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Hib antibody responses in infants following diphtheria, tetanus, acellular pertussis, and conjugated *Haemophilus influenzae* type b (Hib) combination vaccines with decreasing amounts of tetanus toxoid

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ABSTRACT

Background: While combination vaccines have contributed to improved vaccine uptake rates in children, studies have documented varied immunogenicity to specific vaccine components. We studied whether varying the amount of tetanus toxoid (TT) in a DTaP and Hib combination vaccine would result in immunogenicity comparable with separate, concurrent administration.
Methods: We evaluated the immunogenicity of Massachusetts Biologic Laboratories (MBL) diphtheria, tetanus, and acellular pertussis (mDTaP) vaccine combined with tetanus-conjugated MBL *Haemophilus influenzae* type b vaccine (mHib) in a single injection (DTaPH). We compared four DTaPH vaccines containing varying concentrations of TT. We also evaluated the immune response to the DTaP vaccine manufactured by Connaught Laboratories (now known as Sanofi Pasteur) given with mHib and with Wyeth Hib-CRM_{197} (HbOC) as separate injections. Vaccines were administered to 240 healthy infants at 2, 4, and 6 months of age, and blood specimens for antibody determination were obtained before each immunization and one month after the third immunization.
Results: We found no significant differences in immune response to the vaccines between the four DTaPH groups. Hib antibody responses were similar in the mHib and the HbOC groups but significantly lower in the DTaPH groups, as measured by Chinese Hamster Ovary (CHO) cell neutralization titers and filamentous hemagglutinin antigen (FHA) geometric mean concentrations (GMC) of anti-Hib antibodies. There were no significant differences between the groups in pertussis or tetanus toxoid antibody levels.
Conclusion: Reducing tetanus toxoid amounts did not produce comparable immunogenicity for Hib. The nature of the interaction between immune responses to DTaPH components should be explored further to enable the development of better Hib-containing combination vaccines.

1. Introduction

Combination vaccines reduce the number of vaccine injections given, resulting in both increased coverage and improved timeliness of vaccination in children [1,2]. Studies of combination vaccines that include Hib have reported a mixed impact on immunogenicity. Combination diphtheria, tetanus, and whole-cell pertussis (DTwP) and tetanus-conjugated *Haemophilus influenzae* type b vaccine (Hib-T) showed no impairment of immunogenicity; in fact, whole cell pertussis antigen had an adjuvant effect on tetanus toxoid (TT), resulting in heightened responses to conjugate vaccines that use TT as a carrier [3–6]. With the licensure of acellular pertussis vaccines combined with diphtheria and tetanus toxoid (DTaP), combination vaccines with DTaP and Hib were anticipated. However, fewer infants attained the anti-Hib capsular

Abbreviations: Hib, *Haemophilus influenzae* type b; TT, tetanus toxoid; MBL, Massachusetts Biologic Laboratories; mDTaP, MBL diphtheria, tetanus, and acellular pertussis; mHib, tetanus-conjugated MBL *Haemophilus influenzae* type b vaccine; DTaPH, diphtheria, tetanus, and acellular pertussis vaccine combined with tetanus-conjugated *Haemophilus influenzae* type b vaccine; HbOC, Wyeth Hib-CRM_{197} vaccine; CHO, Chinese Hamster Ovary; FHA, filamentous hemagglutinin antigens; GMC, geometric mean concentration; DTwP, diphtheria, tetanus, and whole-cell pertussis vaccine; Hib-T, tetanus-conjugated *Haemophilus influenzae* type b vaccine; DTaP, diphtheria, tetanus, and acellular pertussis vaccine; PRP, polyribose ribitol phosphate; IPV, inactivated polio vaccine; DTaP-Conn, Connaught Diphtheria-Tetanus-acellular Pertussis vaccine; Lf, limit of flocculation units; PT, pertussis toxoid; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; CI, confidence intervals.

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polysaccharide polyribose ribitol phosphate (PRP) GMC long-term protection benchmark of 1 μg/mL when DTaP and Hib were combined [7–9]. It was postulated that the amount of tetanus toxoid may have contributed to the reduced Hib immune responses as a result of carrier-induced epitopic suppression, whereby antibody responses to antigens conjugated to a carrier protein are blunted by prior or simultaneous immunization with that carrier [10–12]. Reductions in the amount of TT in the combination vaccine resulted in a more vigorous Hib immune response in guinea pigs [13]. Further, a dose-ranging study of a pneumococcal vaccine conjugated to TT and co-administered with a pentavalent DTP-IPV (inactivated polio vaccine)-PRP-T vaccine to infants found that anti-PRP antibody concentrations increased as the load of pneumococcal-conjugated TT decreased [14].

Our objective was to evaluate the immunogenicity of the Massachusetts Biologic Laboratories (MBL) diphtheria, tetanus, and acellular pertussis vaccine combined with MBL tetanus-conjugated *Haemophilus influenzae* type b vaccine in a single injection administered to infants as the primary series at 2, 4, and 6 months of age. We studied whether reducing the amount of TT in the combination vaccine would improve its immunogenicity compared with separate administration of DTaP and Hib vaccines.

2. Materials and methods

2.1. Vaccines

The antigenic composition of each 0.5 mL dose of vaccine varied. DTaPH-1, DTaPH-2, and the DTaP vaccine (DTaP-Conn) manufactured by Connaught Laboratories (now known as Sanofi Pasteur) each maintained a 5 limit of flocculation units (Lf) of TT while DTaPH-3 had 1 Lf and DTaPH-4 had 2.5 Lf. Additionally, DTaPH-1 had 25 μg of pertussis toxoid (PT) while DTaPH-2, DTaPH-3, and DTaPH-4 had 50 μg of PT compared to the 23 μg in DTaP-Conn. Each DTaPH contained 10 Lf of diphtheria toxoid while DTaP-Conn had 6.7 Lf, and each DTaPH had 3 μg of FHA while DTaP-Conn had 23 μg. The DTaPH vaccines were combined during manufacturing with mHib, which consisted of 10 μg of PRP conjugated to TT. Infants in the Connaught DTaP groups received either HbOC, which contained 10 μg of PRP covalently linked to approximately 25 μg of diphtheria CRM_{197} protein, or mHib. The vaccines and their antigenic composition are summarized in Table 1.

2.2. Study design

Healthy infants 6–12 weeks of age were recruited from an urban, hospital-based pediatric practice and three private suburban pediatric practices in Massachusetts. Exclusion criteria were: (1) birth weight less than 2.5 kg; (2) infants with an acute febrile illness (>38.7 °C) or signs of serious respiratory, gastrointestinal, or other infectious diseases; (3) infants with a serious chronic disease or known or suspected immunodeficiency; (4) infants with neurologic disorders including seizures; (5) infants receiving chronic systemic medication other than iron, vitamins, or short courses of antibiotics; (6) infants who had received immunoglobulin therapy, whole blood, or blood products in the previous 2 months, or who were expected to receive these therapies during the course of the trial; and (7) infants who had pertussis confirmed by culture, polymerase chain reaction assay, or direct fluorescent antibody assay of nasopharyngeal secretions. The Institutional Review Board of Boston Children's Hospital approved the study.

Written informed consent was obtained, and a physical examination was performed prior to each set of immunizations. Infants were randomized to one of six study groups. Four of these study groups each received one of four DTaPH vaccines with differing amounts of tetanus and pertussis toxoids as a single intramuscular injection at 2, 4, and 6 months of age. The remaining participants received the Connaught DTaP and either mHib or HbOC as separate intramuscular injections at 2, 4, and 6 months of age. Each infant received the same vaccines at each subsequent visit. Other routine immunizations were given according to the American Academy of Pediatrics and the Advisory Committee on Immunization Practices recommended childhood immunization schedule at the time of this trial in 1997.

2.3. Serologic methods

Blood specimens for antibody determination were obtained before each immunization and one month after the third immunization. MBL personnel blinded to the subjects' immunization group performed serologic testing. The Farr radioimmunoassay was used to measure anti-PRP antibody. The FDA provided reference sera (lot #1983), and the assay was standardized according to OoBRR. Anti-tetanus toxin antibody and anti-diphtheria toxin antibody levels were determined by direct immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) standardized with a standard globulin containing a known concentration of precipitating antibody. An indirect ELISA method was used to determine the IgG antibody response to PT, utilizing PT directly adsorbed to microtiter plates with standard serum calibrated in micrograms per milliliter. This assay was correlated with OoBRR standard sera. Antibody to FHA was measured by direct IgG ELISA utilizing FHA directly adsorbed to microtiter plates using standard sera calibrated in micrograms per milliliter. PT neutralizing antibody was measured by CHO cell neutralization assay using standard sera.

2.4. Statistical analysis

Comparisons were deemed statistically significant when the *P* value was <.05. Geometric mean titers of serum antibody were transformed to their natural logarithm, and comparisons between

Table 1
Antigenic composition of vaccines per 0.5 mL dose.

	Diphtheria (Lf)	Tetanus (Lf)	Pertussis toxoid (μg)	FHA (μg)	PRP (μg)
DTaPH-1	10	5	25	3	10
DTaPH-2	10	5	50	3	10
DTaPH-3	10	1	50	3	10
DTaPH-4	10	2.5	50	3	10
DTaP-Conn	6.7	5	23	23	[a]
mHib	[a]	[a]	[a]	[a]	10
Hib-CRM_{197}	[a]	[a]	[a]	[a]	10

Abbreviations: DTaPH, Massachusetts Diphtheria-Tetanus-acellular Pertussis vaccine combined with *Haemophilus influenzae* type b (Hib)-tetanus toxoid conjugate vaccine; DTaP-Conn, Connaught Diphtheria-Tetanus-acellular Pertussis vaccine; mHib, Massachusetts Hib-tetanus conjugate vaccine; Hib-CRM_{197}, Wyeth-Lederle Hib-CRM_{197}; Lf, limit of flocculation units; FHA, filamentous hemagglutinin antigens; PRP, polyribose ribitol phosphate.

[a] Not Applicable.

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