Challenge with Hepatitis B Vaccine in Children Previously Vaccinated with a Hepatitis B-Containing Combination Vaccine

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ABSTRACT

Introduction: The World Health Organization (WHO) recommends universal infant hepatitis B virus (HBV) vaccination as the most effective preventive measure against HBV-induced disease in endemic areas. More than 160 countries have followed the WHO recommendations to incorporate HBV vaccination in their national infant immunization programs. While antibodies to hepatitis B surface antigen (anti-HBs) concentrations progressively decrease following vaccination in infancy, protection persists, probably due to lasting immune memory. Hence, it is thought that future exposure to wild-type virus or HBV vaccine will induce a protective secondary immune response. **Methods:** Children aged 7–8 years old

who had been vaccinated in infancy with a hexavalent DTaP-IPV-HB-Hib vaccine (Hexavac®; Sanofi Pasteur MSD, Lyon, France) and children aged 7–9 years vaccinated in infancy with a DT-HB vaccine (Primavax®; Sanofi Pasteur MSD), whose anti-HBs concentrations had fallen below the seroprotective threshold of 10 mIU/mL at age 4.5–6 years, received a challenge dose of monovalent HBV vaccine (HBVAXPRO®; Sanofi Pasteur MSD) to assess persistence of protection. **Results:** One month postchallenge, 54 of 61 (88.5%) Hexavac-primed seronegative children and 34 of 40 (85.0%) Primavax-primed seronegative children had anti-HBs concentrations ≥ 10 mIU/mL. The percentage of protected children would have been even higher if the children who still had protective antibody levels (who were not included in this challenge study) had been assessed (42.1% with Hexavac, 55.4% with Primavax). **Conclusion:** Vaccination with a HBV-containing multivalent vaccine during infancy induces a lasting immune memory that can be boosted, even in children with a decline in anti-HBs concentrations. The present results confirm that the full primary vaccination schedule in infancy seems to confer long-term protection via immune memory and that an additional HBV dose is not generally required.

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INTRODUCTION

Hepatitis B virus (HBV) is a major global public health problem. More than 350 million of the 2 billion people infected worldwide have chronic HBV infections and are at serious risk of developing cirrhosis and liver cancer.¹ Safe and effective vaccines exist and the World Health Organization (WHO) recommends universal infant HBV vaccination as the most effective preventive measure against HBV-induced disease in endemic areas. More than 160 countries have followed the WHO recommendations to incorporate HBV vaccination in their national infant immunization programs.² However, to be fully effective, the protection provided by vaccination in infancy needs to last until adolescence and early adulthood when exposure to HBV and risk of infection once again increases with the onset of sexual activity and high-risk behaviors such as intravenous drug abuse.³

A decrease in titers of antibodies to hepatitis B surface antigen (anti-HBs) over time following HBV vaccination has been documented.^{4–7} Despite this, there is currently no evidence to recommend the routine use of boosters in children or adolescents provided that they have received and responded to a full, three-dose, primary course (ie, an anti-HBs concentration ≥ 10 mIU/mL 1 month after the third dose, which is considered seroprotective).^{7–10} Children whose anti-HBs concentrations fall below 10 mIU/mL are still considered protected against symptomatic HBV infection and chronic carriage for up to 15 years as a result of their initial seroconversion and subsequent immune memory.^{4,5,11,12}

The mechanism for the persistence of protection beyond the waning of detectable antibody

concentrations against HBV is not fully understood. In addition, there is no good marker of persistent protection in the absence of antibody persistence. HBV vaccine efficacy trials have demonstrated an association between initial postvaccination anti-HBs concentrations ≥ 10 mIU/mL and protection against clinically significant breakthrough HBV infection and chronic carriage. When an individual, who has previously been seropositive, is exposed to HBV, a rapid activation of specific memory T and B lymphocytes should occur, and a significant and clinically protective increase in anti-HBs concentrations should be observed within 5–10 days. This rapid secondary immune response contrasts with the 4–12-week incubation period of HBV, and thus contributes to protection prior to the development of significant HBV viremia.^{13,14}

Only a few studies have examined anti-HBs persistence and most of these were carried out in areas of high endemicity.^{4,5,15–17} Furthermore, much of the data on long-term protection against HBV infection were initiated with plasma-derived or recombinant monovalent HBV vaccines rather than the combination vaccines containing recombinant hepatitis B surface antigen (HBsAg) that have been developed in recent years.^{18–24} The extent of long-term protection against HBV infection in low-risk infants vaccinated from birth is not as well documented as that for infants in high endemicity areas, although protection clearly persists beyond the waning of detectable antibody.

MATERIALS AND METHODS

In two separate studies we examined long-term anti-HBs persistence and immune memory to HBV in children aged between 7 and 9 years of age who were resident in areas of low (France) or low to intermediate (Italy) endemicity and who had responded to vaccination

(anti-HBsAb ≥ 10 mIU/mL) in infancy after a primary series of a combination vaccine containing a HBV component—either DTacP-IPV-HB-Hib (Hexavac®; Sanofi Pasteur MSD, Lyon, France) or DT-HB (Primavax®, Sanofi Pasteur MSD) and whose antibody concentrations had fallen below 10 mIU/mL between 4.5 and 6 years of age. In both studies, the children were challenged with a single injection of HBV vaccine (HBVAXPRO®; Sanofi Pasteur MSD) to simulate exposure to wild-type HBV infection, and postchallenge anti-HBs levels were measured to determine the characteristics of the anamnestic immune response.

Design of the Studies

The two study protocols were approved by the relevant ethics committees, and the studies were conducted in accordance with the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) (ICH Topic E6 Note for Guidance on Good Clinical Practice CPMP/ICH/135/95) and the relevant national laws and regulations. Informed consent forms signed by the child's parents or legal guardian were required for participation in the study. In France, the child's agreement to participate was also required.

Hexavac-Primed Children

An open-label, multicenter, single-arm study was performed at 24 centers in France. Eligible children (aged 7–8 years old) had previously participated in a phase 3 study investigating the safety and immunogenicity of a three-dose primary series and booster of Hexavac (containing 5 μ g of HBsAg) at 2, 3, 4, and 15–17 months or 2, 4, 6, and 15–17 months, and had an anti-HBs concentration <10 mIU/mL during a subsequent antibody persistence study at 5.5–6 years of age (Figure 1).

Primavax-Primed Children

An open-label, single center, single-arm study was performed in Italy. Eligible children (aged 7–9 years old) had previously participated in a phase 3 study investigating the safety and immunogenicity of three doses of Primavax (containing 5 μ g of HBsAg) at 2, 4, and 11 months or 3, 5, and 11 months, and had an anti-HBs concentration <10 mIU/mL in a subsequent antibody persistence study at 4.5–5.5 years of age (Figure 1).

Exclusion Criteria

In both studies children were excluded if they had a vaccination scheduled during the course of the study; if they were receiving treatment likely to alter the immune response (administration of immunoglobulin, long-term systemic corticosteroids, or blood products); if they had been vaccinated against HBV since the end of the antibody persistence study; or if they were allergic to one or more of the vaccine components. Children were also ineligible to participate in the studies if they: had a known history of thrombocytopenia or any coagulation disorder that would contraindicate intramuscular injection; had any medical condition that, in the opinion of the investigator, might interfere with the evaluation of the study objectives; or were participating in another clinical study scheduled during the course of the study.

Study Procedures

In both studies, the subjects attended three visits and they were followed for up to 37 days. A challenge dose of HBV vaccine was administered at visit 1 (day 1). No blood sample was taken on day 1; the baseline anti-HBs concentration was taken to be <10 mIU/mL, from the antibody persistence study. Blood samples were taken at

Figure 1. Study sequences for Hexavac-primed and Primavax-primed children. Anti-HBs=antibodies to hepatitis B surface antigen; PPS=per-protocol set.

visit 2 (days 7-10) and visit 3 (days 30-37). Safety parameters were assessed at visit 1 and visit 3.

Study Vaccine

A single, 0.5 mL dose of monovalent HBVAXPRO® vaccine containing 5 µg recombinant HBsAg was administered by intramuscular injection into the deltoid muscle at visit 1.

Antibody Measurements

All antibody measurements were performed at Merck Research Laboratories (Wayne, PA, USA). Anti-HBs concentrations were measured

by a solid-phase sandwich radioimmunoassay technique, using quantitative AUSAB® ABBOTT test kits with a detection limit of 0.06 mIU/mL (Abbott Laboratories North, Chicago, IL, USA). The counts per minute of the sample were compared with the mean counts per minute from a group of negative control sera, and anti-HBs concentrations expressed as mIU/mL calculated as previously recommended.²⁵

Immunogenicity Endpoints

The primary immunogenicity endpoint in both studies was the seroprotection rate defined as the percentage of tested subjects

with an anti-HBs titer ≥ 10 mIU/mL 1 month after administration of the challenge dose. The secondary immunogenicity endpoints were the seroprotection rate 1 week after administration of the challenge dose, and the 1-week and 1-month postchallenge geometric mean concentrations (GMCs).

Safety Assessment

Diary cards were given to the parents or legal guardian to record specific adverse events. Solicited injection-site reactions (redness, induration, and swelling) and other spontaneously reported reactions were recorded on the diary card for 3 days following vaccination or at any time between the study visits if they led to a medical consultation. All injection-site reactions ≥ 2 cm were reported by the investigator. Fever (equivalent rectal temperature $\geq 38.0^{\circ}\text{C}$) and other spontaneously reported systemic events were recorded in the same manner.

Statistical Methods

Statistical analyses were performed using Statistical Analysis System® (SAS, version 8.2; SAS Inc., Cary, NC, USA). Three sets of subjects were defined: the safety analysis set consisted of all children who received the study challenge vaccine; the full analysis set (FAS) consisted of all children whose anti-HBs concentration was ≥ 10 mIU/mL at completion of the initial study, who received the study challenge vaccine, and for whom at least one post-dose immunogenicity evaluation was available; the per-protocol set (PPS) was defined as those participating in the study without protocol violations that could have interfered with the immunogenicity evaluation.

Descriptive statistics (mean, standard deviation, minimum, median, and maximum) were

calculated for quantitative variables, and were used to display qualitative variables. GMCs were calculated for anti-HBs concentrations, and within-group 95% confidence intervals (CIs) were calculated for response rates and GMCs. The 95% CIs for response rates were based on the one-sample Wilson score method and those for GMCs were based on Student's *t* distribution after $\log_{10}$ transformation of individual concentrations. A further analysis was performed, according to the level of the subjects' responses in the initial study with the primary series plus booster.

RESULTS

Hexavac-Primed Children

Of 106 eligible children, 95 were screened for inclusion in the study and 71 children were included (parents or legal guardian gave consent) and completed the study. One of these children, who received the vaccine, had an anti-HBs concentration ≥ 10 mIU/mL in the antibody persistence study and was excluded from the immunogenicity analyses; no blood samples were taken and the child was followed for safety only. Four children were excluded from the FAS and the PPS because their anti-HBs concentrations were either unknown ($n=1$) or <10 mIU/mL ($n=3$) at the end of the initial Hexavac study. The FAS therefore consisted of 66 children. A further five children were excluded from the PPS because their blood samples were taken outside the 30–37-day window after visit 1; the PPS therefore consisted of 61 children (Figure 1).

The demographic and baseline characteristics were similar for the PPS and the FAS (FAS data in Table 1). The recorded medical histories and the previous or concomitant medications were not judged likely to interfere with the conduct of the study. No concomitant, nonstudy vaccines were administered.

Table 1. Baseline demographic characteristics for the children in the full analysis sets in both hepatitis challenge studies.

	Hexavac-primed children (<i>n</i> =66)	Primavax-primed children (<i>n</i> =40)
Mean age, years (SD)	7.7 (0.1)	7.9 (0.4)
Male sex, <i>n</i> (%)	27 (40.9)	22 (55.0)
Mean weight, kg (SD)	26.7 (4.2)	30.8 (6.1)
Normal physical examination, <i>n</i> (%)	64 (97.0)	40 (100)

Immunogenicity

One month after the challenge dose, 54 children (88.5%) in the PPS had anti-HBs concentrations ≥ 10 mIU/mL (Table 2). The results for the FAS were similar to those for the PPS, with 89.4% of children achieving seroprotective anti-HBs concentrations. At 7 days after the challenge dose, 34 (55.7%) children in the PPS were seroprotected (Table 2). The anti-HBs GMC increased from 9.5 mIU/mL at 7 days after the challenge dose to 170.3 mIU/mL at 1 month postchallenge dose (Table 2).

Results from a further analysis according to children's antibody levels 1 month after the completion of the primary vaccination series and booster dose are shown in Table 3. Six of

the seven nonresponders to the challenge dose after 1 month had an anti-HBs concentration between 10 and 100 mIU/mL after the primary immunization, while the seventh had an anti-HBs concentration of 285 mIU/mL. It was also noted that two of the three subjects considered as nonresponders after the primary course of vaccination had anti-HBs concentrations ≥ 10 mIU/mL after the challenge dose (16 and 1045 mIU/mL, respectively).

Safety

Safety was evaluated for all 71 children who received the challenge dose of vaccine. At least one adverse event was experienced by 22 of these children (31.0%), and a total of 37 adverse events were reported. Nine children (12.7%) experienced an injection-site reaction, and a total of 13 injection-site reactions (mostly mild in intensity) were reported. At least one systemic adverse event was reported for 14 children (19.7%), and a total of 24 systemic adverse events were reported. Five of these were assessed as being related to the vaccination and 19 as being unrelated. All adverse events unrelated to the vaccination were clearly related to intercurrent diseases (mostly respiratory tract infections, which were not unexpected in the study

Table 2. Geometric mean concentrations (GMCs) and seroprotection rates after challenge dose for Hexavac-primed children (per-protocol-set [PPS]) and for Primavax-primed children (full analysis set and PPS).

	Concentrations (mIU/mL)		Seroprotection rates (≥ 10 mIU/mL)
	GMC (95% CI)	Median (min-max)	<i>n</i> (%) (95% CI)
Hexavac-primed children (<i>n</i>=61)			
Time after challenge			
1 week (days 7–10)	9.5 (4.5–20.1)	12.0 (0.3–4203.0)	34 (55.7) (43.3–67.5)
1 month (days 30–37)	170.3 (90.4–320.6)	260.0 (0.3–8171.0)	54 (88.5) (78.2–94.3)
Primavax-primed children (<i>n</i>=40)			
Time after challenge			
1 week (days 7–10)	2.5 (1.3–5.0)	3.0 (0.3–137.0)	13 (32.5) (20.1–48.0)
1 month (days 30–37)	107.9 (45.3–257.1)	236.5 (0.3–8898.0)	34 (85.0) (70.9–92.9)

Table 3. One-month postchallenge dose immune response according to anti-HBs titers at the end of the initial study; per-protocol set (PPS) for Hexavac-primed children and full analysis set/PPS for Primavax-primed children.

Postbooster concentration in initial study	<i>n</i>	Concentration _{PC} (mIU/mL)			SPR
		GMC	Median	Min; max	<i>n</i> (%)
Hexavac-primed					
10 mIU/mL ≥ concentration _{S1} < 100 mIU/mL	9	3.5	7.0	0.3; 45.0	3 (33%)
100 mIU/mL ≥ concentration _{S1} < 1000 mIU/mL	24	158.2	205.0	0.3; 3709.0	23 (96%)
Concentration _{S1} ≥ 1000 mIU/mL	28	630.0	626.0	32.0; 8171.0	28 (100%)
Primavax-primed					
10 mIU/mL ≥ concentration _{S1} < 100 mIU/mL	5	1.3	0.3	0.3; 16.0	2 (40%)
100 mIU/mL ≥ concentration _{S1} < 1000 mIU/mL	19	157.7	236.0	0.3; 4322.0	17 (89%)
Concentration _{S1} ≥ 1000 mIU/mL	16	270.7	333.0	5.0; 8898.0	15 (94%)

Concentration_{SI}=postbooster-dose concentration in initial study; Concentration_{PC}=postchallenge-dose concentration (present study); GMC=geometric mean concentration; SPR=seroprotection rate (anti-HBs concentration ≥ 10 mIU/mL).

population). No serious or significant adverse events were observed during the study.

Primavax-Primed Children

Of 48 subjects who were eligible for inclusion in the study, 42 were enrolled. Two children were not vaccinated and withdrew prematurely from the study. The remaining 40 children completed the study according to the protocol, and therefore were included in the FAS, the PPS, and the safety data set. The baseline demographic details of the FAS are summarized in Table 1.

Immunogenicity

One month after the challenge dose of HBsAg, 34 (85.0%) of the children had anti-HBs concentrations ≥ 10 mIU/mL, and 7 days post-challenge dose 13 (32.5%) children had anti-HBs concentrations ≥ 10 mIU/mL (Table 2). The anti-HBs GMC was 2.5 mIU/mL 7 days after the challenge dose and increased to 107.9 mIU/mL at 1 month (Table 2). Results from a further analysis according to children's antibody levels 1 month after the completion of the primary

vaccination series and booster dose are shown in Table 3.

Safety

Eight of the 40 children (20%) experienced at least one adverse event, and a total of 11 adverse events were reported. Two children (5%) had one mild injection-site reaction each. At least one systemic adverse event was reported in six children (15%) and a total of nine systemic adverse events were reported, of which three (moderate fever) were assessed as related to vaccination. Adverse events unrelated to the vaccination were clearly related to intercurrent diseases (mostly respiratory tract infections). No serious adverse events were reported during the study.

DISCUSSION

It is thought that subjects who respond to HBV vaccination (ie, anti-HBs concentration ≥ 10 mIU/mL at any time), even if their anti-HBs concentrations fall below 10 mIU/mL, will be protected against severe acute symptomatic or chronic HBV disease through an

immune memory mechanism. The exact duration of protection is currently unknown, but in one effectiveness study in a country with high endemicity immunized subjects were reported to be protected for up to 18 years.²⁶ However, in countries with lower endemicity, such as France and Italy, it is not known if subjects vaccinated during their infancy are protected to the same degree via this immune memory mechanism, since, in high endemicity areas, it has been suggested that repeated exposure to wild-type virus results in the boosting of immunity. In the studies reported here, in children fully immunized in infancy with a HBV-containing combination vaccine, and whose anti-HBs concentrations had subsequently fallen below 10 mIU/mL at 4.5–6 years of age, we investigated HBsAg immune memory, as shown by the ability to mount an anti-HBs response. We observed that 88.5% and 85.0% of the Hexavac- and Primavax-primed children, respectively, mounted a secondary immune response when a challenge dose of monovalent HBV vaccine was used as a surrogate for exposure to wild-type HBV.

The children enrolled in these studies were a subset of the original study cohorts, and were selected because their anti-HBs concentrations had fallen below 10 mIU/mL in the antibody persistence study. All children with anti-HBs ≥ 10 mIU/mL after 5–6 years (42.1% with Hexavac; 55.4% with Primavax) would be predicted to have anti-HBs ≥ 10 mIU/mL after the challenge dose. Hence, the percentage of all children vaccinated in infancy with Hexavac or Primavax who remain protected against HBV in childhood, would have been higher than that observed in these studies, which included only those children who had become seronegative 4.5–6.0 years after initial vaccination.

Our results demonstrate a secondary immune response to a challenge dose of HBV vaccine in children primed 6–7 years previously with an

HBV-containing multivalent vaccine. A study conducted in a region in which HBV is endemic showed that the lack of a secondary immune response to a booster does not necessarily imply a lack of protection. Despite a correlation between protection from infection and initial response, none of the vaccinated children showing evidence of postvaccination HBV infection (anti-HBc positive) had a chronic infection, regardless of anti-HBs titers following the primary series.²⁴ Furthermore, a study in 76 healthcare workers who had failed to mount a humoral response to HBV vaccination found that 75% had developed specific cellular immune responses, which the investigators considered would protect them against viral infection.²⁷

Several studies of long-term antibody persistence following HBV vaccination and response to a booster dose have been performed in children who were vaccinated at birth with a monovalent HBV vaccine.^{18,19,21,22} The majority of these studies were performed in countries or populations of moderate or high HBV endemicity, where higher levels of natural exposure may influence the persistence of protection.^{17,28,29} Both the timing of the anti-HBs testing and the specific definition of anamnestic response differed between studies, which showed a range of 89%–100% of HBV-vaccinated infants with anti-HBs titers ≥ 10 mIU/mL following a booster/challenge dose of monovalent HBV vaccine. While the potential effects of the differences in methodology and setting cannot be ignored, these values are comparable with those seen in the current studies.

Recent studies have reported anti-HBs persistence data for HBV-containing combination vaccines used according to European schedules and strong immune response after a booster vaccination.^{30–33} In one study, 79.6% of children primed with Hexavac at 2, 4, and 6 months of age had seroprotective anti-HBs titers prior to a

booster vaccination at 12–19 months of age.³⁰ One month following a booster dose of the hexavalent DTaP-HBV-IPV/Hib vaccine, 98.1% had anti-HBs titers ≥ 10 mIU/mL. A substantial increase of anti-HBs concentration from prebooster to postbooster suggests that an anamnestic response was induced. Another analysis of anti-HBs persistence in Italian children who received a primary series of either Hexavac or Infanrix at 3, 5, and 12 months of age found that seroprotection rates 12 months after the third dose were 69% and 96% respectively (relative risk 7.7; 95% CI 3.1–18.9).³¹ One month after children with anti-HBs titers < 10 mIU/mL had received a booster dose of monovalent HBV vaccine corresponding to the recombinant HBV component included in the priming vaccine, five of five children primed with Infanrix and 24 of 26 Hexavac-primed children were seroprotected, demonstrating persistence of immune memory following priming with both hexavalent vaccines.³¹ As part of another Italian study, 342 children, who received a primary series of Engerix B at 3, 5, and 11 months of age had anti-HBs concentrations lower than 10 mIU/mL or undetectable concentrations, more than 10 years later were given a booster vaccination with Engerix.³² Two weeks after the booster 97% ($n=332$) of the children had anti-HBs concentrations greater than 10 mIU/mL. Postbooster anti-HBs concentrations were higher in children whose prebooster concentrations were detectable but below 10 mIU/mL than in children whose concentrations were undetectable. In another two studies in Germany, children who had been vaccinated with four doses of Infanrix and whose anti-HBs concentrations had fallen below 10 mIU/mL received one dose of Engerix B at 4–6 years old in one study and 7–9 years in the other.³³ One month later the seroprotection rates were 88.5% and 95.1%, respectively. These response rates are comparable with those seen in the present studies in French and Italian children.

Our data do not allow us to assess the relative effect of the vaccines used in the studies on the postchallenge results, either for the primary series and first booster or the challenge dose. Additionally, we cannot anticipate the level of protection of vaccinated nonresponders against clinical HBV infection and its clinical after-effects. Therefore, we are unable to judge whether similar results would have been obtained in an equivalent cohort who had been vaccinated 10, 15, or 20 years earlier, or whether the percentage of nonresponders to a challenge dose of a HBV vaccine would have increased over time. Nevertheless, the present results confirm that the full primary vaccination schedule in infancy seems to confer long-term protection via immune memory and that an additional HBV dose is not generally required.

CONCLUSION

Analysis of responses to an HBV challenge in children vaccinated in infancy with an HBV-containing combination vaccine, but whose anti-HBs concentrations had subsequently fallen below seroprotective levels, found that the majority mounted an anamnestic response to a challenge dose of monovalent HBV vaccine. In children who responded to the HBV component of Hexavac or Primavax in infancy (anti-HBs ≥ 10 mIU/mL) and whose anti-HBs concentrations subsequently fell below 10 mIU/mL by age 4.5–6 years, 88.5% and 85.0%, respectively, mounted an anamnestic response. If all children in the original cohorts, irrespective of their anti-HBs antibody concentrations at age 4.5–6 years had been included in this challenge study, we can assume that the percentage of children who would have mounted an anamnestic response to the challenge dose would have been higher than that observed, since we can assume that all those children whose antibodies were ≥ 10 mIU/mL

at age 4.5–6 years would have responded. These results are consistent with those of other published studies, despite differences in vaccines used, vaccination schedules, and HBV endemicity in the study settings.

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