1995; 96(Pt 2):580–4. [PubMed: 7659480]
34. Forsyth K, Tan T, von Konig CH, Caro JJ, Plotkin S. Potential strategies to reduce the burden of pertussis. *Pediatr Infect Dis J*. 2005; 24(Suppl):S69–74. [PubMed: 15876930]

APPENDIX

Exclusion criteria included children who (1) were born at <37 gestational weeks of age; (2) had complicated neonatal courses that included admission to the Neonatal Intensive Care Unit, infants requiring resuscitation in the delivery room, infants remaining in the hospital after the mother was discharged, and infants requiring antibiotic therapy while in the nursery; (3) had received gamma immune globulin at any time before enrollment; (4) had received any investigational drug or vaccine other than the study vaccines during the study period; (5) had known or suspected impairment of immunologic functions including family history of congenital immunodeficiency, receiving immunosuppressive therapy, or mother with human immunodeficiency virus; (6) had an evolving neurologic disorder or history of seizures; (7) had major congenital malformations; (8) had developmental disorders or serious chronic disorders; (9) had a known hypersensitivity to any components of the study vaccine; (10) had a history of idiopathic thrombocytopenic purpura or other bleeding disorders; (11) had a contraindication to vaccination that was included in the manufacturers' package inserts for DTaP, *Haemophilus influenza* type b vaccine (Hib), inactivated polio vaccine (IPV), 7-valent conjugated pneumococcal vaccine, or hepatitis B vaccines (Hep B); (12) had a mother younger than 18 years of age; (13) had a parent/legal guardian who was a drug abuser; (14) had previously been vaccinated with Hep B or had been born to a mother who was positive for Hep B surface antigen at birth or whose Hep B status was unknown; or (15) whose mother had a complicated pregnancy.

Grading scale for system and local reactions

Grades	Fever	Injection site redness, limb swelling, and thigh diameter:
0	<37.5° C	Absent
1	37.5° C to 38° C	>0 to <5 mm
2	>38° C to 38.5° C	5 mm to 20 mm
3	>38.5° C to <39.5° C	>20 mm to <35 mm
4	39.5° C	35 mm

COMPONENTS OF THE VACCINE

Hepatitis B vaccine (HepB) (Recombivax HB, Merck)— each 0.5 mL contains Hepatitis B surface Ag 5 μ g.

Inactivated trivalent poliovirus vaccine (IPV) (IPOL, sanofi pasteur)— each 0.5-mL dose of trivalent vaccine is formulated to contain 40D antigen units of type 1, 8D antigen units of type 2, and 32D antigen units of type 3 poliovirus.

Haemophilus influenzae, type b Conjugate (Hib) (ActHIB, sanofi pasteur)— each 0.5-mL dose contains purified capsular polysaccharide of *Haemophilus influenzae* type b 10 μ g covalently bound to tetanus protein 20 μ g.

Pneumococcal Conjugate Vaccine 7-valent (PCV7) (Prennar, Wyeth)— each 0.5-mL dose contains 2 μ g of each saccharide for serotypes 4, 9V, 18C, 19F, and 23F, and 4 μ g of serotype 6B per dose (16 μ g total saccharide); approximately 20 μ g of CRM₁₉₇ carrier protein, and 0.125 μ g of aluminum per 0.5-mL dose as aluminum phosphate adjuvant.

Varicella Virus Vaccine Live (V) (Varivax, Merck)—each 0.5-mL dose contains a minimum of 1350 PFU (plaque forming units) of Oka/Merck varicella virus when reconstituted and stored at room temperature for 30 minutes, sucrose approximately 25 mg, hydrolyzed gelatin 12.5 mg, sodium chloride 3.2 mg, monosodium L-glutamate 0.5 mg, sodium phosphate dibasic 0.45 mg, potassium phosphate monobasic 0.08 mg, potassium chloride 0.08 mg; residual components of MRC-5 cells including DNA and protein; and trace quantities of sodium phosphate monobasic, ethylenediamine tetraacetic acid, neomycin, and fetal bovine serum.

Measles Mumps Rubella (MMR), (M-M-R, Merck)—each 0.5-mL dose contains not less than 1 000 TCID₅₀ (tissue culture infectious doses) of measles virus; 12 500 TCID₅₀ of mumps virus; and 1 000 TCID₅₀ of rubella virus. Each dose of the vaccine is calculated to contain sorbitol (14.5 mg), sodium phosphate, sucrose (1.9 mg), sodium chloride, hydrolyzed gelatin (14.5 mg), recombinant human albumin (0.3 mg), fetal bovine serum (<1 ppm), other buffer and media ingredients and approximately 25 μ g of neomycin.

RESCUE PLAN

As a requirement from the Food and Drug Administration (FDA), if the antibody concentrations for the subjects were below the minimum protective levels outlined above for each antigen, an additional dose of the vaccine was required. Specifically for Hib: if the ELISA antibody to PRP was below minimum protective level of 0.15 μ g/mL at 7 months, an additional dose of Hib was required at 12 month visit and if the antibody to PRP was 1.0 μ g/mL at 18 months, an additional dose of the Hib was required. For hepatitis B: if the antibody levels were below the minimum protective antibody level 10 mIU/mL at 7 months, then an additional dose of the hepatitis B vaccine was required. For diphtheria and tetanus toxoid: if the antibody levels to either antigen were below the minimum protective level (0.01 IU/mL) at the 7 months, the fourth dose of DTaP would be administered at 12 months, and if the antibody levels to either antigen were below the minimum protective level (0.1 IU/mL) at the 18 months, an additional dose of the antigen (DT or T only) was required. For polio, if the neutralization levels for each serotype were below the minimum protective concentrations for each serotype (<1:8) at the 18 months, an additional dose of IPV was required.

Table 1

Vaccination and phlebotomy schedule for experimental and control groups

Group	Age at vaccination						
	2-14 Days	2 Mos	4 Mos	6 Mos	12 Mos	17 Mos	18 Mos
Experimental group (25 subjects)	Hep B (LLAT)	DTaP (RAT)	DTaP (RAT)	DTaP (RAT)	MMR (RUAT)	DTaP (RAT)	
	DTaP (RAT)	IPV (LLT)	IPV (LLT)	PCV (LUT)	Varicella (LLT)	IPV (LLT)	
		PCV (LUT)	PCV (LUT)	Hep B (LLAT)	PCV (LUT)	Hib (RLT)	
		Hep B (LLAT)	Hib (RLT)	Hib (RLT)			
		Hib (RLT)					
Control group (25 subjects)	Hep B (LLAT)	DTaP (RAT)	DTaP (RAT)	DTaP (RAT)	MMR (RUAT)	DTaP (RAT)	
		IPV (LLT)	IPV (LLT)	PCV (LUT)	Varicella (LLT)	IPV (LLT)	
		PCV (LUT)	PCV (LUT)	Hep B (LLAT)	PCV (LUT)	Hib (RLT)	
		Hep B (LLAT)	Hib (RLT)	Hib (RLT)			
		Hib (RLT)					
Blood Draws	x		x	x		x	x

Location of injections: *RAT*, right anterolateral thigh; *RUAT*, right upper anterolateral thigh; *RLT*, right lower thigh; *LLAT*, left lower anterolateral thigh; *LLT*, left lower thigh; *LUT*, left upper thigh.

Table II

Comparison of GMTs and 95% confidence intervals of the 4 pertussis antigens (Pertussis Toxin, Pertactin, Fimbriae and Filamentous hemagglutinin) at 6, 7, 17, and 18 months of age between the experimental group versus the control group

Antigen	Experimental group GMC (95% CI)	Control group GMC (95% CI)	P value
Pertussis toxin			
Birth	9 (6, 12)	11 (8, 16)	.526
6 Months	12 (9, 17)	18 (13, 25)	.0367
7 Months	17 (12, 23)	27 (20, 38)	.0394
17 Months	5 (4, 7)	6 (4, 9)	.4008
18 Months	12 (8, 16)	29 (20, 41)	.0006
Pertactin			
Birth	27 (17, 43)	26 (16, 41)	.7185
6 Months	51 (32, 80)	104 (64, 167)	.0086
7 Months	161 (102, 253)	442 (275, 713)	.0029
17 Months	25 (16, 39)	35 (22, 58)	.1073
18 Months	176 (110, 280)	508 (308, 837)	.0020
Fimbriae			
Birth	31 (19, 50)	22 (14, 37)	.1337
6 Months	57 (35, 92)	101 (35, 167)	.0204
7 Months	113 (70, 181)	264 (160, 453)	.0022
17 Months	22 (13, 36)	33 (20, 56)	.0308
18 months	149 (91, 243)	447 (264, 757)	.0001
Filamentous hemagglutinin			
Birth	11 (7, 16)	12 (8, 19)	.9238
6 Months	18 (12, 26)	18 (12, 27)	.6272
7 Months	25 (17, 36)	26 (17, 38)	.7136
17 Months	2 (2, 4)	3 (2, 4)	.6057
18 Months	21 (14, 30)	33 (21, 49)	.0426

The percentage of subjects who achieved a 4-fold rise in antibody concentration when compared with prevaccination levels to the 4 pertussis antigens at 6, 7, 17, and 18 months of age

Table III

Experimental group										Control group			
Number of infants		22	22	22	22	22	22	22	22	22	22	20	20
Time point in months		6 mos	7 mos	17 mos	18 mos	18 mos	6 mos	7 mos	7 mos	17 mos	18 mos	18 mos	18 mos
PT		13%	17%	0%	9%*	9%	23%	32%	32%	10%	40%*		
FHA		26%	30%	5%	27%	27%	27%	36%	36%	5%	35%		
PRN		43%	52%*	14%	64%	59%	59%	82%*	82%*	35%	80%		
FIM		43%	57%	9%	55%*	59%	59%	73%	73%	30%	85%*		

* $P < .05$ comparing control group versus experimental group.

Table IV

Geometric mean concentrations to other vaccines at the 7- and 18-month visits

	Antibody									
	Diphtheria	Tetanus	PRP	Polio 1	Polio 2	Polio 3	PnP6B	PnP14	PnP23F	HBS
7 Months										
Experimental group (n = 23)	1.64	4.25	3.49	126	81	153	1.96	4.12	1.49	427
Control (n = 22)	3.00	3.76	1.77	104	84	165	3.92	7.39	2.33	439
<i>P</i> value	.002	.584	.141	.512	.935	.788	.088	.035	.106	.959
18 Months										
Experimental group (n = 22)	3.92	7.25	18.86	1256	1108	2719	1.85	2.38	0.77	
Control (n = 20)	5.97	6.33	7.82	763	1042	1525	2.24	2.68	1.06	
<i>P</i> value	.078	.602	.078	.039	.819	.041	.553	.701	.230	