Safety and Immunogenicity of Six Acellular Pertussis Vaccines and One Whole-Cell Pertussis Vaccine Given as a Fifth Dose in Four- to Six-Year-Old Children

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ABSTRACT. *Objective.* **To evaluate the safety and immunogenicity of 6 different acellular pertussis vaccines combined with diphtheria and tetanus toxoids (DTaP) and with 1 licensed whole-cell pertussis vaccine (DTwP) as a fifth dose in children who had previously received the same DTaP, a different DTaP, or DTwP as primary and fourth-dose vaccinations.**

Methods. **Healthy 4- to 6-year-old children were enrolled at 5 National Institute of Allergy and Infectious Diseases Vaccine Treatment and Evaluation Units to receive a fifth dose of a DTaP or DTwP vaccine. All had been randomly assigned to receive 3 primary doses of DTaP or DTwP at 2, 4, and 6 months and a fourth-dose booster at 15 to 20 months of age as part of earlier National Institutes of Health multicenter acellular pertussis vaccine trials. Parents recorded the occurrence and magnitude of fever, irritability, and injection site redness, swelling, and pain for 3 days after vaccination. Sera obtained before and 1 month after the booster vaccination were analyzed by enzyme-linked immunosorbent assay for antibody to pertussis toxin, filamentous hemagglutinin, fimbriae, pertactin, and diphtheria and tetanus toxoid. Safety and/or immunogenicity data are reported for 317 children who received DTaP and 10 children who received DTwP.**

Results. **Fever and moderate or severe irritability were uncommon following the fifth dose of DTaP vaccine and were generally less frequent than following the fourth dose. However, for the DTaP vaccine groups, redness, swelling, and pain increased in prevalence compared with the fourth dose. The time course and frequency of reactions following DTaP vaccination were generally similar in children who received the same DTaP, a different DTaP, or DTwP for previous doses in the 5- dose series. No significant differences among the DTaP vaccines were detected in the occurrence of reactions, but the statistical power to detect differences was limited by sample size.**

Significant increases in antibodies directed against the included antigens were observed for all DTaP vaccines in paired pre- and post-fifth dose sera. Post-fifth dose antibody concentrations differed significantly among the DTaP vaccines. Some children in the study showed an antibody response to an antigen not reported to be in the DTaP vaccine.

Conclusion. **All the studied DTaP vaccines performed similarly with regard to reactions, whether given as a fifth sequential dose of the same vaccine, a mix of different DTaP vaccines in the 5-dose sequence, or after 3 DTwP and 1 DTaP vaccinations. Large injection site reactions occurred more frequently after the fifth dose of DTaP than after the previous 4 doses. A fifth dose of all DTaP vaccines induced an antibody response to those antigens contained in the vaccine. No DTaP was consistently most or least reactogenic or immunogenic.** *Pediatrics* **2000;105(1). URL: http://www.pediatrics.org/cgi/content/full/105/1/e11;** ***acellular, adverse reactions, antibody, diphtheria-tetanus-pertussis vaccine, pertussis vaccine, whole cell, whooping cough.***

ABBREVIATIONS. DTaP, diphtheria and tetanus toxoids combined with acellular pertussis vaccine; DTwP, diphtheria and tetanus toxoids combined with whole-cell pertussis vaccine; DTP, DTwP or DTaP; NIAID, National Institute of Allergy and Infectious Diseases; PT, pertussis toxin; FHA, filamentous hemagglutinin; PRN, pertactin; FIM, fimbriae; ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; GMC, geometric mean concentration.

During the past 2 decades, several acellular pertussis vaccines combined with diphtheria and tetanus toxoids (DTaP) have been developed and evaluated for safety and efficacy, and recently 4 DTaP vaccines have been licensed for use in the United States. In the routine childhood immunization schedule, DTaP vaccines are a preferred alternative to traditional whole-cell pertussis vaccine combined with diphtheria and tetanus toxoids (DTwP) because they are less reactogenic.[1–6] DTaP vaccines have been in use in the United States as the fourth- and fifth-dose boosters for children 15 to 20 months of age and 4 to 6 years of age, respectively, for several years.[5,6] Initially, these products were approved for administration after a primary series of DTwP injections; however, with the recent licensure of DTaP vaccines for use in infants, there will be, in

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the near future, children eligible for fifth-dose immunization for whom all previous immunizations were DTaP. This is the first and only study to evaluate comparatively the safety and immunogenicity of a fifth consecutive dose of different DTaP vaccines.

We conducted a double-blinded, multicenter clinical trial of 6 DTaP vaccines and 1 US licensed DTwP vaccine when given as a fifth-dose booster. All children enrolled had already received 4 doses of 1 of the 6 DTaP vaccines included in this study, 1 of 7 other DTaP vaccines that had been included in earlier National Institute of Allergy and Infectious Diseases (NIAID) multicenter acellular pertussis vaccine trials,[7–9] or a licensed DTwP vaccine. The primary goal was to evaluate the safety and immunogenicity of a fifth dose in those children who had received 4 previous doses of the same DTaP product. Additionally, data on the safety and immunogenicity of DTaP vaccines when given as a fifth-dose booster after previous vaccinations with a variety of DTaP products were generated.

METHODS

Vaccines

DTaP vaccines containing 2 to 4 pertussis components plus diphtheria and tetanus toxoids were included in the study (Table 1). All the DTaP vaccines included inactivated pertussis toxin (PT) and filamentous hemagglutinin (FHA), and some included pertactin (PRN) and/or fimbriae (FIM).

Subjects

Healthy children 4 to 6 years of age who had completed earlier NIAID multicenter acellular pertussis vaccine trials[7–9] that compared 2 DTwP and several DTaP vaccines, administered at 2, 4, 6, and 15 to 20 months of age were offered participation. Institutional review boards at each collaborating institution approved the protocol. Informed consent was obtained from parents or guardians before enrollment. No subjects with contraindications or precautions to immunization as specified in the *Report of the Committee on Infectious Diseases* of the American Academy of Pediatrics[6] were enrolled, and only those who received all 3 primary study doses in the earlier trial[7] were eligible to participate. Some subjects had not participated in the fourth-dose study[9]; all these individuals had received a licensed DTaP or DTwP vaccine for their fourth-dose booster.

Study Design

The vaccines were evaluated at 5 study sites: Baylor College of Medicine, Houston, Texas; University of Maryland School of Medicine, Baltimore, Maryland; University of Rochester, Rochester, New York; St Louis University School of Medicine, St Louis, Missouri; and Vanderbilt University School of Medicine, Nashville, Tennessee. When determining the vaccine to be administered at fifth dose, if possible, children received the same product as they had at fourth dose. When the product given for the fourth dose was not available for fifth dose, children who had received the Connaught Laboratories Ltd 3-component vaccine[7,9] at fourth dose, received CLL-4F_2 and children who received the SmithKline Beecham 2-component vaccine,[7,9] at fourth dose, received SKB-3P. All other children were randomly assigned to receive CLL-4F_2, LPT-4F_1, CB-2, PM-2, or BSc-3P. Thus, there were children who received 5 doses of the same DTaP product (same AAA group), children who received DTaP for all 5 doses but not all 5 were the same product (mixed AAA group), children who received DTwP for all 5 doses (WWW group), and children who received a priming series with DTwP followed by DTaP at fourth and fifth dose (WAA group) (Table 2). A small number of children received uncommon immunization schedules (WAW or AWA) and were excluded from additional analysis.

Double-blinding procedures were as previously described.[7,9] Study vaccinations were given in the arm (.5 mL. with 1-inch needles). Children were given oral polio vaccine concurrently.

Reaction Assessment

Reactions were assessed as previously described,[8,9] including temperature (oral); drowsiness (defined as unusually sleepy or inactive); irritability (scored as normal, periodically more irritable than usual but had normal activity [mild], prolonged crying and refused to play [moderate], or persistent crying and could not be comforted [severe]); anorexia (defined as an unusually poor appetite); vomiting; injection site redness and swelling (each measured in millimeters using the provided gauge); and injection site pain (scored as none, minor reaction to touch [mild], cried or protested to touch [moderate], or cried when arm moved [severe]).

Serology

For all sera, antibodies to PT, FHA, PRN, and FIM were measured by enzyme-linked immunosorbent assay (ELISA) at the University of Rochester using methods adapted from those used for the primary series trial.[10] Using the reference line calculation method,[11] results were expressed in ELISA units/mL (EU/mL) based on US reference pertussis antiserum (human), lots 3 and 4. Lower limits of detection for the University of Rochester laboratory were 2 EU/mL for PT and FHA and 3 EU/mL for PRN and FIM antibody assays. For consistency, the same lower limit of detection (6 EU/mL) was used as in previous publications when calculating fourfold increases in PRN antibody.[7,9] The 4 ELISAs also were performed at the Center for Biologics Evaluation and Research on a subset of 125 sera. When comparing the 4 ELISAs performed at the University of Rochester and the Center for Biologics Evaluation and Research laboratories, the geometric mean of ratios of individual values ranged from .67 to 1.5, indicating generally similar quantitative results. Spearman rank correlation

TABLE 1. Sources and Characteristics of Evaluated Vaccines

Vaccine Manufacturer	Vaccine Type	Abbreviation*	Inactivated PT (μg/Dose)	FHA (μg/Dose)	Pertactin (μg/Dose)	Fimbriae (μg/Dose)	Aluminum (mg/Dose)	Diphtheria Toxoid (Lf/Dose)	Tetanus Toxoid (Lf/Dose)
Pasteur Merieux Connaught (United States)/Biken	DTaP	CB-2	23.4	23.4	—	—	.17	6.7	5
Pasteur Merieux Connaught (France)	DTaP	PM-2	25	25	—	—	.30	25	10
Chiron	DTaP	BSc-3P	5	2.5	2.5	—	.35	25	10
SmithKline Beecham Biologicals	DTaP	SKB-3P	25	25	8	—	.50	25	10
Pasteur Merieux Connaught (Canada)	DTaP	CLL-4F_2	10	5	3	5	.30	15	5
Wyeth Lederle/Takeda	DTaP	LPT-4F_1	3.5	35	2	.6	.23	7.5	5
Wyeth Lederle	DTwP	WCL	NA	NA	NA	NA	.10	12.5	5

NA indicates not applicable.
* Abbreviations for DTaP vaccines same as for previous publications. Some manufacturers have changed corporate names.
† Limit of flocculation units.

TABLE 2. Five-dose Vaccination Schedules and Fifth-Dose DTaP Vaccines Studied

Vaccination Schedule	Group Code†	Primary	Dose 4	Dose 5	Vaccine Administered at Fifth Dose*							Totals	
					CB-2	PM-2	SKB-3P	BSc-3P	LPT-4F$_1$	CLL-4F$_2$	WCL		
5 doses of the same DTaP	Same AAA	A1‡	A1	A1	18	18	22	22	29	12		121	121
		A1	A1	A2	14	15	23[a]	17	16	28[b]		113	
5 doses of DTaP (Not all doses with same DTaP)	Mixed AAA	A1	A2	A2	0	0	0	0	30[c]	0		30	
		A1	A2	A1	0	1	0	1	0	2		4	147
Primary immunization: DTwP Doses 4 and 5: DTaP	WAA	WCL§	A1	A1	5	4	4	5	6	5		29	
		WCL	A1	A2	4	6	1	2	0	7		20	49
5 doses of DTwP	WWW	WCL	WCL	WCL							5	5	
		WCL	WCX[d]	WCL							4	4	
		WCM[d]	WCL	WCL							1	1	10
		TOTALS			41	44	50	47	81	54	10	327	327

* For abbreviations of vaccine names, see Table 1. Numbers in table indicate number of children receiving the specified vaccine at fifth dose.
Data for 24 individuals who participated in this study are not included in these analyses (see "Methods" section).
[a] All 23 individuals received the following schedule: SmithKline Beecham 2-component vaccine for the primary series[7] and fourth dose booster[9] and SKB-3P for fifth dose. [b] Seventeen of the 28 received the following schedule: Connaught Laboratories, Ltd. three component vaccine for the primary series[7] and fourth-dose booster[9] and CLL-4F$_2$ for fifth-dose booster. [c] All 30 individuals received the following schedule: Lederle Praxis Biologicals three component vaccine for the primary series[7] and LPT-4F$_1$ for fourth-[9] and fifth-dose boosters.
† See "Methods" section for a description of vaccine study groups and codes.
‡ A1 refers to the first DTaP product and A2 to the second DTaP received by a child.
§ WCL refers to Lederle DTwP, WCM to Massachusetts DTwP, and WCX to US licensed nonstudy DTwP.

coefficients for the 4 ELISAs were .88 (PT), .97 (FHA), .95 (PRN), and .90 (FIM).

Diphtheria and tetanus antibody levels were measured at the University of Rochester by ELISA using a method adapted from Melville-Smith and Balfour[13] and Melville-Smith et al,[14] respectively. Tetanus and diphtheria antitoxin concentrations were expressed in U/mL using international reference sera that had been calibrated using tetanus Antitoxin human immunoglobulin, reagent 76/589 and diphtheria antitoxin human serum, reagent 91/534. Both were obtained from the National Institute for Biological Standards and Control (London, UK).

Statistical Analysis

Reaction Data

Data are presented for 5 common reactions (fever, irritability, injection site redness, swelling, and pain) that were used previously in comparisons of DTaP and DTwP vaccines[15] using methods of tabulation described previously.[8] Incidence and severity of reactions in different groups were compared using appropriate nonparametric tests.[12] Within-group comparisons of reactions after the fourth and fifth vaccinations in the same individuals used the Wilcoxon signed-rank test.[12] For these nonparametric tests, ranks were calculated as described previously.[8]

Serology Data

Serologic analyses included children who had been vaccinated at 4 to 6 years of age and from whom serum had been collected both preimmunization and postimmunization. For calculations of geometric mean antibody concentrations (GMCs) and 95% confidence intervals, results lower than the minimum detectable level for the assay were assigned a value of one half of the minimum detectable level. For calculation of fold-rises, results lower than the minimum detectable level were assigned the minimum detectable level. Within-group comparisons of preimmunization and postimmunization values included both a sign test[12] and a paired *t* test on the logarithms of antibody levels. The 2 methods gave comparable results, and we report the results of the paired *t* test. Among-group comparisons used Duncan's multiple-range test to determine groups that did not differ significantly.[16]

Calculations were performed with SAS software (SAS Institute, Inc, Cary, NC). Unless stated otherwise, *P* values were not adjusted for multiple testing beyond any adjustment inherent in the test procedures; however, a Bonferroni adjustment was used in pairwise comparisons of reaction occurrences between recipients of a specific acellular vaccine at the fifth dose who did and did not receive that same vaccine at all previous injections.[16] A P value $<.05$ was considered statistically significant.

RESULTS

A total of 351 children received a fifth dose of DTaP or DTwP in the current study. Of these children, 327 fell into 4 groups of primary interest: same AAA (5 doses of the same DTaP; $n = 121$); mixed AAA (5 doses of DTaP, but not all with the same product; $n = 147$); WAA (DTwP primary series, DTaP boosters; $n = 49$); and WWW (5 doses of DTwP, but not necessarily all with the same product; $n = 10$). The distribution of the vaccinees in these groups is shown in Table 2. Of the children, 24 received a dose of DTaP or DTwP but could not be placed into 1 of the above categories; these children were not included in this analysis. Two thousand three hundred forty-two children were enrolled in the primary NIAID series trial[7,8] and 1374 participated in the fourth-dose trial.[9] The attrition of enrollees in this study occurred primarily because children had already received their fifth dose of DTaP or DTwP before initiation of the study or were not enrolled by the parent because of objections to blood collections (with several licensed DTaP vaccines available as an option). All children in the current study received the fifth vaccination at 4 to 6 years of age and all postvaccination sera were obtained between 21 and 81 days after immunization.

Adverse Reactions

For the 4 vaccination sequences of interest, Table 3 gives rates of systemic (fever and irritability) and local (redness, swelling, and pain) reactions after the fifth dose. There was very little fever >100°F. Any irritability, redness, swelling, and pain, including moderate and severe pain, tended to occur more frequently in the WWW group. However, there were no distinct differences by vaccine group in the occurrence of large areas of injection site redness and swelling. Large swelling or redness among those children who received 5 doses of the same DTaP

TABLE 3. Percent of Children With the Indicated Reaction by the Third Evening After Their Fifth Vaccination*

Vaccine Groups†	*N*	Temperature		Irritability			Redness			Swelling			Pain‡		
		≥100.1°F	≥101.1°F	>102°F	Any	Moderate or Severe	Severe	Any	>20 mm	>50 mm	Any	>20 mm	>50 mm	Any	Moderate or Severe	Severe
Same AAA	121	2.5	.8	.8	23.1	.8	.0	47.1	28.1	16.5	40.5	24.0	12.4	52.9	15.7	2.5
Mixed AAA	146	2.1	.7	.0	26.0	4.1	.7	44.5	26.7	18.5	41.1	21.9	15.8	59.6	17.8	4.8
WAA	49	6.1	6.1	2.0	20.4	2.0	2.0	42.9	18.4	10.2	28.6	8.2	4.1	67.4	20.4	8.2
WWW	10	.0	.0	.0	40.0	10.0	.0	90.0	30.0	10.0	70.0	20.0	10.0	100.0	50.0	10.0

* See "Methods" section for definitions of moderate and severe irritability and moderate and severe pain.
† See Table 2 and "Methods" section for description of study groups.
‡ Significant differences among 4 groups for pain ($P < .05$).

vaccine was not uncommon with swelling >50 mm occurring in 15 of 120 (12.5%) children and redness >50 mm in 20 of 120 (16.7%) children.

Adverse reactions developed in a similar pattern with respect to time after injection for the 4 groups. The temporal pattern was similar to that reported after fourth dose.[9] Irritability and pain tended to be reported most frequently on the first evening, whereas redness and swelling >20 mm were reported most frequently on the second or third evening. Fever was infrequent and generalizations regarding temporal pattern cannot be made. In general, the rates and severity of reactions were decreasing by the end of the third day.

In the primary series[8] and fourth-dose booster[9] trials, a significant trend toward an increased prevalence of injection site reactions with successive vaccinations for both DTaP and Lederle DTwP was observed. To assess whether this trend for intensifying local reactions would continue with the fifth-dose booster, reaction rates between the fourth-dose booster[9] and the fifth-dose boosters were compared (Table 4). Because the incidence of reactions from dose to dose for an individual is not independent,[17] only the subset of participants from the fourth-dose trial who participated in the current study and who received the same DTaP product at fourth and fifth doses and for whom complete reaction data were available were included. For the same AAA group, there were significant differences for all reaction categories between the fourth and the fifth doses. There were decreases in the 2 systemic reactions, temperature and irritability, but increases for the injection site reactions, swelling, redness, and pain. In the mixed AAA group, there was a decrease in fever and increases for redness and swelling. In the WAA group, there were increases in redness and in pain.

Among the 121 children in the same AAA group, there were between 12 and 29 who received 5 doses of any particular DTaP product. The reaction rates for the 6 individual DTaP products are shown in Table 5 (upper half). Significant differences among the 6 vaccine groups were observed for pain but not for the other 4 reactions. Table 5 (lower half) reports for each DTaP the reactions for those children who had received other products during the 5-dose series (mixed AAA or WAA). Product specific analyses were performed comparing reactions in the same AAA group (Table 5 upper half) to those in the mixed schedule groups (mixed AAA or WAA groups, Table 5 lower half). There were no significant differences when adjustment was made for the multiple comparisons. The occurrence of anorexia, vomiting, and drowsiness was uncommon after a fifth dose of DTaP or DTwP vaccine, and no differences were observed when vaccine groups were analyzed in a like manner.

Special attention was focused on monitoring for more severe reactions. None of the children in our study experienced a seizure, hypotonic/hyporesponsive episode, or other uncommon events associated with pertussis vaccination. No child had a fever >103.0°F. Severe irritability was reported for only 2 children. As noted above, redness >50 mm, swelling >50 mm, and severe pain occurred after DTaP im-

TABLE 4. Percent of Children Who Received the Same DTaP Vaccine at Fourth and Fifth Dose and Were Reported To Have Had the Indicated Reaction by the Third Evening After Their Fourth Vaccination of Pertussis Vaccine at 15 to 20 Months of Age or Fifth Vaccination at 4 to 6 Years of Age*

Study Group		Temperature			Irritability		Redness			Swelling			Pain	
		≥100.1°F	≥101.1°F	>102°F	Any	Moderate or Severe	Severe	Any	>20 mm	>50 mm	Any	>20 mm	>50 mm	Any	Moderate or Severe	Severe
Reaction rates after fourth dose																
Same AAA	$n = 120$	17.5	2.5	.0	35.8	6.7	.8	23.3	6.7	3.3	24.2	5.8	4.2	20.0	5.0	.0
Mixed AAA†	$n = 27$	25.9	7.4	.0	29.6	7.4	.0	18.5	11.1	.0	14.8	3.7	.0	33.3	7.4	.0
WAA	$n = 28$	10.7	.0	.0	17.9	7.1	3.6	7.1	3.6	.0	10.7	.0	.0	17.9	7.1	3.6
Reaction rates after fifth dose																
Same AAA‡	$n = 120$	2.5	.8	.8	23.3	.8	.0	47.5	28.3	16.7	40.8	24.2	12.5	53.3	15.8	2.5
Mixed AAA§	$n = 27$	3.7	.0	.0	25.9	.0	.0	51.9	22.2	11.1	37.0	18.5	11.1	44.4	7.4	.0
WAA‖	$n = 28$	10.7	10.7	3.6	21.4	.0	.0	39.3	14.3	10.7	21.4	7.1	.0	75.0	17.9	.0

* Table only includes data for those individuals who participated in the fourth- and fifth-dose booster studies and received the same DTaP product at both doses. See "Methods" section definitions of moderate and severe irritability and moderate and severe pain.
† For this analysis, all 27 children in mixed AAA group received Lederle-Praxis 3-component in primary series with LPT-4F$_1$ as fourth and fifth dose.
‡ Dose 5 significantly different from dose 4 for all 5 reactions ($P < .05$).
§ Dose 5 significantly different from dose 4 for temperature, redness, and swelling ($P < .05$).
‖ Dose 5 significantly different from dose 4 for redness and pain ($P < .05$).

munization (Tables 3–5), including whole-arm swelling in 5 children (Rennels MB, Deloria MA, Pichichero ME, et al, unpublished data).

Serologic Response

The primary immunogenicity data for this study are provided by those individuals in the same AAA groups (Table 6). Antibody concentrations before the fifth-dose immunizations (data available on request) were lower than those observed 1 month after the fourth-dose vaccination,[9] although quantitative comparisons must be made cautiously because assays after the fourth dose were performed in a different laboratory. For all 6 DTaP vaccines, the postimmunization GMCs were significantly higher than the preimmunization GMCs for all antigens reported to be in the vaccines. Table 6 also presents the proportion of children with at least a fourfold increase in antibody concentration. In those children who received different DTaP products, an antibody response was observed to the pertussis antigens contained in the DTaP vaccine received at the fifth dose (Table 5). For all 6 DTaP vaccines, the postimmunization GMCs were significantly higher than the preimmunization GMCs for those antigens reported to be in the vaccines.

In mixed AAA groups, the composition of the vaccine received at fifth dose was often different from that received for previous doses. As a consequence, there were some children who received a specific antigen at fifth dose without receiving that antigen in the 4 previous immunizations. To gain insight into the response that would occur in this circumstance, we separated the mixed AAA group into those children who received a particular antigen for the first time and those children who had received that antigen in 1 or more of the preceding DTaP immunizations. These results are presented for FHA, PRN, and FIM in Table 7. Analyses were not performed for PT antibody because all children had received inactivated PT in their previous doses. As anticipated, the antibody response was generally higher in children who had been exposed to that antigen in 1 or more of the previous vaccinations.

As reported previously,[7,9] some children in the study showed an antibody response to an antigen not reported to be in the DTaP vaccine. For the CB-2 vaccine, 8 of 18 (44%) children in the same AAA group, 2 of 14 (14%) in the mixed AAA group, and 6 of 9 (67%) in the WAA group had a fourfold or greater increase in antibodies to PRN, suggesting that CB-2 contained some PRN. Any quantity of PRN in CB-2, seems to be low however, because none of the children immunized with 3 doses of CB-2[7] and only ~10% of the children immunized with 4 doses of CB-2[9] had at least a fourfold rise in PRN antibody. Two of the 80 (3%) children in the same AAA group who received a vaccine without FIM showed a fourfold rise in FIM antibody; 1 had been immunized with CB-2 and 1 with PM-2.

A significant increase in diphtheria and tetanus antibodies was observed in all study groups, including those children who received different products throughout the schedule. All children in this study had antibody concentrations that exceeded the presumed minimum protective levels of .1 U/mL for

diphtheria[18] and .01 U/mL for tetanus[19] 1 month after the fifth dose.

DISCUSSION

Four DTaP products are currently licensed in the United States for primary immunization of infants; the first vaccine was licensed in July 1996. A large number of children who have received DTaP vaccines for the first 4 doses will soon come of age for their fifth dose in the recommended schedule. Several studies have evaluated the safety and immunogenicity of a fifth dose of DTaP vaccines after primary immunization with DTwP vaccines and 2 DTaP vaccines were licensed in 1991 and 1992 for this indication. However, there are very few reports of the safety and immunogenicity of a fifth dose after 4 previous doses of the same DTaP vaccine. Reflecting this absence of data, 3 of the 4 US licensed products have not yet been approved for a fifth dose when the first 4 doses were DTaP. This study took advantage of the opportunity to evaluate the safety and immunogenicity of a fifth dose of 6 different DTaP products in children who had been evaluated in previous studies. This is 1 of few reports on the safety and immunogenicity of a fifth dose of DTaP, and the first to evaluate multiple DTaP products in a schedule that included more than 1 product. Larger product specific studies are being conducted by vaccine manufacturers so that more information can be available before the cohort of children immunized with DTaP for their primary series reach the age for fifth dose.

Combining the results of this study with our earlier publications[8,9] suggests the overall safety profile for the 5-dose series of DTaP vaccines for the common reactions was better than that observed for 5 doses of DTwP vaccine. No DTaP vaccine was consistently the most or least reactogenic after the fifth dose of a 5-dose series, but sample size limited the statistical power to detect potentially meaningful differences. The fifth-dose booster showed a trend for intensifying injection site reactions but not systemic reactions from dose 4 to dose 5. Large injection site reactions, which usually did not limit activity, were not uncommon with overall rates of ~1 of every 6 for redness >50 mm, 1 of every 8 for swelling >50 mm, and 1 of every 40 for severe pain. These rates were for the cohort of children in the same AAA group combining data from all products. Larger studies may demonstrate that the rates vary for the different DTaP products. Nevertheless, parents should be forewarned about this possibility after fifth-dose boosters with DTaP vaccines.

In those children who received 5 doses of the same DTaP product, each of the evaluated DTaP vaccines produced significant increases in antibodies directed against included antigens, but postvaccination antibody concentrations differed significantly among the DTaP vaccines. Because serologic correlates of protection are not known, it is not currently possible to determine whether a greater quantity of antibody generated by 1 vaccine compared with another has clinical relevance.

It is currently recommend that, when feasible, the same DTaP should be used throughout the series. However, there will be circumstances when use of the same product will be impractical, if not impossi-

TABLE 5. Reactions Reported After Fifth Dose of the Individual DTaP Vaccines*

Study Group	Vaccine Received for Fifth Dose	*n*	Temperature			Irritability			Redness			Swelling			Pain†		
			≥100.1°F	≥101.1°F	>102°F	Any	Moderate or Severe	Severe	Any	>20 mm	>50 mm	Any	>20 mm	>50 mm	Any	Moderate or Severe	Severe
Same AAA	CB-2	18	5.6	.0	.0	11.1	.0	.0	33.3	22.2	5.6	27.8	16.7	.0	66.7	11.1	.0
	PM-2	18	.0	.0	.0	38.9	5.6	.0	55.6	38.9	22.2	66.7	33.3	16.7	77.8	27.8	.0
	BSc-3P	22	.0	.0	.0	31.8	.0	.0	50.0	18.2	9.1	27.3	18.2	9.1	27.3	4.6	.0
	SKB-3P	22	4.6	4.6	4.6	22.7	.0	.0	59.1	40.9	36.4	50.0	45.5	27.3	54.6	27.3	4.6
	LPT-4F$_1$	29	3.5	.0	.0	17.2	.0	.0	44.8	24.1	10.3	34.5	13.8	10.3	51.7	13.8	3.5
	CLL-4F$_2$	12	.0	.0	.0	16.7	.0	.0	33.3	25.0	16.7	44.7	16.7	8.3	41.7	8.3	8.3
Mixed AAA or WAA	CB-2	23	4.4	4.4	4.4	39.1	.0	.0	43.5	17.4	13.0	39.1	4.4	4.4	60.9	17.4	.0
	PM-2	26	7.7	3.9	.0	15.4	.0	.0	42.3	26.9	19.2	34.6	15.4	15.4	65.4	19.2	7.7
	BSc-3P	25	.0	.0	.0	36.0	4.0	4.0	40.0	24.0	16.0	36.0	20.0	12.0	60.0	20.0	8.0
	SKB-3P	27	3.7	3.7	.0	18.5	7.4	.0	55.6	33.3	25.9	59.3	29.6	25.9	63.0	25.9	7.4
	LPT-4F$_1$	52	1.9	.0	.0	21.2	3.9	.0	36.5	19.2	9.6	30.8	15.4	7.7	51.9	7.7	.0
	CLL-4F$_2$	42	2.4	2.4	.0	23.8	4.8	2.4	50.0	28.6	19.1	35.7	23.8	14.3	71.4	26.2	11.9

* Percentage of children with indicated reaction by the third evening after fifth dose. See "Methods" section for definitions of moderate and severe irritability and moderate and severe pain.
† For same AAA groups, significant differences among the six vaccines for pain ($P < .05$).

TABLE 6. Antibody Response to Pertussius Antigens After a Fifth Dose of DTaP or DtwP Vaccine: Comparison of the Same DTaP Vaccine for All 5 Doses, a Mix of Different DTaP Vaccines and a Mix of DtwP and DTaP Vaccines

Vaccine at Fifth Dose	Study Group	*n*	PT		FHA		PRN		FIM	
			Post GMC (95% CI)	Percent Response*	Post GMC (95% CI)	Percent Response	Post GMC (95% CI)	Percent Response	Post GMC (95% CI)	Percent Response
CB-2	Same AAA	18	175 (111–275)	100	319 (219–466)	94	36[a] (18–68)	44	3[b] (2–5)	6
	Mixed AAA	14	248 (145–426)	93	73 (17–308)	64	10[a] (4–27)	14	6 (2–22)	0
	WAA	9	596 (356–999)	100	202 (87–469)	78	75[a] (47–120)	67	74 (30–185)	11
PM-2	Same AAA	18	180 (117–276)	100	682 (522–892)	89	6 (3–12)	0	2 (1–4)	6
	Mixed AAA	16	155 (107–223)	94	195 (79–478)	81	6 (4–11)	0	4 (2–9)	0
	WAA	10	307 (178–528)	90	182 (73–457)	90	25[c] (11–56)	0	33 (15–73)	10
BSc-3P	Same AAA	22	126 (98–164)	100	146 (115–184)	82	339 (224–515)	91	2 (1–2)	0
	Mixed AAA	18	87 (44–171)	89	60 (20–182)	67	26 (14–58)	67	6 (2–15)	0
	WAA	7	320 (147–694)	100	29 (5–158)	57	167 (50–560)	71	10 (2–42)	14
SKB-3P	Same AAA	22	105 (74–150)	100	503 (376–672)	86	849 (536–1346)	86	2 (1–4)	0
	Mixed AAA	23	169 (130–220)	100	755 (585–974)	91	44 (23–85)	52	2 (1–2)	0
	WAA	5	132 (49–354)	100	345 (167–713)	80	224 (71–709)	80	7 (1–73)	0
CLL-4F$_2$	Same AAA	12	61 (35–108)	92	59 (31–112)	83	444 (189–1041)	92	583 (335–1017)	100
	Mixed AAA	29	111 (84–148)	100	69 (39–122)	62	42 (22–82)	52	436 (212–895)	83
	WAA	12	225 (120–424)	100	65 (27–156)	75	614 (308–1222)	100	892 (597–1332)	100
LPT-4F$_1$	Same AAA	29	21 (13–33)	72	146 (100–212)	86	263 (168–411)	76	48 (33–70)	76
	Mixed AAA	46	32 (24–43)	89	218 (168–282)	80	186 (117–295)	80	34 (20–58)	59
	WAA	6	80 (17–380)	83	144 (58–357)	100	200 (62–648)	100	222 (58–859)	83
WCL	WWW	9	92 (46–182)	100	36 (20–66)	56	80 (34–192)	56	343 (231–509)	89

* Percentage of children with fourfold or greater increase in antibody concentration.
For all vaccine groups (same AAA, mixed AAA, and WAA), when the vaccine contained an antigen, the post was significantly greater than pre and when the antigen was not present, the post was not significantly greater. The exceptions are: [a] for CB-2, significant increase in PRN for WAA groups, same AAA, mixed AAA; [b] for CB-2, significant increase in FIM for GMC antibody from pre to post booster was observed in the same AAA group; and [c] for PM-2, significant increase in PRN f for GMC antibody from pre to post booster was observed in the same WAA group.
For the study groups, the GMCs in preimmunization samples ranged from 2 to 25 EU/mL for PT, 5 to 41 EU/mL for FHA, 4 to 58 EU/mL for PRN, and 2 to 49 EU/mL for FIM.

ble. In such cases the Advisory Committee on Immunization Practices recommends continuing the vaccination schedule with the administration of a different DTaP product. There are no published data that report the safety or immunogenicity of schedules that involve the use of more than 1 DTaP product. Several of the experimental DTaP products evaluated in the NIAID Vaccine Treatment and Evaluation Units acellular pertussis trials were not available for the fifth-dose study, giving rise to the opportunity to collect data on the safety and immunogenicity of schedules that involved more than 1 DTaP product. Our study was not designed to evaluate specific mixed schedules and our sample size was small; thus few formal analyses have been performed in this report. Nevertheless, the safety data

TABLE 7. Postbooster Geometric Mean Antibody Concentrative and Percentage of Children With Fourfold Increase in Antibody Concentration to Fifth Dose in the Mixed AAA Groups, Separating Those Individuals Who Had Received the Antigen in Previous Vaccines From Those Who Had Not Received the Antigen in Previous Vaccines

Fifth Dose	FHA			PRN			FIM		
	n	Post	% Response	*n*	Post	% Response	*n*	Post	% Response
CB-2									
Without*	10	46	50	14	10	14	11	2	0
With†	4	230	100	0	—	—	3	337	0
PM-2									
Without	9	99	78	15	6	0	13	2	0
With	7	466	86	1	29	0	3	64	0
BSc-3P									
Without	9	39	56	17	26	65	12	2	0
With	9	92	78	1	175	100	6	49	0
SKB-3P									
Without	0	—	—	23	44	52	23	2	0
With	23	755	91	0	—	—	0	—	—
CLL-4F$_2$									
Without	6	30	50	28	35	50	6	47	83
With	23	86	65	1	198	100	23	777	83
LPT-4F$_1$									
Without	7	204	57	16	41	63	11	11	36
With	39	220	85	30	416	90	35	49	66

* Antigen was not contained in any of the DTaP vaccines received for first 4 doses.
† Antigen was contained in at least 1 of the DTaP vaccines received for first 4 doses.
For the study groups, the GMCs in preimmunization samples ranged from 2 to 25 EU/mL for Pt, 5 to 41 EU/mL for FHA, 4 to 58 EU/mL for PRN, and 2 to 49 EU/mL for FIM.

are generally encouraging in that the nature and rates of reactions to DTaP at fifth dose were similar whether the same or different products were used at previous doses. Few definitive conclusions can be made from the immunogenicity of a mixed schedule; however, the data from Tables 6 and 7 suggest that most individuals will respond to the antigens in a DTaP vaccine, although the response for each antigen is likely to be higher if that antigen was contained in previously received doses.

In previous publications,[7,9] evidence was provided that some children produced an antibody response to antigens not reported to be contained in the vaccine. In the current study, a fourfold or greater antibody rise to PRN was observed in some children after 5 doses of CB-2 vaccine, although PRN is not reported to be present in this vaccine. The PRN content of CB-2 seems to be low because of the limited response after 3 or 4 doses.[7,9] Among the children who were immunized with 1 of the four DTaP vaccines without FIM, fourfold responses to FIM were infrequent, occurring in only 5 of 182 (3%) of subjects. These results suggest that some DTaP products that were reported to lack PRN and/or FIM contain sufficient amounts of these antigens to stimulate an immune response in some individuals that have been primed.

This study has limitations. Although we present *P* values from formal statistical tests, several factors dictate that they be interpreted with caution. First, the children in the present study represent a small proportion of those in the original randomized trial;[7,8] thus the results presented herein could in some cases be influenced by selection biases of unknown direction and magnitude. Uncertainty about the representativeness of the study population attributable to the high dropout rate causes a concern about generalization of the findings. Second, small numbers in 1 or more of the groups being compared limit the statistical power of the tests, especially with regard to comparisons of individual DTaP vaccines. Third, primary antibody assays were performed in 3 different laboratories, making precise comparisons of postimmunization antibody concentrations after the fifth dose to those after the third and fourth doses difficult because of variation in absolute quantitation of antibody in different laboratories.[20]

This study reports the completion of a series of NIAID acellular pertussis vaccine trials that began with 2342 infants. Of these, 121 children received 5 consecutive doses of the same DTaP product. Following these children over the full series of 5 doses provides insight on the safety and immunogenicity the immunization series. We reach the following conclusions: 1) over the full five-dose series,[7,9] all common reactions occur less frequently after DTaP compared with DTwP; 2) there is an increase in local injection site reactions with each subsequent dose, particularly redness and swelling, but such reactions to the fifth dose do not seem to exceed those typically observed with DTwP vaccines;[21,22] and 3) DTaP vaccines are immunogenic when administered as a fifth-dose booster. Because serologic correlates and minimum protective levels of antibody to the studied pertussis antigens have not been established, the clinical relevance of differences in magnitude of antibody responses remains unknown.

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REFERENCES

1. Mortimer EA Jr. Pertussis and pertussis vaccines: 15 years of change. *Semin Pediatr Infect Dis*. 1991;2:82–90
2. Cherry JD. Pertussis: the trials and tribulations of old and new pertussis vaccines. *Vaccine*. 1992;10:1033–1038
3. Edwards KM. Acellular pertussis vaccines–a solution to the pertussis problem? *J Infect Dis*. 1993;168:15–20
4. Pichichero ME. Acellular pertussis vaccines: toward an improve safety profile. *Drug Safety*. 1996;15:311–324
5. Centers for Disease Control and Prevention. Pertussis vaccination: acellular pertussis vaccine for the fourth and fifth doses of the DTP series: update to supplementary ACIP statement, recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morb Mortal Wkly Rep CDC Surveill Summ*. 1992;41:1–5
6. American Academy of Pediatrics, Committee on Infectious Diseases. *Report of the Committee on Infectious Diseases*. Elk Grove Village, IL: American Academy of Pediatrics; 1994
7. Edwards KM, Meade BD, Decker MD, et al. Comparison of thirteen acellular pertussis vaccines: overview and serologic response. *Pediatrics*. 1995;96:548–557
8. Decker MD, Edwards KM, Steinhoff MC, et al. Comparison of thirteen acellular pertussis vaccines: adverse reactions. *Pediatrics*. 1995;96:557–566
9. Pichichero ME, Deloria MA, Rennels MB, et al. A comparison of 12 acellular pertussis vaccines with one whole cell pertussis vaccine when given as a fourth dose in 15 to 20 month-old children. *Pediatrics*. 1997; 100:772–788
10. Meade BD, Deforest A, Edwards KM. Methodology and evaluation of the serologic assays employed in a multicenter trial of acellular pertussis vaccines. *Pediatrics*. 1995;96:570–575
11. Reizenstein E, Hallander HO, Blackwelder WC, Kuhn I, Ljungman M, Mollby R. Comparison of five calculation modes for antibody ELISA procedures using pertussis serology as a model. *J Immunol Methods*. 1995;183:279–290
12. Lehmann EI. *Nonparametrics: Statistical Methods Based on Ranks*. San Francisco, CA: Holden-Day; 1975
13. Melville-Smith M, Balfour A. Estimation of *Corynebacterium diphtheriae* antitoxin in human sera: a comparison of an enzyme-linked immunosorbent assay with the toxin neutralization test. *J Med Microbiol*. 1988;25:279–283
14. Melville-Smith ME, Seagroatt VA, Watkins JT. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for the estimation of tetanus antitoxin in human sera. *J Biol Stand*. 1983;11:137–144
15. Pichichero ME, Christy C, Decker MD, et al. Defining the key parameters for comparing common reactions among acellular and whole cell DTP vaccines. *Pediatrics*. 1995;96:588–592
16. Miller RG. *Simultaneous Statistical Inference*. New York, NY: Springer-Verlag; 1981
17. Deloria MA, Blackwelder WC, Decker MD, et al. Association of reactions after consecutive acellular or whole-cell pertussis vaccine immunizations. *Pediatrics*. 1995;96:592–594
18. Ipsen J. Circulating antitoxin at the onset of diphtheria in 425 patients. *J Immunol*. 1946;54:325–347
19. McComb JA. The prophylactic dose of homologous tetanus antitoxin. *N Engl J Med*. 1964;270:175–178
20. Lynn F, Reed GF, Meade BD. Collaborative study for the evaluation of enzyme-linked immunosorbent assays used to measure human antibodies to *Bordetella pertussis* antigens. *Clin Diagn Lab Immunol*. 1996;3:689–700
21. Barkin RM, Pichichero ME. Diphtheria-pertussis-tetanus vaccine: reactogenicity of commercial products. *Pediatrics*. 1979;63:256–260
22. 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;68:650–658

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