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Beschreibung:

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

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ABSTRACT. *Objective.* **To evaluate the effect of maternally derived antibody on the immunogenicity and reactogenicity of acellular (DTaP) or whole-cell (DTP) pertussis vaccine with diphtheria and tetanus toxoids combined.**

Methods. **A total of 2342 infants were randomized to receive one of 13 DTaP or 2 DTP vaccines at 2, 4, and 6 months of age. The correlation between preimmunization and postimmunization antibody after three doses of vaccine and the relation between preimmunization antibody and adverse reactions after the first immunization were modeled by linear regression.**

Results. **After DTP but not DTaP, higher levels of preexisting antibody were associated with substantial (28% to 56%) reductions in the subsequent antibody response to pertussis toxin (PT). For other pertussis antibodies, modest inverse correlations were seen between preexisting antibody concentrations and most postimmunization antibody responses (resulting in 8% to 18% reductions in postimmunization antibody) for both DTP and DTaP. There was no consistent association in any DTP or DTaP group between adverse reactions and preimmunization antibody levels.**

Conclusion. **The PT antibody response to DTaP, unlike DTP, is not adversely affected by preexisting antibody to PT. Inhibitory effects with respect to other antibodies, seen with both DTP and DTaP, were relatively modest. Our data suggest that the use of acellular pertussis vaccines in adults, which could confer higher levels of antibody in women before pregnancy, would be unlikely to adversely affect pertussis antibody responses after DTaP among infants born to mothers with high antibody levels.** *Pediatrics* **1995;96:580–584;** ***acellular, antibody, diphtheria-tetanus-pertussis, maternal, pertussis, whole-cell, vaccine, whooping cough.***

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ABBREVIATIONS. DTP, diphtheria and tetanus toxoids combined with whole-cell pertussis vaccine; DTaP, diphtheria and tetanus toxoids combined with acellular pertussis vaccine; PT, pertussis toxin; FHA, filamentous hemagglutinin; FIM, fimbrial protein; PRN, pertactin; NIAID, National Institute of Allergy and Infectious Diseases; ELISA, enzyme-linked immunosorbent assay; AGG, agglutinin; CHO, Chinese hamster ovary; MLD, minimum level of detection; EU, ELISA unit; WCM, Massachusetts whole-cell; DIP, diphtheria toxin/toxoid; TET, tetanus toxin/toxoid. For the abbreviations used to identify individual vaccines, see Table 1.

The effect of maternal antibody on the reactogenicity and immunogenicity of vaccines administered to young infants is an important factor to be considered. Previous investigations have suggested that higher levels of transplacental antibody may decrease the immune response to conventional pertussis vaccines combined with diphtheria and tetanus toxoids (DTP) given to infants younger than 6 months of age.[1–4] Indeed, one comparative trial[5] of DTP with an acellular pertussis combined with diphtheria and tetanus toxoids vaccine (DTaP) showed that, among the infants given DTP, those with higher preimmunization levels of antibody to pertussis toxin (PT) had lower final postimmunization PT antibody levels than did those who began with lower preimmunization antibody levels. This effect was not seen among the recipients of DTaP, all of whom had comparably high postimmunization PT antibody titers regardless of their preimmunization antibody levels.

In this study, we analyze the effect of preexisting antibody to the pertussis antigens PT, filamentous hemagglutinin (FHA), fimbriae types 2 and 3 (FIM), and pertactin (PRN) on the immunogenicity and reactogenicity of various DTP and DTaP vaccines in a large population of US infants. We also investigate the effects on infant immune response of maternal antibody to two nonpertussis antigens, diphtheria toxin (DIP) and tetanus toxin (TET).

METHODS

As previously described,[6,7] 2342 2-month-old infants were enrolled and followed in the National Institute of Allergy and Infectious Diseases (NIAID) Multicenter Acellular Pertussis Trial. Parents were responsible for entering reaction data during the first 48 hours after vaccination in a reaction diary provided by the investigators; direct telephone follow-up was used to promote compliance and to ascertain the development of serious adverse events. Reactions recorded included daily temperatures (recorded in degrees Fahrenheit), redness and swelling (recorded in millimeters),

TABLE 1. Source and Composition of Studied Vaccines

Abbreviation	Manufacturer	Components
BSc-1	Biocine	PT
SSVI-1	Swiss Serum and Vaccine Institute	PT
CB-2	Connaught (US)/Biken	PT, FHA
Mich-2	Michigan Department of Public Health	PT, FHA
PM-2	Pasteur Merieux	PT, FHA
SKB-2	SmithKline Beecham	PT, FHA
BSc-3P	Biocine	PT, FHA, PRN
LPB-3P	Lederle-Praxis Biologicals	PT, FHA, PRN
SKB-3P	SmithKline Beecham	PT, FHA, PRN
CLL-3F_2	Connaught (Canada)	PT, FHA, FIM2, FIM3
Por-3F_2	Porton Products*	PT, FHA, FIM2, FIM3
LPT-4F_1	Lederle Praxis/Takeda	PT, FHA, PRN, FIM2
CLL-4F_2	Connaught (Canada)	PT, FHA, PRN, FIM2, FIM3
WCL	Lederle Praxis	Whole-cell vaccine
WCM	Massachusetts Public Health Biologic Laboratories	Whole-cell vaccine

* Now Speywood Pharmaceuticals Ltd.

and fussiness and pain (recorded on a scale of none, 0; mild, 1; moderate, 2; and severe, 3).[7]

Infants were excluded from analyses of serologic responses or adverse reactions as previously detailed;[6] in addition, because preimmunization antibody levels were a focus of the present analyses, an additional 36 infants were excluded because of uncertain preimmunization specimen quality (eg, specimen hemolysis). Thus, 1906 infants were available for analysis of associations between preimmunization and postimmunization serology, and 2096 were available for analysis of associations between preimmunization serology and adverse reactions after the first immunization.

Vaccines

The compositions of the vaccines administered in this study have been described previously (Table 1).[6] In total, 13 DTaP and 2 DTP vaccines were administered. The DTaP vaccines contained from one to four different pertussis antigens and varying amounts of DIP and TET.

Serology

Blood samples from infants were processed at the participating institutions, and the frozen sera were shipped to a central receiving site. Immunoglobulin G antibodies to PT, FHA, PRN, and FIM were measured by enzyme-linked immunosorbent assay (ELISA) at the Food and Drug Administration.[6,8,9] The agglutination assay was used to determine the agglutinin (AGG) titer, and PT neutralizing antibody was measured by the Chinese hamster ovary (CHO) cell pertussis toxin neutralization assay. The minimum level of detection of antibody (MLD) was 2 ELISA units (EU) for PT and FHA, 3 EU for FIM, 6 EU for PRN, 40 reciprocal dilutions for CHO cells, and 8 reciprocal dilutions for AGG. For calculations, any results falling below the MLD were arbitrarily assigned a value of half the MLD. An antibody level in the postimmunization specimen collected at age 7 months that was at least four times the MLD was defined arbitrarily to represent an immune response to the relevant antigen.

Diphtheria and tetanus antibody levels were measured on a 10% sample of all serum pairs. Diphtheria antibody was assayed by toxin neutralization in Vero cells[10] and tetanus antibody by a modified passive hemagglutination assay.[11]

Statistical Methods

Simple linear regression was used to model the relationship between log-transformed (base 10 for the ELISA assays and base 2 for AGG and CHO) antibody levels measured before and after vaccination. The regression coefficient thus obtained represented the amount (in log units) by which the postimmunization antibody level declined for each log unit increase in the preimmunization antibody level. Regression models were built for each combination of the three major vaccine groups (DTaP, Lederle [Pearl River, NY] whole-cell vaccine [WCL], and Massachusetts whole-cell vaccine [WCM]) and the eight evaluated antibodies (with the exception that for FHA, WCL was excluded, because it did not stimulate antibody to that antigen[6]), as well as for each of the individual DTaP vaccines and antibodies to their included antigens (Table 1). The t test was used to determine whether a regression coefficient was significantly different from zero. To compare coefficients derived from different vaccines or vaccine groups, the difference between the coefficients was divided by the square root of the sum of their squared SEs to form a z statistic.[12]

For each assay, the preimmunization antibody levels that equaled two and four times the MLD were determined, and the corresponding postimmunization antibody levels were calculated from the regression equation and then converted to the arithmetic scale (eg, ELISA units). The predicted change in postimmunization antibody level associated with a twofold increase in preimmunization antibody level, from twice the MLD to four times the MLD, was expressed as a percentage.

To examine the effect of a preexisting antibody on reactions

TABLE 2. Association Between Preimmunization and Postimmunization Antibody Levels by Vaccine Type, as Measured by Regression Coefficients

Antibody	Aggregated DTaP		WCL		WCM	
	Coefficient*	P Value	Coefficient	P Value	Coefficient	P Value
PT	−0.0402	.26	−1.1902	<.001	−0.4645	.027
FHA	−0.1173	<.001	NA†	NA†	−0.2424	.003
PRN	−0.1922	<.001	0.1572	.11	−0.0316	.81
FIM	−0.2686	<.001	−0.2871	<.001	−0.1109	.42
AGG	−0.1470	.006	−0.2344	.003	−0.0805	.56
CHO	−0.1141	.002	−0.6940	<.001	−0.2663	.075
DIP	−0.4093	<.001	−0.1858	.21	−0.6340	.002
TET	−0.0703	.20	−0.1692	.18	−0.0786	.80

* Negative numbers imply that postimmunization antibody levels decline with increasing preimmunization antibody levels; positive numbers, that postimmunization levels rise with increasing preimmunization levels. The coefficients are derived from the regression equation: postimmunization antibody level = constant + (coefficient × preimmunization antibody level).
† Not applicable; WCL did not induce antibody to FHA.[6]

after the first vaccination, a simple linear regression equation with the log of the preimmunization antibody level as the independent variable and the reaction score as the dependent variable was fit for each of the combinations of four antibodies (PT, FHA, PRN, and FIM) and five reactions (temperature, redness, swelling, fussiness, and pain). The reaction score used for a given reaction was the maximum recorded to occur in the 48-hour period after the first vaccination. Because adverse reactions generally were infrequent for the DTaP vaccines, these vaccines were grouped according to their contained antigens, and the data from these grouped vaccines were aggregated in the analysis. Thus, all DTaP vaccines were aggregated for the analysis of PT antibody versus reactions, 11 vaccines were aggregated for the FHA analysis, 5 for the PRN analysis, and 5 for the FIM analysis. Results for WCL were analyzed separately. The slope estimate from the regression measured the strength of the association, and the t test was used to determine whether the slope differed significantly from zero.

All tests of significance were two sided, and P values greater than .05 were considered not significant. P values were not adjusted to correct for multiple statistical tests.

RESULTS

Serology

Infants were categorized into one of three vaccine groups (DTaP, WCL, and WCM). Eight antibody assays were evaluated for each group (Table 2), for a total of 24 analyses. For 23 of these 24 analyses, there was a negative effect of preexisting antibody on postimmunization antibody levels at 7 months of age; that is, higher levels of maternally derived antibody were associated with lower levels of the antibody after immunization. However, there was substantial variation in the magnitude of these effects, and not all were statistically significant.

There was a strong and significant negative effect of preexisting PT antibody on postimmunization PT antibody levels for WCL (Figure, A) and WCM recipients, but not for DTaP recipients (Figure, B). Results for the other antibodies were mixed. Statistically significant negative effects were seen among DTaP recipients for FHA, PRN, FIM, AGG, and DIP; among WCL recipients, for FIM and AGG (for WCL, FHA was not applicable); and among WCM recipients, for FHA and DIP. As expected, the effect of preexisting antibody on CHO cell assay results generally mirrored the effect seen with PT antibody. There was a positive, rather than negative, correlation between preimmunization and postimmunization PRN antibody levels for WCL, but this effect was not statistically significant (Table 2).

The statistical significance of differences between WCL, WCM, and DTaP in the magnitude of these effects is shown in Table 3. Compared with DTaP, both WCL and WCM were associated with significantly greater negative effects of preexisting PT antibody on postimmunization PT antibody levels. The three vaccine groups did not significantly differ with respect to the effects of preexisting FIM or AGG antibody. For PRN, a positive association that was not statistically significant ($P = .11$) was found for WCL (Table 2); these WCL results differed significantly from those found for DTaP but not WCM (Table 3).

Examples of the results of these negative effects of preexisting antibody are presented in Table 4, which shows the predicted percent reduction in postimmunization antibody associated with an increase in preimmunization antibody from twice the MLD to four times the MLD. The strongest effect by far is seen for antibody to PT, which declines by 56% for WCL and by 28% for WCM (each $P < .05$), but only by 3% (P = NS) for DTaP recipients. The magnitude of significant declines in other postimmunization antibody levels ranged from 8% to 17% for DTaP recipients and from 15% to 18% for DTP recipients.

Regression coefficients for the individual DTaP vaccines are shown in Table 5. Although the sample sizes for the individual vaccine groups are comparatively small, the coefficients are surprisingly uniform among the DTaP vaccines. For PRN and AGG, a few vaccines have substantially larger negative coefficients than do the remaining vaccines.

Sixty-one (3.2%) of 1906 infants had postimmunization PT antibody levels less than twice the MLD: 30 (10%) of 300 WCL recipients, 21 (23%) of 90 WCM recipients, but only 10 (0.6%) of 1516 DTaP recipients ($P < .0001$). Regardless of vaccine group, these poor postimmunization PT antibody responses could not, in general, be attributed to high levels of maternally derived PT antibody; among infants with undetectable postimmunization PT antibody, preimmunization PT antibody levels did not exceed twice the MLD for 7 (70%) of 10 DTaP recipients, 20 (68%) of 30 WCL recipients, and 13 (62%) of 21 WCM recipients ($P = .8$).

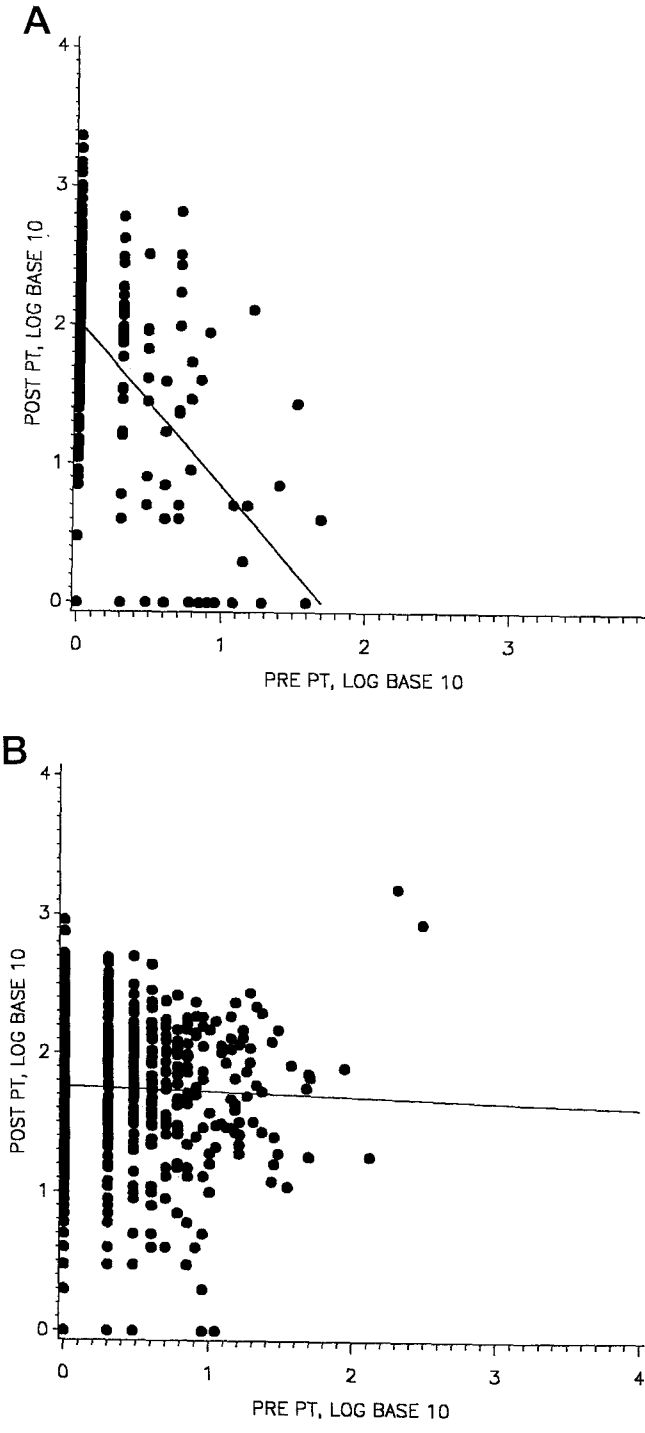

Figure. Relationship between preimmunization and postimmunization PT antibody levels after WCL (A) and DTaP (B). The slope of the linear regression for preimmunization versus postimmunization antibody is −0.04 for DTaP ($P = .26$), indicating no significant effect of preimmunization antibody on the postimmunization response. In contrast, the slope of the regression line is −1.19 for WCL ($P < .001$), indicating a significant negative effect of preimmunization antibody on the postimmunization antibody level. See Table 2 for the various regression coefficients (slopes).

Reactions

No consistent correlation was found between the preimmunization level of pertussis antibody and the occurrence of adverse reactions after the first immunization. For the DTaP vaccines as a group, the evaluation of five reactions (fever, pain, redness, swell-

TABLE 3. *P* Values for Comparisons Among DTaP, WCL, and WCM of Regression Coefficients Relating Preimmunization and Postimmunization Antibody Levels*

Antibody	DTaP vs WCL	DTaP vs WCM	WCL vs WCM
PT	<.001	.043	.003
FHA	NA†	.14	NA†
PRN	.001	.25	.24
FIM	.82	.28	.24
AGG	.36	.65	.33
CHO	<.001	.32	.020
DIP	.15	.17	.033
TET	.47	.98	.79

* The coefficients are derived from the regression equation: postimmunization antibody level = constant + (coefficient × preimmunization antibody level).
† Not applicable; WCL did not induce antibody to FHA.[6]

TABLE 4. Predicted Percent Decrease in Postimmunization Antibody Level with Increase in Preimmunization Antibody Level from Twice to Four Times the Minimum Level of Detection*

Antibody	Aggregated DTaP	WCL	WCM
PT	2.7	56.2†	27.5†
FHA	7.8†	NA‡	15.5†
PRN	12.5†	−11.5	7.2
FIM	17.0†	18.0†	7.4
AGG	9.7†	15.0†	5.4
CHO	7.6†	38.2†	16.9

* DIP and TET are excluded from this table because their minimum levels of detection varied from assay to assay.
† $P \leq .05$.
‡ Not applicable; WCL did not induce antibody to FHA.[6]

ing, and irritability) versus four antibody concentrations (PT, FHA, FIM, and PRN) produced 20 regression equations, only one of which resulted in a statistically significant association (a direct association between swelling and preimmunization PT antibody; $P = .014$). For WCL, 1 of 15 regression equations relating preexisting PT, FIM, or PRN antibody with adverse reactions revealed a significant correlation (a direct association between pain and PRN; $P = .022$).

A similar analysis of the subset of specimens assayed for antibody to DIP or TET toxin found no significant correlations between preimmunization concentrations of those antibodies and adverse reactions after the first immunization.

DISCUSSION

A suppressive effect of maternal pertussis antibody on the subsequent immune response of the infant after DTP was noted by investigators in the 1940s,[1-4] an era when maternal antibody presumably was higher than at the present time. It subsequently was shown that immunoparalysis to pertussis AGG lasting up to 15 months could occur when DTP was given during the first 24 hours of life.[13] More recently, infants with high PT antibody levels were shown to have poor serologic responses to PT when immunized with DTP.[5,14,15] Our results confirm a substantial negative effect of maternally derived PT antibody on postimmunization antibody levels in 7-month-old infants who had received three doses of DTP. Our results also support previous evidence that no similar inhibitory effect of preexisting antibody to PT is seen after immunization with a two-component DTaP.[5] This study extended those findings to additional DTaP vaccines containing many different combinations of antigens and to the antibodies raised in response to these various antigens, using contemporary specific serologic methods.

We assessed the effect of transplacentally acquired antibodies to three additional pertussis antigens (FHA, FIM, and PRN) that are not known to have been studied previously. In general, there was an inverse association between preimmunization and postimmunization levels of these antibodies, but the effects were much smaller in magnitude than for PT and were similar for acellular and whole-cell vaccines. Analysis by specific DTaP revealed substantial similarity among the acellular vaccines, although a few vaccines were associated with relatively stronger negative correlations between preimmunization and postimmunization antibody levels for PRN and AGG.

Transplacental transfer of maternal antibody to the infant potentially may result in transient protection from childhood diseases,[16-18] but this same transplacental antibody also may interfere with active immunization to diseases such as pertussis[1-4] or measles.[19] In this study, the rare occurrences of poor antibody response to PT were much more likely to be seen with DTP than with DTaP. This lack of antibody response could not be attributed to high levels of preexisting antibody, because approximately 70% of infants who failed to produce PT antibody after receiving DTaP or DTP had low levels (less than twice the MLD) of the preexisting antibody. Thus, for infants who received whole-cell vaccine, elevated preexisting levels of antibody were associated with a reduction in the magnitude of the PT antibody response but were not associated with a complete failure to respond. Of course, the correlation between any of the immune responses detailed in this article and vaccine efficacy remains uncertain at this time.

In contrast to these findings regarding the serologic response to immunization, there did not seem to be any relationship between preimmunization antibody levels and the occurrence of adverse reactions after the first immunization (the finding of two apparently significant results is not unexpected when conducting 35 analyses, given no adjustment of the *P* values for multiple tests).

The highest rates of pertussis disease, hospitalization, and death are in young infants, both in countries that administer pertussis vaccine routinely to infants and in countries that do not.[20] However, there is growing appreciation of the role of adults as a reservoir for pertussis, and the increasing rates of pertussis in young adults,[21] combined with the development of acellular pertussis vaccines of low reactogenicity, have stimulated interest in the routine booster immunization with DTaP of health care workers, adolescents, and young adults.[22] The potential impact of such immunization strategies on transplacentally transferred maternal

TABLE 5. Association Between Preimmunization and Postimmunization Antibody Levels for Individual DTaP Vaccines, as Measured by Regression Coefficients

Vaccine	N	PT	FHA	PRN	FIM	AGG	CHO
BSC-1	98	0.001					−0.229†
SSVI-1	119	−0.003					−0.078
CB-2	118	−0.100	−0.185†				−0.015
Mich-2	110	−0.051	−0.060				−0.100
PM-2	120	−0.061	−0.071				−0.026
SKB-2	164	−0.103	−0.202†				−0.057
BSC-3P	90	0.148	−0.034	−0.083		−0.056	−0.089
LPB-3P	93	−0.073	−0.148†	−0.273†		0.085	−0.348†
SKB-3P	105	−0.115	−0.130†	−0.478†		−0.027	−0.198
CLL-$3F_2$	100	−0.067	−0.033		−0.282†	−0.206	−0.018
Por-$3F_2$	101	−0.272†	−0.166†		−0.394†	−0.509†	−0.435
LPT-$4F_1$	183	−0.164	−0.169†	−0.185†	−0.304†	−0.340†	−0.499†
CLL-$4F_2$	115	0.013	−0.086	−0.073	−0.170†	−0.131	−0.125

* For explanation of individual vaccines, see Table 1. For explanation of coefficients, see footnote to Table 2.
† For *t* test of null hypothesis that coefficient does not differ from zero, $P \leq .05$.

antibody and on subsequent infant immune responses should be considered carefully. This study evaluated the impact of transplacental antibody originating from mothers who, in general, had been immunized with a DTP vaccine in the distant past; the quality and type of antibody that is potentially transferrable after adult acellular pertussis immunization is not known. These data suggest that the use of acellular pertussis vaccines in adults, which could confer higher levels of antibody in women and, thus, in their infants, would be unlikely to affect adversely the pertussis antibody responses of those infants after the administration of DTaP.

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Effect of Gender, Race, and Parental Education on Immunogenicity and Reported Reactogenicity of Acellular and Whole-Cell Pertussis Vaccines

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ABSTRACT. *Objective.* **To determine whether gender, race (black or white), or level of parental education influenced serologic responses or reporting of clinical reactions after immunization with acellular (DTaP) or whole-cell (DTP) pertussis vaccine with diphtheria and tetanus toxoids combined.**

Methods. **Healthy infants were prospectively randomized to receive one of 13 DTaP, Lederle DTP, or another DTP. Parents recorded the occurrence of adverse reactions for 2 weeks after each inoculation. Sera obtained before the first immunization and 1 month after the third immunization were analyzed for antibody to pertussis toxin, filamentous hemagglutinin, fimbriae, and pertactin (PRN). Chinese hamster ovary cell pertussis toxin**

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neutralization assays were performed, and levels of agglutinating antibodies determined.

Results. **Prevaccination antibody levels did not differ by race, gender, or parental education. Postimmunization geometric mean titers (GMTs) were strongly and consistently associated with race. For both DTaP and DTP and for every included antigen, postimmunization GMTs were about twice as high for black as for white infants. Among DTaP recipients, these differences were significant for pertussis toxin, Chinese hamster ovary cell pertussis toxin neutralization assay, filamentous hemagglutinin, PRN, and agglutinins; among the much smaller sample of WCL recipients, the differences achieved or approached statistical significance for agglutinins, PRN, and fimbriae. These findings were confirmed by regression analyses that controlled for gender, parental education, study site, and preimmunization antibody level. Reported reactions were not correlated with parental education level and showed no material correlation with gender. Black infants were reported to have had more pain than white infants after receiving WCL and DTaP and were reported to be more fussy after receiving WCL.**

Conclusions. **The consistently higher postimmunization GMTs among black infants seems to be a real finding for which we have no explanation; the infants did not significantly differ by race in vaccine assignment, preimmunization antibody levels, age at immunization, or interval from immunization to phlebotomy. These observations should be confirmed and further evaluated in future pertussis vaccine trials. Reported differences by race in pain and fussiness after receiving WCL might reflect chance, differences by race in the occurrence of reactions, or differences by race in the reporting of reactions.** ***Pediatrics* 1995;96:584–587;** ***acellular, antibody, diphtheria-tetanus-pertussis, vaccine, whole-cell, whooping cough.***

ABBREVIATIONS. DTP, diphtheria and tetanus toxoids combined with whole-cell pertussis vaccine; DTaP, diphtheria and tetanus toxoids combined with acellular pertussis vaccine; PT, pertussis toxin; FHA, filamentous hemagglutinin; PRN, pertactin; FIM, fimbrial protein; CHO, Chinese hamster ovary; AGG, agglutinin; MLD, minimum level of detection; GMT, geometric mean titer or concentration. For the abbreviations used to identify individual vaccines, see Table 1.

The US Public Health Service Task Force on Minority Health Data has emphasized the need for additional data on race and ethnicity in health-related research, because of the projected increases in racial and ethnic diversity in the United States.[1] It has been proposed that differences in responses to vaccines must be examined to determine whether vaccines are equally effective in different subgroups of the population. If clinically relevant differences are found to exist, vaccine trial study design and entry criteria need to accommodate such differences.

Susceptibility to and severity of pertussis infection are known to vary with gender and racial group.[2–5] The attack rate of pertussis is higher in female infants and children, who experience more severe disease and a higher case fatality rate;[3,4] a higher incidence is seen also in adolescent and adult women.[5] In the prevaccine era, pertussis fatality rates were elevated threefold to fivefold for black patients and sixfold for Native American patients compared with white patients. The reasons for these differences by gender and race in pertussis incidence and mortality are unclear; they may represent socioeconomic, biological, or other factors. It is possible that these differences reflect differences in immune response, which might be mirrored in responsiveness to pertussis vaccination.

Cultural, ethnic, socioeconomic, educational, and racial group differences may exist that could influence parental perception and reporting of infant reactions to vaccinations. If so, then differences among studies in the distribution of these parental characteristics may result in differences in the rates of reactions reported from the studies.

In this article, we describe the effects of infant gender, race (black or white), and parental education on serologic responses and reported adverse reactions after immunization with acellular (DTaP) or whole-cell (DTP) pertussis vaccine with diphtheria and tetanus toxoids combined.

METHODS

Infants

Detailed descriptions of vaccines, infants, study design, data management, and serologic methods can be found elsewhere.[6–8] Briefly, 2342 healthy infants 6 to 12 weeks of age were recruited from private pediatric offices and vaccine clinics in protocols approved by institutional review boards at each of the six participating vaccine treatment and evaluation units for this randomized, prospective, double-blinded study of 13 DTaP and 2 DTP vaccines (Table 1). Participants were recruited from suburban, middle– to upper–middle-class private practices, as well as from urban practices serving families of low to moderate incomes. Parents or guardians gave informed consent before enrollment and provided basic demographic information, including the highest level of education attained by each parent, the gender of the infant, and the race or ethnic group of the infant. Infants were vaccinated at 2, 4, and 6 months of age. An oral trivalent polio virus vaccine was given concomitantly with a study vaccine at 2 and 4 months of age; after licensure, the *Haemophilus influenzae* type b conjugate vaccine also was administered to most infants.

In addition to applying exclusion criteria detailed elsewhere,[6,7,9] we excluded from analysis infants who received the Massachusetts whole-cell vaccine and infants who reported races or ethnicities other than black or white, because there were too few infants in these excluded groups to perform the present analyses.

TABLE 1. Source and Composition of Studied Vaccines

Abbreviation	Manufacturer	Components
BSc-1	Biocine	PT
SSVI-1	Swiss Serum and Vaccine Institute	PT
CB-2	Connaught (US)/Biken	PT, FHA
Mich-2	Michigan Department of Public Health	PT, FHA
PM-2	Pasteur Merieux	PT, FHA
SKB-2	SmithKline Beecham	PT, FHA
BSc-3P	Biocine	PT, FHA, PRN
LPB-3P	Lederle-Praxis Biologicals	PT, FHA, PRN
SKB-3P	SmithKline Beecham	PT, FHA, PRN
CLL-3F_2	Connaught (Canada)	PT, FHA, FIM2, FIM3
Por-3F_2	Porton Products*	PT, FHA, FIM2, FIM3
LPT-4F_1	Lederle Praxis/Takeda	PT, FHA, PRN, FIM2
CLL-4F_2	Connaught (Canada)	PT, FHA, PRN, FIM2, FIM3
WCL	Lederle Praxis	Whole-cell vaccine
WCM	Massachusetts Public Health Biologic Laboratories	Whole-cell vaccine

* Now Speywood Pharmaceuticals Ltd.

Adverse Reactions

Parents evaluated adverse reactions at 3 hours, 6 hours, and the first evening after each vaccination (unless these coincided), then each evening for 6 additional days, with a final evaluation recorded on day 14 after vaccination (which included any reactions occurring since day 7).[8] Parents were asked to record rectal temperatures, to measure redness and swelling at the injection site, and to gauge the degree of pain at the injection site. Parents also assessed their infants for changes in drowsiness, fussiness, appetite, or vomiting. Pain and fussiness were scored from 0 (no reaction) to 3 (severe reaction).[7] For each infant, each category of reaction was summarized by a single value representing the maximal reaction observed by the end of the second evening after any of the three immunizations.[10]

Serology

Serum was obtained before immunization and approximately 1 month after the third immunization. Enzyme-linked immunosorbent assays were performed as previously described[6,8] to measure immunoglobulin G antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and a mixture of fimbrial serotypes 2 and 3 (FIM). PT-neutralizing antibodies were measured by the Chinese hamster ovary (CHO) cell pertussis toxin neutralization assay. The agglutinin (AGG) titer was determined by the agglutination assay. For each assay, the minimum level of detection (MLD) was estimated; for calculations, any result falling below the MLD was arbitrarily assigned a value of half the MLD.

Statistical Analysis

Parental education was summarized by assigning a score to each parent, based on the highest educational level begun (grade school, 1; high school, 2; college, 3; postgraduate, 4) and then summing the scores for both parents. Data were missing for three infants concerning education of both parents and for another 15 infants concerning education of the father; these 18 infants were excluded from analyses of parental education.

Analyses of adverse reactions by age and gender were based on the *t* test. The relation between parental education and each adverse reaction was evaluated by using Spearman rank correlations. Serologic data were log transformed for analysis, reporting the antilogarithms as geometric mean titers or concentrations (GMTs). Serologic response was defined as achieving a postimmunization antibody level at least four times the MLD. Because sample sizes for the individual DTaP vaccines were small relative to that of WCL, the DTaP recipients were considered as a single group. Analyses of prevaccination DTaP GMTs included all the DTaP vaccines; analyses of postvaccination DTaP GMTs included only those vaccines containing the antigen relevant to the antibody being analyzed (Table 1).

The *t* test was used for comparisons of prevaccination GMTs by race or gender. For comparisons of postvaccination GMTs, analysis of covariance was used to adjust for the prevaccination antibody level. Parental education, gender, study site, and preimmunization antibody level were evaluated as potential confounders of the relationship between race and postimmunization antibody level by linear regressions for each antibody, with postimmunization antibody level as the dependent variable. Simple linear regression with the prevaccination serologic response as the dependent variable and the education score as the independent variable was used to produce a regression coefficient that measured the strength of the trend in prevaccination antibody level with education. For analysis of parental education versus the postvaccination antibody level, the prevaccination level was included as an independent variable for covariant adjustment. All statistical testing was two-sided, and *P* values were not adjusted for multiple tests.

RESULTS

Of 2342 enrolled infants, 2143 received DTaP or WCL and were reported to be either black or white. Of these 2143, 103 (4.8%) were black; 54% of black and 49% of white infants were female. Parental education was related to race, but not to parental gender: 55% of mothers and 52% of fathers of black infants reported at least some college education, compared with 75% of mothers and 74% of fathers of white infants. There were no significant differences in distribution of gender, race, or parental educational level among the 15 vaccine groups.

Serology

Of the 2143 eligible infants, 1768 had both preimmunization and postimmunization sera available for analysis. Prevaccination GMTs did not differ significantly between male and female infants, between black and white infants, or by parental education score.

Postimmunization GMTs were strongly and consistently associated with race (Table 2). For both DTaP and DTP and for every included antigen, postimmunization GMTs of black infants were about twice as high as those in white infants. Among DTaP recipients, these differences were significant for PT, CHO, FHA, PRN, and AGG; among the much smaller sample of WCL recipients, the differences achieved or approached statistical significance for AGG, PRN, and FIM. These findings were confirmed by regression analyses that controlled for gender, parental education, study site, and preimmunization antibody level (Table 3). The infants did not significantly differ by race in vaccine assignment, preimmunization antibody levels, age at immunization, or interval from immunization to phlebotomy (data not shown).

For most antigens, postimmunization antibody titers at least four times the MLD for included antigens developed in almost all infants, regardless of race or vaccine group (Table 2). Consequently, the proportion of infants achieving that level did not differ significantly by race except for AGG among DTaP recipients ($P = .04$) and PRN among WCL recipients ($P = .04$).

Postimmunization GMTs did not differ by gender for any antibody. There was no correlation between parental education and serologic responses for DTaP recipients, but among WCL recipients, postimmunization antibody levels decreased with increasing parental education for PT ($P = .02$) and CHO ($P = .05$).

Adverse Reactions

Of the 2143 eligible infants, 2129 had adverse reaction data reported after at least one immunization. Adverse reactions did not significantly differ by gender, except that redness at the injection site after receiving DTaP was slightly more prominent among girls than boys (mean, 4.3 vs 3.5 mm; $P = .03$). The reporting of reactions did not differ by parental education score. Black infants

TABLE 2. Effect of Black or White Race on Serologic Response to DTaP and DTP Vaccines

Antigen*	GMTs						Infants with Postvaccination Antibody Level ≥4 Times MLD					
	DTaP			WCL			DTaP			WCL		
	Black	White	P†	Black	White	P†	Black	White	P‡	Black	White	P‡
PT	94	56	<.001	107	68	.27	57/58	1353/1418	.51	12/13	237/279	.81
CHO	497	274	<.001	480	253	.31	48/54	1037/1298	.13	9/12	175/262	.81
FHA	106	67	.002	4	3	.43	52/52	1190/1216	.67	3/13	53/279	.94
PRN	196	88	.007	129	60	.07	18/18	494/554	.26	13/13	206/279	.04
FIM	304	141	.095	347	184	.07	15/15	439/469	.75	13/13	260/279	.82
AGG	98	41	.005	167	80	.03	23/28	454/739	.036	13/13	228/278	.16

* For DTaP, each analysis includes only those DTaP vaccines containing the indicated antigen (see Table 1).
† Two-tailed *P* values from analysis of covariance, adjusting for the preimmunization antibody level.
‡ Fisher's exact test, two-tailed.

TABLE 3. Statistical Significance of Race Effect in Linear Regression of Postimmunization Antibody Level Versus Race, Gender, Parental Education, and Preimmunization Antibody Level

Antigen	*P* value, DTaP	*P* value, WCL
PT	.001	.32
CHO	<.001	.44
FHA	.001	.55
PRN	.006	.07
FIM	.28	.14
AGG	.007	.06

were reported to have more injection-site pain than white infants (mean pain score: WCL, 2.4 vs 1.3; $P < .001$; DTaP, 0.6 vs 0.4; $P = .01$), and black infants were reported to be fussier after WCL (mean fussiness score, 1.9 vs 1.4; $P = .03$).

DISCUSSION

Our finding that postimmunization antibody levels were consistently about twofold higher among black than white infants, both for DTaP and WCL recipients, was unexpected. These differences by race were not attributable to differences in vaccine allocation, preimmunization antibody levels, or in the timing of vaccination or phlebotomy, and persisted in analyses that controlled simultaneously for gender, parental education, study site, and preimmunization antibody level. The fact that these findings attained statistical significance for nearly all the tested antibodies in the DTaP group and several in the smaller WCL group, despite the fact that only 5% of infants were black, indicates that the distributions of results by race were distinct.

These results are made even more intriguing by the relative absence of differences by race in adverse reactions, although black infants were reported to experience more injection-site pain and to be more fussy after receiving WCL than white infants. If these differences in reactions are real, rather than a random effect, it remains unknown whether they represent differences in the infants' response to vaccine, differences in the parents' perceptions of their infants, or differences in the way parents report otherwise similar reactions.

Others have shown differences in antibody responses among groups defined by race, ethnic group, geography, or genetic characteristics for polysaccharide and conjugate *Haemophilus influenzae* b vaccines,[11,12] measles vaccine,[13] and hepatitis B vaccine.[14] Ours may be the first demonstration of such a difference for pertussis antigens. Do black and white infants differ in their physiologic responses to pertussis vaccine, or are these differences attributable to differences in the social and environmental circumstances of black and white children? Like race, educational attainment may serve as a surrogate measure of other social factors; yet in the DTaP group, there was no correlation between parental education and either antibody response or reports of adverse reactions. We did not have the necessary data to evaluate directly the possible roles of such factors as breast-feeding practices, family size, crowding, or community exposures.

Caution must be applied when attempting to analyze vaccine safety and immunogenicity data by race in the absence of secure data on socioeconomic, cultural, and other societal factors. In addition, race is not measurable and has no validated classification system, and racial categories are not exclusive. Some investigators choose to use the term ethnic group, which implies a recognition of social influences on health outcomes. Regardless, both race and ethnicity are imperfect predictors of health outcomes.[1]

Our study did not find serologic evidence to explain previous findings[3–5] of higher attack rates or increased severity of pertussis in girls. Perhaps the higher risk in adult women[5] simply reflects the fact that women are much more likely than men to have prolonged close contact with young children, who have the highest incidence of disease.[5]

Our results raise a host of fascinating questions that we cannot answer; we must await confirmation and further exploration of our findings in subsequent studies. Currently, DTaP efficacy trials are underway in Italy, Sweden, Germany, and Senegal.[15] It will be of interest to compare the reactogenicity and immunogenicity results from these diverse cultural, ethnic, and racial groups.

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