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Hepatitis B and pertussis antibodies in 4- to 5-year-old children previously vaccinated with different hexavalent vaccines

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ABSTRACT

In randomized active-comparator controlled studies, DTaP5-HB-IPV-Hib showed comparable immunogenicity and safety to other licensed vaccines. This study assessed persistence of anti-hepatitis B surface antigen (HBs) and anti-pertussis antibodies, when children were 4 to 5 years of age, 3 to 4 years after initial infant/toddler hexavalent vaccination. This was an extension of 2 European studies in which infants/toddlers received either DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib on a 2 + 1 or 3 + 1 schedule. Primary endpoints included percentages with anti-HBs ≥10 mIU/mL, and anti-pertussis toxin (PT), anti-filamentous hemagglutinin (FHA), anti-pertactin (PRN), and anti-fimbriae types 2 & 3 (FIM) greater than or equal to the lower limit of quantitation (LLOQ). One month after 2 + 1 or 3 + 1 dosing, nearly all toddlers had anti-HBs ≥10 mIU/mL, and responded to the received pertussis antigens. Approximately 3 to 4 years later, 65.8%-70.2% in the DTaP5-HB-IPV-Hib and 82.0%-83.7% in the DTaP3-HB-IPV/Hib groups, respectively, had anti-HBs ≥10 mIU/mL. Percentages of children with pertussis antibodies above LLOQ after 2 + 1 dosing were 58.4% and 41.5% (anti-PT), 80.9% and 88.3% (anti-FHA), 66.1% and 72.6% (anti-PRN), and 94.4% and 3.3% (anti-FIM), in the DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib groups, respectively. This study demonstrated, as expected, waning of hepatitis B and pertussis antibodies during the 3 to 4 years after completion of a 3 + 1 or 2 + 1 hexavalent vaccination schedule. Nonetheless, anti-HBs levels ≥10 IU/mL and detectable antibodies against acellular pertussis antigens persisted in most study participants. The implications of these findings for the long-term prevention of hepatitis B and pertussis are further discussed.

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Introduction

The complex recommended vaccination schedule for children younger than 2 years of age can be cumbersome for both healthcare professionals and parents, and may lead to missed opportunities for vaccination that increases the risk of epidemic outbreaks of otherwise preventable diseases.[1–3] To help reduce the number of injections at office visits, 5- and 6-valent childhood vaccines have been long introduced in Europe, and more recently, in the United States. Numerous studies have shown that the use of combination vaccines increases coverage and on-time vaccination rates.[4–7]

Currently, 3 hexavalent vaccines are licensed in the European Union: Infanrix® hexa (combined diphtheria, tetanus toxoids, acellular pertussis, hepatitis B, inactivated poliomyelitis, adsorbed conjugated *Haemophilus influenzae* [DTaP3-HB-IPV/Hib]; 950 µg aluminum salts per 0.5-mL dose; GlaxoSmithKline Biologicals, Rixensart, Belgium), approved in 2000; Hexyon® (fully liquid diphtheria, tetanus, pertussis [acellular, component], hepatitis B [rDNA], poliomyelitis [inactivated] and *Haemophilus influenzae* type b [Hib] conjugate vaccine adsorbed, [DTaP2-HB-IPV-Hib]; 600 µg aluminum salts per 0.5-mL dose; Sanofi Pasteur Europe, Lyon, France), approved in 2013; and Vaxelis® (fully liquid diphtheria, tetanus, pertussis [acellular component], hepatitis B [rDNA], poliomyelitis [inactivated] and *Haemophilus influenzae* type b conjugate vaccine adsorbed DTaP5-HB-IPV-Hib]; 314 µg aluminum salts per 0.5-mL dose; MCM Vaccine B. V., Leiden, The Netherlands), approved in 2016. These vaccines are indicated for the vaccination of infants and toddlers against the diseases caused by these pathogens.[8–10] DTaP5-HB-IPV-Hib differs from DTaP3-HB-IPV/Hib and DTaP2-HB-IPV-Hib, as it contains 5 acellular pertussis antigens and utilizes a meningococcal outer membrane protein as the conjugate for the Hib antigen. DTaP5-HB-IPV-Hib was approved in Europe in February 2016 and in the United States in December 2018 based on its similar immunogenicity and safety compared with the other licensed comparator vaccines. To meet a request from the European Medicines Agency (EMA), this study was conducted to assess the long-term persistence of anti-hepatitis B surface antigen (HBs) and anti-pertussis antibodies 3 to 4 years after initial vaccination with the DTaP5-HB-IPV-Hib. The EMA had previously requested persistence studies for the other hexavalent vaccines, DTaP3-HB-IPV/Hib and DTaP2-HB-IPV-Hib.[11,12]

Methods

Study design

The clinical portion of this study was conducted in Finland from late April to early August 2016, as an extension of 2

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Supplemental data for this article can be accessed on the publisher's website.

European pivotal studies: a study evaluating a 3 + 1 schedule conducted in Belgium, Finland, and Germany from late May 2011 to mid-March 2013 (NCT01341639)[13] and a study evaluating a 2 + 1 schedule conducted in Finland, Italy, and Sweden from late November 2011 to early October 2013 (NCT01480258).[14] In these randomized, double-blind trials, infants received either a 3-dose primary series of DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib at 2, 3, and 4 months of age and a toddler dose at 12 months of age or a 2 dose primary series of DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib at 2 and 4 months of age and a toddler dose at 11 to 12 months of age.

Four groups were defined according to previous vaccination schedule (3 + 1 or 2 + 1) and type of vaccine (DTaP5-HB -IPV-Hib or DTaP3-HB-IPV/Hib) received during each study

- **Group 1**: DTaP5-HB-IPV-Hib (3 + 1), those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib
- **Group 2**: DTaP3-HB-IPV/Hib (3 + 1), those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib
- **Group 3**: DTaP5-HB-IPV-Hib (2 + 1), those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib
- **Group 4**: DTaP3-HB-IPV/Hib (2 + 1), those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib

All participants in the 3 + 1 and 2 + 1 studies received concomitant conjugate pneumococcal vaccine (PCV13) and rotavirus vaccine. Participants in the 3 + 1 study also received concomitant measles-mumps-rubella-varicella vaccine.

No vaccine (eg, booster or challenge dose) was administered as part of this extension study. The long-term persistence of antibody to hepatitis B surface antigen (anti-HBs) was assessed approximately 3 to 4 years after completion of a 3 + 1 or 2 + 1 schedule when children were 4 to 5 years of age. The long-term persistence of pertussis antibodies was assessed in the 2 + 1 study only and not the 3 + 1 study. The 3 + 1 study started and finished earlier than the 2 + 1 study. Because the school-entry pertussis-containing booster vaccine is given at approximately 4 years of age in Finland, many of the children in the 3 + 1 study had already received their pertussis school-entry booster. They were therefore not eligible to undergo the anti-pertussis antibody measurments in this study.

Study population

This study enrolled boys and girls who had received a complete 3-dose primary series or a complete 2-dose primary series, followed by a toddler dose with DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib as part of the pivotal 3 + 1 or the 2 + 1 studies. Exclusion criteria included receipt of any dose of a hepatitis B- or pertussis-containing vaccine other than the vaccines of the initial studies, or a diagnosis of hepatitis B infection or pertussis after the initial studies. Children were also excluded if they received immunoglobulins, blood, or blood-derived products within 3 months of study initiation or any immunosuppressive therapy or immune-modifying drugs at any time.

A child could withdraw at any time or be withdrawn at the request of parent(s) or legal representative(s), or if the investigator thought that continuation in the study was not in the interest of the child. Informed consent was signed by each child's parent(s) or legal representative(s). The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practices standards, the Declaration of Helsinki, and all applicable regulatory requirements, as well as any European and/or local applicable laws and regulations relating to the conduct of clinical trials. It is registered in ClinicalTrials.gov with an identifier of NCT02759354.

Immunogenicity measurements

The primary endpoints were the percentage of children with anti-HBs concentrations ≥10 mIU/mL, and anti-pertussis toxin (PT), anti-filamentous hemagglutinin (FHA), anti-pertactin (PRN), and anti-fimbriae types 2&3 (FIM) concentrations greather than or equal to the lower limit of quantitation (LLOQ), ≥2× LLOQ, and ≥4× LLOQ, with a LLOQ of 4 enzyme-linked immunosorbent assay (ELISA) units (EU)/mL for PT, PRN, FIM, and of 3 EU/mL for FHA. Secondary endpoints included anti-HBs, anti-PT, anti-FHA, anti-PRN and anti-FIM geometric mean concentrations (GMCs). Endpoint analysis was performed on the persistence analysis set approximately 4 years after the initial vaccination schedule. The persistence analysis set was defined as all children previously vaccinated with a complete 3 + 1 or 2 + 1 schedule who were not excluded per the previously noted criteria, and for whom immunogenicity results were available in the persistence study.

Anti-HBs was assessed using a hepatitis B-enhanced chemiluminescence (HepB ECiQ) assay at Focus Diagnostics Clinical Trials, San Juan Capistrano, California, USA. Anti-PT, FHA, PRN, and FIM antibodies were assessed using an ELISA at the Sanofi Pasteur Global Clinical Immunology platform, Swiftwater, Pennsylvania, USA.

Safety assessments

No safety analyses were performed in this study, as no additional vaccinations were administered. Only serious adverse events (SAEs) related to study procedures (blood sampling) were collected.

Statistics

It was estimated that with a sample size of approximately 180 evaluable children, the half width of the 2-sided 95% confidence interval (CI) was not to exceed 8% if the observed percentages of persistence were ≥60%. This was deemed acceptable to provide an appropriate estimate for the primary endpoints. The statistical analyses were performed using SAS® software version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

For categorical variables (except antibody concentrations), descriptive statistics of sample size, count by category, and proportion by category are presented. For continuous variables (except antibody concentrations), descriptive statistics of sample size, mean and standard deviation are presented. For

antibody concentrations, descriptive statistics of sample size, count greater than or equal to the threshold, proportion greater than or equal to the threshold, geometric mean, and 2-sided 95% CIs are presented.

Pertussis antibody concentrations reported by the laboratory as below the LLOQ were replaced by half of the LLOQ; those above the upper limit of quantitation were replaced by this limit. The 2-sided 95% CIs were provided for proportions and GMCs were calculated for immunogenicity endpoints. CIs for proportions were based on the exact method for binary variables.[15] CIs for GMCs were calculated using the *t* distribution and sample variance of logarithms of individual concentrations.

Results

Study population

In the original 3 + 1 study, 1217 children were randomized, and in the original 2 + 1 study, 1315 children were randomized. Of these children (n = 2532), 760 were screened for possible inclusion into this study, with 754 children enrolled and 752 included in the persistence analysis set. No child screened was found to have had either hepatitis B or pertussis infection. All 752 children were evaluable for anti-HBs analysis; however, only those who had participated in the 2 + 1 study (n = 371) were included in pertussis follow-up (see Methods). Demographic data are presented in Table 1. The 3 + 1 and 2 + 1 persistence study cohorts were very similar to the original study cohorts in terms of female:male ratio and mean age at toddler dose.

Hepatitis B immunogenicity

All of the children (100%) in the persistence study cohort had seroconverted (ie, achieved anti-HBs ≥10 mIU/mL) 1 month after the 3 + 1 vaccination and 2 + 1 schedules with DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib, reflecting robust hepatitis B responses for both vaccine groups. These high seroconversion rates in the persistence study cohort were consistent with the anti-HBs seroconversion rates (>99% in both vaccine groups following the 3 + 1 schedule and >98% in in both vaccine groups following the 2 + 1 schedule) in the overall study populations (Supplemental Tables 1 and 2). The percentages of toddlers with anti-HBs ≥100 mIU/mL were also similar between DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib in the 3 + 1 schedule (96.8% and 98.4%, respectively, Table 2) and in the 2 + 1 schedule (97.6% and 97.7%, respectively, Table 2), with overlapping 95% CIs. The anti-HBs GMCs after the toddler dose were lower for DTaP5-HB-IPV-Hib compared with DTaP3-HB-IPV/Hib, with overlapping 95% CIs for the 3 + 1 schedule, but non-overlapping 95% CIs for the 2 + 1 schedule (Table 2).

The persistence of anti-HBs was lower after DTaP5-HB-IPV-Hib than DTaP3-HB-IPV/Hib. Approximately 4 years after completion of a 3 + 1 schedule, the percentage of children with anti-HBs ≥10 mIU/mL was 70.2% (95% CI, 63.1%–76.5%) in Group 1 and 82.0% (95% CI, 75.8%–87.2%) in Group 2. Of those who received the 2 + 1 schedule, the percentage of children with anti-HBs ≥10 mIU/mL was 65.7% (95% CI, 58.3%–72.6%) in Group 3 and 83.7% (95% CI, 77.6%–88.6%) in Group 4. The persistence of anti-HBs GMCs across study groups were consistent with the initial GMC results, with lower levels at 4 years after completion of the original schedules of DTaP5-HB-IPV-Hib (Table 2).

Pertussis immunogenicity

Approximately 4 years after completion of the 2 + 1 schedule, the proportions of children with antibodies above LLOQ were 58.4% and 41.5% for anti-PT, 80.9% and 88.3% for anti-FHA, 66.1% and 72.6% for anti-PRN, and 94.4% and 3.3% for anti-FIM in the DTaP5-HB-IPV-Hib and the DTaP3-HB-IPV/Hib groups, respectively (Table 3). The 95% CIs overlapped for DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib for FHA and PRN but were non-overlapping for PT and FIM. In the original 2 + 1 study, all of these percentages were 100% 1 month after the 2 + 1 vaccination schedule with DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib, except for FIM, of which 100% of children in the DTaP5-HB-IPV-Hib group had anti-FIM antibodies above LLOQ versus 8.0% in the DTaP3-HB-IPV/Hib group.

For the secondary endpoint of GMCs assessed at 4 years after completion of the original 2 + 1 vaccination schedule, persistence of pertussis-related antibodies was generally consistent with post-primary immunization levels. Anti-PT and anti-FIM GMCs were higher, anti-FHA was lower, and anti-PRN GMCs were similar in the DTaP5-HB-IPV-Hib group compared with the DTaP3-HB-IPV/Hib group (Table 4).

Safety

No SAEs related to study procedures were reported during the study period.

Table 1. Demographic characteristics of the persistence analysis set.

	3 + 1			2 + 1			
Parameter	Group 1 (N = 191)	Group 2 (N = 189)	All (N = 380)	Group 3 (N = 181)	Group 4 (N = 191)	All (N = 372)	All (N = 752)
Gender, n (%)							
Female	98 (51.3)	86 (45.5)	184 (48.4)	84 (46.4)	94 (49.2)	178 (47.8)	362 (48.1)
Male	93 (48.7)	103 (54.5)	196 (51.6)	97 (53.6)	97 (50.8)	194 (52.2)	390 (51.9)
Mean age at toddler dose in previous study, months (SD)	12.2 (0.4)	12.2 (0.4)	12.2 (0.4)	11.2 (0.4)	11.2 (0.4)	11.2 (0.4)	11.7 (0.6)
Mean age at the persistence time point, months (SD)	4.8 (0.2)	4.8 (0.2)	4.8 (0.2)	3.9 (0.1)	3.9 (0.1)	3.9 (0.1)	4.4 (0.4)
Mean weight at the persistence time point, kg (SD)	19.2 (2.5)	19.3 (2.6)	19.2 (2.6)	16.9 (2.1)	17.3 (2.4)	17.1 (2.3)	18.2 (2.6)

SD, standard deviation.
Group 1: DTaP5-HB-IPV-Hib (3 + 1): those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib.
Group 2: DTaP3-HB-IPV/Hib (3 + 1): those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib.
Group 3: DTaP5-HB-IPV-Hib (2 + 1): those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib.
Group 4: DTaP3-HB-IPV/Hib (2 + 1): those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib.

Table 2. Summary of anti-HBs responses: persistence analysis set.

		Post-infant		Post-toddler		Persistence	
Schedule	Parameter	DTaP5-HB-IPV-Hib	DTaP3-HB-IPV/Hib	DTaP5-HB-IPV-Hib	DTaP3-HB-IPV/Hib	DTaP5-HB-IPV-Hib	DTaP3-HB-IPV/Hib
3 + 1	n	162	165	186	186	**Group 1**: 191	**Group 2**: 189
	% ≥10 mIU/mL	98.8	97.0	100.0	100.0	70.2	82.0
	(95% CI)	(95.6, 99.9)	(93.1, 99.0)	(98.0, 100.0)	(98.0, 100.0)	(63.1, 76.5)	(75.8, 87.2)
	% ≥100 mIU/mL	79.6	84.2	96.8	98.4	18.3	39.7
	(95% CI)	(72.6, 85.5)	(77.8, 89.4)	(93.1, 98.8)	(95.4, 99.7)	(13.1, 24.6)	(32.7, 47.0)
	GMC, EU/mL	230	271	3085	4157	24.4	51.3
	(95% CI)	(190, 278)	(225, 326)	(2529, 3762)	(3403, 5077)	(19.5, 30.6)	(40.2, 65.5)
2 + 1	n	104	110	124	131	**Group 3**: 181	**Group 4**: 190
	% ≥10 mIU/mL	99.0	98.2	100	100	65.7	83.7
	(95% CI)	(94.8, 100)	(93.6, 99.8)	(97.1, 100)	(97.2, 100)	(58.3, 72.6)	(77.6, 88.6)
	% ≥100 mIU/mL	80.8	86.4	97.6	97.7	17.7	45.3
	(95% CI)	(71.9, 87.8)	(78.5, 92.2)	(93.1, 99.5)	(93.5, 99.5)	(12.4, 24.0)	(38.0, 52.6)
	GMC, EU/mL	255	450	2413	4271	19.4	71.0
	(95% CI)	(199, 327)	(348, 580)	(1946, 2992)	(3334, 5470)	(15.5, 24.4)	(54.9, 91.8)

Anti-HBs, anti-hepatitis B surface antigen; CI, confidence interval; EU, ELISA units; GMC, geometric mean concentration; IU, international units.

Table 3. Summary of pertussis antibodies persistence (responses rate) following a 2 + 1 schedule: persistence analysis set.

		Group 3			Group 4		
Antibody	Concentration Endpoint[b]	N	n (%)	95% CI[a]	N	n (%)	95% CI[a]
Anti-PT	≥LLOQ[b]	178	104 (58.4)	50.8, 65.8	188	78 (41.5)	34.4, 48.9
	≥2xLLOQ		72 (40.5)	33.2, 48.1		41 (21.8)	16.1, 28.4
	≥4xLLOQ		26 (14.6)	9.8, 20.7		7 (3.7)	1.5, 7.5
Anti-FHA	≥LLOQ	173	140 (80.9)	74.3, 86.5	188	166 (88.3)	82.8, 92.5
	≥2xLLOQ		81 (46.8)	39.2, 54.5		133 (70.7)	63.7, 77.1
	≥4xLLOQ		45 (26.0)	19.7, 33.2		85 (45.2)	38.0, 52.6
Anti-PRN	≥LLOQ	180	119 (66.1)	58.7, 73.0	190	138 (72.6)	65.7, 78.8
	≥2xLLOQ		79 (43.9)	36.5, 51.5		97 (51.1)	43.7, 58.4
	≥4xLLOQ		28 (15.6)	10.6, 21.7		35 (18.4)	13.2, 24.7
Anti-FIM	≥LLOQ	177	167 (94.4)	89.9, 97.3	183	6 (3.3)	1.2, 7.0
	≥2xLLOQ		156 (88.1)	82.4, 92.5		4 (2.2)	0.6, 5.5
	≥4xLLOQ		123 (69.5)	62.1, 76.2		2 (1.1)	0.1, 3.9

[a]Confidence intervals were calculated based on the exact binomial method of D. Collett.[15]
[b]Lower limit of quntification (LLOQ) = 4 EU/mL for PT, PRN, and FIM; LLOQ = 3 EU/mL for FHA.
CI, confidence interval; FHA, filamentous hemagglutinin; FIM, fimbriae; PRN, pertactin; PT, pertussis toxin.

Table 4. Summary of pertussis antibodies persistence (GMCs) following a 2 + 1 schedule: persistence analysis set.

	Group 3 (N = 173–180)	Group 4 (N = 183–190)
Anti-PT		
GMC, EU/mL (95% CI)	5.3 (4.6, 6.1)	3.6 (3.2, 4.1)
Anti-FHA		
GMC, EU/mL (95% CI)	6.6 (5.5, 7.9)	11.1 (9.1, 13.4)
Anti-PRN		
GMC, EU/mL (95% CI)	5.9 (5.1, 6.9)	7.2 (6.2, 8.3)
Anti-FIM		
GMC, EU/mL (95% CI)	26.0 (21.9, 30.9)	2.1 (2.0, 2.3)

CI, confidence interval; EU, ELISA units; FHA, filamentous hemagglutinin; FIM, fimbriae; GMC, geometric mean concentration; PRN, pertactin; PT, pertussis toxin.

Discussion

This immunogenicity study demonstrated the long-term persistence of hepatitis B antibodies following the administration of DTaP5-HB-IPV-Hib as a 3-dose primary series given at 2, 3, and 4 months of age and a toddler dose at 12 months of age, or a 2-dose primary series given at 2 and 4 months of age and a toddler dose at 11 to 12 months, and the persistence of pertussis antibodies after the latter vaccination schedule. In this study, the threshold for seroprotection was anti-HBs concentrations ≥10 mIU/mL, which is a widely accepted immune correlate of protection against hepatitis B virus infection.[16] In contrast, pertussis lacks a simple correlate of protection, although logistic regression analysis of immune responses from household-exposed cohorts within acellular pertussis vaccine efficacy studies demonstrated statistically significant associations between the presence of antibodies against pertactin, fimbriae 2/3, and pertussis toxin, and clinical protection from pertussis.[17,18]

Anti-HBs results in this persistence study were consistent with those from the original studies, which showed similarly high rates of children with anti-HBs ≥10 and ≥100 mIU/mL for both DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib, with higher GMCs for the DTaP3-HB-IPV/Hib vaccine. All anti-HBs levels are expected to wane over time, regardless if from infection or vaccination, with anti-HBs protective level concentrations declining rapidly within the first year (down to 15%–50%) and more slowly thereafter (low or undetectable after 5–15 years). However, lower anti-HBs concentrations years after vaccination do not indicate the absence of protection against clinically important hepatitis B disease. It is well documented and accepted by international public health authorities that initial seroprotection following a complete series of hepatitis B vaccination determines long-term protection, and robust seroprotection rates following vaccination were observed in the original studies. After disappearance of vaccine-induced circulating antibodies, protection against hepatitis B results from an immune memory response.[19–29] This immune memory or anamnestic response years after vaccination with hepatitis B-containing vaccine can be demonstrated with a challenge dose of hepatitis B vaccine,

which is composed of hepatitis B surface antigen and serves as a model of exposure to hepatitis B virus itself. Years after primary hepatitis B vaccination, children and adults demonstrate high rates of seroprotective antibody responses following a challenge dose of hepatitis B vaccine, including individuals with anti-HBs <10 mIU/mL prior to the challenge.[30] Such anamnestic responses have been demonstrated as long as 22 years after infant and childhood vaccination with recombinant or plasma-derived hepatitis B vaccine in Taiwan,[19] and 30 years after vaccination with plasma-derived hepatitis B vaccine in Alaska.[31] Immunologic characterization of the 30-year Alaskan cohort showed that immune memory against hepatitis B correlated with the presence of natural killer T-cell responses.[32] Similarly high rates of seroprotection have been demonstrated in response to hepatitis B challenge dosing years after primary vaccination with hepatitis B-containing hexavalent vaccines.[33–36] These immune memory mechanisms are posited to include responses mediated by natural killer T cells. Given that there are no approved clinical tests for cell-mediated immune memory markers against hepatitis B, it is reasonable to monitor for anti-HBs ≥10 mIU/mL after vaccination in the research setting, while understanding that it does not provide the complete view of immune protection against hepatitis B resulting from vaccination. Accordingly, there are no public health recommendations for long-term monitoring of anti-HBs, nor are there routine hepatitis B booster dose recommendations based on anti-HBs levels years after vaccination.

In addition to long-term immune memory, the long-term efficacy and effectiveness of hepatitis B vaccination has been confirmed in cohort studies, and by real-world evidence, respectively. Multiple long-term follow-up cohort studies have demonstrated high rates of protection from chronic carrier state (HBs positivity) and infection (hepatitis B core antigen positivity), following vaccination for 15 to 22 years.[19–27,29] Countries that have adopted universal vaccination against hepatitis B with recombinant HBV have seen dramatic decreases in hepatitis B disease rates[21,37] consistent with the high effectiveness of hepatitis B vaccination during infancy. Given the high long-term efficacy and effectiveness of infant hepatitis B vaccination, routine booster vaccination against hepatits B is not recommended by a number of global organizations, including the World Health Organization, US Centers for Disease Control and Prevention, the Standing Committee on Vaccination at the Robert Koch Institute (STIKO), and the Viral Hepatitis Prevention Board.[38,39]

In a prior study of concomitant vaccination between a hepatitis B-containing hexavalent vaccine (DTaP-IPV-HBV-Hib [Hexavac], Aventis Pasteur MSD, Lyon, France) and a 7-valent pneumococcal conjugate vaccine (Prevnar 7, Wyeth), lower hepatitis B GMCs were noted with concomitant administration as opposed to separate administration.[40] In order to overcome potential immune interference among hexavalent vaccine components, or with concomitant vaccine antigens, DTaP5-HB-IPV-Hib has been designed to optimize hepatitis B immunogenicity, with an increase from 5 to 10 μg of the HBs component, and a modified process aluminum phosphate adjuvant, which allows for greater accessibility of the adsorbed hepatitis B antigen to antigen-presenting cells.[41] Multiple randomized, double-blind, comparator-controlled studies of hepatitis B vaccine with modified process adjuvant, demonstrated similar safety and higher hepatitis B GMCs compared with hepatitis B vaccine with original process adjuvant in a wide range of populations from infants to older adults.[42–46] Accordingly, the hepatitis B responses in the Phase 3 studies of DTaP5-HB-IPV-Hib have been robust, meeting all acceptability and non-inferiority criteria compared with the licensed vaccine regimens in the United States and Europe (pentavalent with separate monovalent hepatitis B, or DTaP3-HB-IPV/Hib, respectively). Indeed, as highlighted in the current manuscript, DTaP5-HB-IPV-Hib resulted in high and similar percentages of children with anti-HBs ≥10 and ≥100 mIU/mL compared with DTaP3-HB-IPV/Hib in the rigorous settings of the European Union (ie, without a hepatitis B birth dose and using a compressed 3-dose [2, 3, 4 months] or 2-dose [2, 4 months] regimen). Note that the increase in the ratio of aluminum phosphate to aluminum hydroxide in the modified process adjuvant does not result in higher total amounts of aluminum salt in DTaP5-HB-IPV-Hib (314 μg) as compared to DTaP3-HB-IPV/Hib (950 μg).

This study also provides information about the long-term persistence of pertussis antibody responses following the administration of DTaP5-HB-IPV-Hib as a 2-dose primary series given at 2 and 4 months of age, with a toddler dose at 11 to 12 months of age. Pertussis antibodies 3 to 4 years following a 2 + 1 hexavalent dosing schedule were higher in DTaP5-HB-IPV-Hib recipients for anti-PT and anti-FIM antibodies compared with those who received the DTaP3-HB-IPV/Hib vaccine. Levels of anti-FHA antibodies were higher in DTaP3-HB-IPV/Hib recipients compared with DTaP5-HB-IPV-Hib recipients, while anti-PRN antibodies were comparable across both vaccine groups. There are differing quantities of pertussis antigens in each 0.5-mL dose of the study vaccines. DTaP5-HB-IPV-Hib contains 20 μg PT, 20 μg FHA, 3 μg PRN and 5 μg FIM. In contrast, DTaP3-HB-IPV/Hib contains 25 μg PT, 25 μg FHA, 8 μg PRN and no FIM. The anti-pertussis anibody levels found in this study generally correlated with the pertussis antigen quantities in the respective vaccines, with the exception of PT antibody. Factors in addition to antigen quantity, such as source and formulation, may explain this somewhat counterintuitive finding.[47]

In this study, anti-PT, anti-FHA, and anti-PRN GMCs had fallen for both vaccine groups back to the pre-vaccination levels in the original 2 + 1 study, while the anti-FIM GMC in the DTaP5-HB-IPV-Hib group persisted well above baseline levels. Although some have suggested that there are no serological correlates of protection, the oposite is actually the case. However, these correlates are multiple and complex.[48] A validated model of pertussis protection,[17,18] as well as other investigations,[49,50] suggest that higher levels of anti-FIM antibodies, as seen in the DTaP5-HB-IPV-Hib group of the present study, may be associated with enhanced pertussis immunity.[17,18,50] In spite of the persistence of anti-FIM antibodies in the DTaP5-HB-IPV-Hib receipients, the overall pertussis antibody findings of this study reinforce the importance of following recommendations for school-entry pertussis booster vaccination at around 5 years of age, as is done in the United States and the majority of countries in the European Union.[51,52]

Limitations of this study include that it was descriptive and not powered for formal between-groups comparisons, and the monitoring of immune memory against hepatitis B was limited to tests approved for clinical use (anti-HBs levels). In addition, the study design only evaluated the persistence of anti-HBs and did not measure the response to a challenge dose of hepatitis B vaccine, which models exposure to hepatitis B.

In conclusion, this study demonstrated that although anti-HBs levels ≥10 IU/mL and detectable antibodies against acellular pertussis antigens persisted in a majority of study participants, there was waning of hepatitis B and pertussis antibodies during the 3 to 4 years after completion of the infant-toddler hexavalent vaccination schedule. These findings have different clinical and public health implications for the long-term prevention of these diseases. Regarding hepatitis B vaccination, responders to a complete series continue to be protected against hepatitis B disease and chronic infection, regardless of the level of circulating anti-HBs at the time of their exposure to hepatitis B virus. For pertussis, the waning of most anti-pertussis antibodies to prevaccination basline levels support pertussis booster vaccination at school-entry and beyond.

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