

Humoral and Cellular Immune Responses to a Hepatitis B Vaccine Booster 15–18 Years after Neonatal Immunization

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Background. Whether hepatitis B (HB) vaccine–conferred immunity persists into adulthood is unknown. We aimed to investigate long-term HB immunity in adolescents.

Methods. In 2004–2005, 6156 high school students (15–21 years old) who had been vaccinated with plasma-derived HB vaccine as infants were recruited for HB seromarker screening. The immune response to an HB vaccine booster was evaluated in 872 subjects who were seronegative. HB surface antibody (anti-HBs) titers and levels of HB surface antigen (HBsAg)–specific interferon (IFN)– γ – or interleukin (IL)–5–secreting peripheral blood mononuclear cells (PBMCs; measured by enzyme-linked immunospot assay) were determined 4 weeks later.

Results. Although the vaccine remained highly efficacious in reducing the HBsAg positivity rate, 63.0% of the vaccinees had no protective anti-HBs. After the booster, anti-HBs remained undetectable in 28.7% (158/551) of the subjects who had received complete HB vaccination (4 doses) during infancy. We estimated that 10.1% of the total population had lost their HB vaccine–conferred booster response. HBsAg-specific IFN– γ – or IL–5–secreting PBMCs remained negative in 27.2% (25/92) of subjects after the booster.

Conclusions. A notable proportion of fully vaccinated adolescents had lost immune memory conferred by a plasma-derived HB vaccine 15–18 years later. This decay of immune memory may raise concerns about the need for a booster vaccine for high-risk groups in the long run.

Universal neonatal and infant hepatitis B (HB) vaccination has been proven to be highly effective in inducing protective antibodies and preventing perinatal and horizontal transmission of HB virus (HBV) [1–4]. Such a program was launched in Taiwan in 1984 [5] and resulted in a significant reduction in the prevalence of chronic HBV infections, fulminant HB, and hepatocellular carcinoma [6–10].

HB vaccine-induced HB surface antibody (anti-HBs) titers wane with time [11–16]. Previous studies conducted in Taiwan showed that the postvaccination seropositivity rate of anti-HBs dropped from 99% at 1 year to 83% at 5 years [17] and from 71.1% at 7 years to 37.4% at 12 years [18]. Clinical observations up to 10 years after neonatal vaccination showed that subclinical infections, as evidenced by the appearance of HB core antibody (anti-HBc), did occur in 1%–9% of subjects, but none became chronic HB surface antigen (HBsAg) carriers [19]. Benign breakthrough HBV infection occurred without leading to chronicity, as indicated by isolated anti-HBc positivity [14, 16–18, 20, 21]. Thus, despite the continued declining of anti-HBs levels to nonprotective or undetectable levels, it is generally believed that vaccinees are protected against clinically significant HB infection through persistent immune memory and anamnestic antibody response. Vaccine-induced immunity appears to last for at least 15 years in immunocompetent individuals who responded adequately to primary immunization [14, 16, 18, 20, 21].

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Therefore, booster vaccinations are not recommended for immunocompetent subjects 15 years after neonatal vaccination [22–26]. Some authorities believe that immunity to HB will probably last for life in fully immunized immunocompetent vaccinees. Booster doses are recommended only for those with impaired immune functions, such as those with chronic renal failure, HIV infection, or organ transplantations [22, 24–26].

Nevertheless, there have also been reports of cases of chronic HBV infections that occurred after vaccine-induced protecting antibodies had disappeared [13]. Studies of the persistence of plasma-derived HB vaccine-induced immunity beyond 15 years after primary vaccination are limited. Uncertainties about whether the protection conferred by HB vaccination can truly span >15 years and the need for a booster dose during adolescence or adulthood exist. This article presents the results of a large-scale study of adolescents who received immunization during infancy.

SUBJECTS, MATERIALS, AND METHODS

Background. A nationwide mass vaccination program was launched in Taiwan in 1984 [5]. During the first 2 years, neonates of HBsAg-carrying mothers were vaccinated. The vaccination program was extended to all newborns in 1986. To ensure a high coverage rate, the government covered all expenses. Infants were vaccinated with plasma-derived HB vaccine (Hevac B; Pasteur-Mérieux), which contained 10 μ g of plasma-derived HBs protein, at birth and at 1, 2, and 12 months of age. Newborns of highly infectious carrier mothers, defined by the presence of HB e antigen or high titers of HBsAg by reverse passive hemagglutination (1:2560 or higher), were also given 100 IU of HB immunoglobulin within 24 h of birth. The plasma-derived vaccine was replaced by a recombinant yeast vaccine with a 3-dose regimen at birth and at 1 and 6 months of age in 1992 [5]. Between 1987 and 1990, preschool and elementary school children who had missed the neonatal vaccination were encouraged to receive the vaccine on a self-pay basis with subsequent complete reimbursement from the government. Catch-up vaccination has been in place since 1991, in which all primary school first graders have their vaccination records checked and are given appropriate doses of vaccine to complete their childhood vaccinations, including for HB. Neonatal vaccination coverage rates, defined as the percentage of children who received 3 or more doses of HB vaccine, were estimated to be 84% for children born between 1984 and 1986 and to be >97% for children born after 1986 [27]. When catch-up vaccinations were taken into account, >94% of preschool children and >98% of primary school students were covered by at least 3 doses of HB vaccine [28].

Study population and design. Students of 3 high schools in the city of Taipei were approached for the present study in 2004. All of the students were born between 1984 and 1989 and were 15–21 years of age. Students who were acutely ill, were immu-

nocompromized, had renal insufficiency, or were not born in Taiwan were excluded from the study. The study was conducted in 2 stages; first, after informed consent was obtained from both the students and their parents, HBV markers, including HBsAg, anti-HBs, and anti-HBc, were checked for all participants. Then, for those born after 1986 (15–18 years of age) and negative for HBsAg, anti-HBs, and anti-HBc, an HB booster vaccination was offered as a stage 2 study. For those who agreed to participate, a booster dose of HB vaccine was given after additional consent was obtained. Serum levels of anti-HBs were checked again 4 weeks after the HB booster. For a randomly sampled subgroup of the boosted cohort, an enzyme-linked immunospot (ELISPOT) assay was used to determine the frequency of HBsAg-specific cytokine-producing T cells before and after receipt of the HB vaccine booster injection.

The students were grouped into 2 cohorts on the basis of the national vaccination programs during the years when they were born. The pre-1986 cohort had received neonatal plasma-derived HB vaccine if their mothers were HBsAg carriers. The post-1986 cohort has been vaccinated regardless of the HB markers of their mothers.

A vaccination recording system was maintained by the Center for Diseases Control (CDC), Taiwan. All vaccine providers in Taiwan were required to submit information on vaccines and recipients after they administered routine childhood vaccinations. Records of HB vaccinations for subjects with a HB vaccine booster were retrieved from the CDC Taiwan for analysis.

HB seromarkers. HBsAg, anti-HBs, and anti-HBc concentrations were determined by enzyme immunoassays (Abbott Laboratories). Anti-HBs concentrations >10 mIU/mL were defined as protective. Subjects who were positive for HBsAg were assumed to be HB carriers. Concentrations between 10 and 100 mIU/mL were considered to be low [22]. The lower and upper limits of anti-HBs detection was 0.1 and 1000 mIU/mL, respectively, by this method. In calculation of geometric mean concentrations (GMCs), a titer of 0.1 mIU/mL was used for those <0.1 mIU/mL and of 1000 mIU/mL for those >1000 mIU/mL.

HBsAg-specific cytokine production by ELISPOT assay. Peripheral blood mononuclear cells (PBMCs) were isolated from 10–20-mL heparinized blood samples by density gradient centrifugation and cultured in RPMI 1640 medium plus 5% human serum. ELISPOT assays were done on freshly isolated PBMCs to measure the secretion of representative cytokines—interferon (IFN)- γ and interleukin (IL)-5, respectively—of the Th1 and Th2 subsets of CD4 cells. The BD ELISPOT human IFN- γ and IL-5 ELISPOT Sets (BD Biosciences) were used in accordance with the manufacturer's instructions. All assays were performed in duplicate. Briefly, 96-well MultiScreen HTS ELISPOT plates (Millipore) were coated with the anti-IFN- γ or anti-IL-5 capture antibodies (5 μ g/mL) and then blocked with RPMI-10% fetal bovine serum (FBS). Then, 100 μ L of phytohemagglutinin (PHA-P; 10 μ g/mL; Sigma), bovine serum albumin (BSA; 20 μ g/mL; Sigma), or recombinant HBsAg (20 μ g/

Table 1. Distribution of hepatitis B (HB) surface antigen (HBsAg), HB core antibody (anti-HBc), and HB surface antibody (anti-HBs) among 6156 high school students 15–21 years old in Taiwan in 2004–2005, according to the time of neonatal immunization with HB vaccine.

Birth cohort	Age at study entry, years	Subjects tested, no.	Subjects, no. (%), positive for		
			HBsAg	Anti-HBc	Anti-HBs
Pre-1986	18–21	175	11 (6.3)	20 (11.4)	109 (62.3)
Post-1986	15–17	5981	96 (1.6)	247 (4.1)	2215 (37.0)
Total	15–21	6156	107 (1.7)	267 (4.3)	2324 (37.8)

NOTE. The differences in the frequency of positive HBsAg, anti-HBc, and anti-HBs responses between the pre- and post-1986 cohorts were significant ($P < .001$ for all).

mL; SmithKline Beecham) was added to the appropriate wells. Cell suspensions (100 μ L in RPMI–5% FBS) of various densities (2.5×10^5 , 5×10^5 , or 1×10^6 cells) were added to each well and incubated for 40–42 h at 37°C. After washing, the cells were incubated with detecting antibodies (100 μ L; 2 μ g/mL in PBS–10% FBS) for 2 h at room temperature. Streptavidin–horseradish peroxidase in PBS–10% FBS (1:100) were added and incubated for 1 h at room temperature. After being washed with PBS–0.05% Tween-20 and PBS, cells were prepared to develop spots by adding 100 μ L 3-amino-9-ethylcarbazole (AEC; BD Biosciences) substrate to each well and incubated for 30 min at room temperature. The reaction was stopped by washing the wells with ddH₂O. The spots were analyzed by using Immunospot software (BD Biosciences). Mean numbers of cytokine-producing cells and spot-forming cells (SFCs) were calculated from duplicated assays. HBsAg-specific SFCs were calculated by subtracting background SFCs induced by BSA from the total SFCs induced by HBsAg.

HB vaccine booster. A booster dose of 20 μ g of the recombinant HB vaccine (Engerix-B; SmithKline Beecham) was given to a subset of 15–17-year-old students who were negative for HBsAg, anti-HBs, and anti-HBc. These students were born after 1986. Anti-HBs was assayed 4 weeks later. ELISPOT assays for cytokine production were performed for a subgroup of 92 subjects before and 4 weeks after the booster.

Statistical analysis. Data were analyzed using SPSS for Windows (version 8.0.0; SPSS). Differences in frequency between groups were examined by the χ^2 test. Pearson correlation coefficients (r) were calculated for determining correlations between HBsAg-specific cytokine-secreting SFCs/ 1×10^6 PBMCs by the ELISPOT assay and anti-HBs titers. The statistical significance of r was tested using a 2-tailed t test.

Ethics approval. The Human Experiment Committee of the National Taiwan University Hospital approved this study.

RESULTS

HB seromarkers and booster response. The study subjects of stage 1 consisted of 6156 (3684 male and 2472 female) high

school students, the majority of whom (5981/6156) were born after 1986. HB seromarkers, according to years of immunization, are shown in table 1. HBV infections continued to happen. Results for anti-HBc were positive in 11.4% of the pre-1986 cohort and in 4.1% in the post-1986 cohort ($P < .001$, χ^2 test). HB vaccination successfully prevented carrier status; results for HBsAg were positive in 6.3% of the pre-1986 cohort and in 1.6% of the post-1986 cohort ($P < .001$, χ^2 test). Anti-HBs was below the protective level in 63.0% of the students born after 1986, the year when universal HB vaccination was instituted. Evidence of natural HBV infections (positive for anti-HBc) was found in 4.1% of the post-1986 cohort ($P < .001$, χ^2 test).

For the subjects who were born after 1986 and were negative for HBsAg, anti-HBc, and anti-HBs, a stage 2 study using an HB vaccine booster was conducted. A total of 872 students gave their consent and participated in this study. The distribution of pre- and postbooster anti-HBs titers are shown in table 2. Twenty-nine percent of the subjects failed to generate protective levels of anti-HBs despite receiving a booster dose of HB vaccine given 15–18 years after the neonatal immunization. Furthermore, 26.0% had a low titer (anti-HBs titer of 10 to 100 mIU/mL; GMC, 38 mIU/mL), and 44.7% showed a robust antibody response (anti-HBs titer of ≥ 100 mIU/mL; GMC, 426.6 mIU/mL) to the booster. When the percentages of the subjects who failed to respond to a booster at different ages were compared, there was no significant difference ($P = .7$, χ^2 test) in subjects ≥ 16

Table 2. Distribution of hepatitis B (HB) surface antibody (anti-HBs) titers before and after HB booster vaccination, 15 years or more after receiving a neonatal vaccination.

Anti-HBs titer	Before booster		After booster	
	Subjects, no. (%)	GMC	Subjects, no. (%)	GMC
<10 mIU/mL	872 (100)	0.75	255 (29.2)	0.48
10–100 mIU/mL	0	...	227 (26.0)	38.0
>100 mIU/mL	0	...	390 (44.7)	426.6

NOTE. GMC, geometric mean concentration.

Table 3. Antibody response after a booster dose of hepatitis B (HB) vaccine, according to HB vaccination during infancy.

HB vaccination record	Anti-HBs		Total
	Positive	Negative	
Record of 4 doses	393 (71.3)	158 (28.7)	551 (100)
Record of <4 doses	165 (72.1)	64 (27.9)	229 (100)
No record available	62 (67.4)	30 (32.6)	92 (100)

NOTE. Data are no. (%) of subjects. No difference in the proportion of subjects with positive HB surface antibody (anti-HBs) responses was found between subjects with a record of 4 doses and the following groups: (1) subjects with a record of <4 doses ($P = .84$, χ^2 test) and (2) subjects with a record of <4 doses plus subjects without any HB vaccination records ($P = .85$, χ^2 test).

years old (28.9% [161/581]) and in subjects <16 years (29.7% [79/273]).

Vaccination history. Vaccination records of 780 (89.4%) of the 872 subjects who received a booster dose of HB vaccine were available for analysis. Of these 780 subjects, 686 (87.9%) had records of 3 or more doses of HB vaccine, and 551 (70.6%) had records of 4 doses of HB vaccine. Of the study subjects who were documented to have received 4 doses of vaccine, 28.7% (158/551) did not gain protective anti-HBs after a booster dose (table 3). This proportion (28.7%) was very close to that for the entire study population (29.2%) (table 2) and was used to calculate the proportion of the population that would have failed to respond to a single booster.

When the proportions of subjects who were positive for anti-HBs were compared between those with records of 4 doses and those with records of <4 doses, no difference was found ($P = .84$, χ^2 test) (table 3). Similarly, a comparison between subjects with HB vaccination records of 4 doses and the rest (including those with records of <4 doses and those without a record) revealed no statistically significant difference ($P = .85$, χ^2 test) (table 3).

HBsAg-specific cytokine production. A subgroup of 92 high school students (15–18 years old; 50 male and 42 female) who were seronegative for HBsAg, anti-HBs, and anti-HBc were tested for the ability of their PBMCs to produce IFN- γ or IL-5 in response to HBsAg. All of them were born after 1986, and all had received 4 doses of HB vaccine during infancy.

The distribution of pre- and post-booster HBsAg-specific cytokine-producing SFCs is shown in table 4. Positive controls using PBMCs from anti-HBs-positive volunteer donors showed hundreds of spots for IFN- γ secretion and from several to 50 spots for IL-5 secretion after HBsAg stimulation, whereas PBMCs from the study subjects typically showed very few spots (figure 1). Very few study subjects were found to have ≥ 1 HBsAg-specific IFN- γ - (17.4%) or IL-5-producing (5.4%) SFCs/ 1×10^6 PBMCs before a booster. Furthermore, no subjects had >5 SFCs/ 1×10^6 PBMCs for either IFN- γ or IL-5 before receiving the booster. Approximately two-thirds (65.2% [60/92]) of the subjects had <1 SFC/ 1×10^6 PBMCs for both HBsAg-specific IFN- γ - and IL-5-producing SFCs before a booster. After the booster vaccination, the number of spots for IFN- γ and IL-5 increased. Nevertheless, 68.5% (63/92) and 44.6% (41/92) of subjects still had <1 SFC/ 1×10^6 PBMCs for IFN- γ and IL-5, respectively. Twenty-five subjects (27.2%) still had <1 SFC/ 1×10^6 PBMCs for both IFN- γ and IL-5 after the booster.

The HBsAg-specific IFN- γ - and IL-5-secreting SFCs/ 1×10^6 PBMCs before and after the booster according to post-booster anti-HBs titers are shown in figure 2. There was no correlation between the prebooster SFC numbers and postbooster anti-HBs titers ($r = 0.039$ and $r = 0.105$ for IFN- γ and IL-5, respectively). However, after the booster, there was a positive correlation between postbooster anti-HBs titers and postbooster cytokine-responding cells ($r = 0.463$ and $r = 0.534$ for IFN- γ and IL-5, respectively).

Estimation of the proportion of the population that would have failed to respond to a single booster. Overall, 63.0% of

Table 4. Distribution of hepatitis B (HB) surface antigen (HBsAg)-specific interferon (IFN)- γ - and interleukin (IL)-5-secreting spot-forming cell (SFC) nos. before and after a booster dose of HB vaccine in 92 high school students who had negative test results for HBsAg, HB surface antibody, and HB core antibody, 15 years or more after receiving a neonatal vaccination.

Response category	IFN- γ		IL-5	
	Before booster	After booster	Before booster	After booster
<1 SFCs/ 10^6 PBMCs	76 (82.6)	63 (68.5)	87 (94.6)	41 (44.6)
>1–5 SFCs/ 10^6 PBMCs	16 (17.4)	13 (14.1)	5 (5.4)	31 (33.7)
>5–10 SFCs/ 10^6 PBMCs	0	9 (9.8)	0	10 (10.9)
>10–100 SFCs/ 10^6 PBMCs	0	5 (5.4)	0	7 (7.6)
>100 SFCs/ 10^6 PBMCs	0	2 (2.2)	0	3 (3.3)

NOTE. Data are no. (%) of subjects.

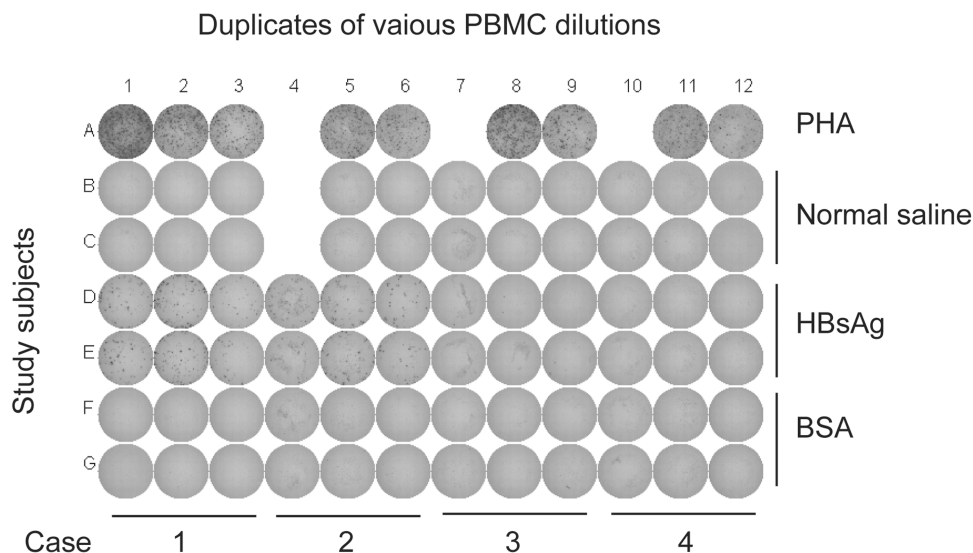


Figure 1. Representative image of the enzyme-linked immunospot assay results for hepatitis B surface antigen (HBsAg)-specific interleukin (IL)-5-secreting peripheral blood mononuclear cells (PBMCs). Depending on the nos. of PBMCs collected, 2 or 3 densities (2.5×10^5 , 5×10^5 , or 1×10^6) of PBMCs from the study subjects were cultured in rows A–G of 3 consecutive columns. Phytohemagglutinin (PHA) or recombinant HBsAg was then used to induce IL-5 secretion from cultured PBMCs. Normal saline and bovine serum albumin (BSA) were used as negative controls. All samples were assayed in duplicate, except those for PHA. Many and a few spots indicating IL-5 secretion were visible in wells stimulated with PHA and HBsAg, respectively. The spots were analyzed by computer software, as described in Methods.

the post-1986 cohort had no protective anti-HBs (table 1). Of these, 29.2% did not respond to a booster (table 2). The percentage was slightly lower (28.7%) for those with documented complete (4 doses) neonatal HB vaccination during infancy (table 3). Being prudent and conservative, we used the response rate of 28.7% for calculations. Thus, 18.1% ($63.0\% \times 28.7\%$) of the entire post-1986 cohort did not respond to a booster dose of HB vaccine.

Because the primary response rate of the current cohort was unknown, we assumed that 8% of the entire study cohort did not respond to primary vaccinations on the basis of an earlier report from Taiwan that the response rate was 92% [3]. They were not expected to have a robust boosted response after a booster dose. Because this 8% was mostly in the nonresponding group, at least 10.1% ($18.1\% - 8\%$) of the population have lost their HB vaccine-conferred immunity.

DISCUSSION

In the present large-scale study, the universal HB vaccination program undertaken in Taiwan has once again been proven successful. During the prevaccine era, the HBsAg carrier rates were $\sim 15\%$ – 20% [29]. In the present study, the partially vaccinated cohorts born from July 1984 through June 1986 had an HBsAg carrier rate of 6.3%, and the rate further dropped to 1.6% in the cohort born after July 1986, when vaccination was extended to all newborns regardless of maternal carrier status.

Several studies have demonstrated an anamnestic antibody response to booster doses in seronegative individuals for as long as 10–15 years after neonatal immunization [21, 23, 24]. We have found that a booster dose given at 10 years of age induced an adequate antibody response in children; although 33% of the children had anti-HBs levels < 10 mIU/mL, all became anti-HBs positive after the booster vaccination, with GMTs rising from ~ 50 mIU/mL to > 4000 mIU/mL [12]. It was therefore a great concern that 29.2% of adolescents seronegative for anti-HBs failed to mount any response to a booster dose and that 26.0% could elicit only a low-titer response in this study.

To understand the real proportion of adolescents who may have lost booster ability and immune memory to HBV, 2 factors must be considered. First, although plasma-derived HB vaccines have been shown to be highly effective, a small proportion of vaccinees would not elicit a protective immune response [1, 30]. Generally, 5%–10% of vaccinees had no response or a poor response to HB vaccines [31]. One earlier large-scale study in Taiwan showed that 8% of vaccinated children who were born during the same period and were from similar geographic areas were negative for all HB markers around the age of 2 years [3]. Accordingly, 8% should be a reasonable estimation of the proportion of our study subjects who had no response to primary HB vaccine.

The second factor was the HB vaccine coverage rate among our study subjects. An earlier epidemiological study has shown that the HB vaccine coverage rate was 97% in Taipei [27], where the current study were performed. The proportion of study sub-

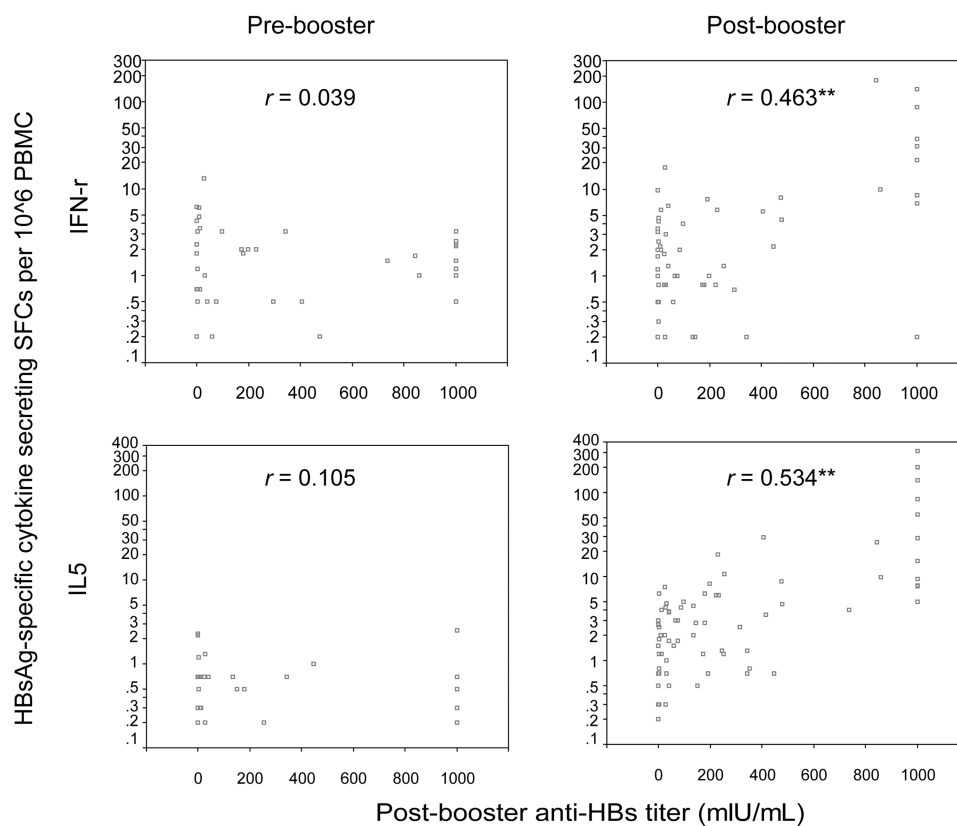


Figure 2. Hepatitis B surface antigen (HBsAg)-specific interferon (IFN)- γ (upper panels) and interleukin (IL)-5 (lower panels) spot-forming cells (SFCs) per 1×10^6 peripheral blood mononuclear cells (PBMCs), before (left panels) and after (right panels) a hepatitis B vaccine booster in relation to postbooster titers of HB surface antibody (anti-HBs). Pearson correlation coefficients (r) for determining the correlations between the SFC nos. and anti-HBs titers are shown. Two asterisks (**) indicate a significant correlation at the 0.01 level (2-tailed). The Y-axis scale is logarithmic.

jects who had received 3 or more doses of HB vaccine was lower (87.9%). This discrepancy might be have resulted from under-registration in the CDC Taiwan database, since the booster response was not different between those with complete vaccination records and those without (table 3). To ensure that the number of vaccination doses did not become a confounding factor, we made our calculations on the basis of subjects with a record of 4 doses. Still, the rate of nonresponse to a booster remained high (28.7%) (table 3). Therefore, immunity conferred by neonatal HB vaccination disappeared in 10.1% of the general population 15–18 years after immunization. Our data provide a concern about HB vaccine-induced immunity 15 years after primary immunization.

Another noteworthy finding was the 26.0% of subjects who mounted a low-titer anti-HBs response (between 10 and 100 mIU/mL). They comprised of 16.4% ($63.0\% \times 26.0\%$) of the total population. Usually, a robust rise in antibody level is readily seen when subjects who were previously vaccinated get boosters. In the above-mentioned study done 10 years after the neonatal vaccination, an average of a 900-fold (range, 0.1–20,805-fold) increase was noted after 1 booster dose of the HB vaccine [12]. In the present study, those subjects with modest antibody re-

sponses (10–100 mIU/mL) were likely to have lost immune memory and manifest a primary response to 1 dose of HB vaccine. Hence, 10.1% is a conservative estimate of the loss of immune memory to HB antigens. The real proportion of adolescents aged 15–18 years and becoming immunologically naive to HB vaccine might be as high as 26.5% (10.1% + 16.4%).

Our estimations, based on serology, are supported by the observation that most of the seronegative students also exhibited a negative or poor T cell IFN- γ or IL-5 response after an HB vaccine booster. ELISPOT assays have been used to evaluate the memory T cell responses elicited by HB vaccines [32, 33]. A direct correlation between the serological and T cell responses has been shown. One recent study by Bauer et al. showed that HBsAg-specific memory T cells could be detected by ELISPOT assay and were enhanced by revaccination in all subjects who had responded to HB vaccination 4–8 years earlier and subsequently lost their anti-HBs [32]. In the present study, we found a positive correlation between postbooster ELISPOT assay results and anti-HBs titers, suggesting that the assay is reliable in detecting anti-HBs immunity. That approximately one quarter (27.2%) of the study subjects had no detectable HBsAg-specific memory T cells by ELISPOT assay provided evidence that these

subjects truly had no immunity against HBV infection. The optimal timing for assaying T cell memory has remained elusive. In a study conducted by Bauer et al., T cell responses were detectable by ELISPOT assay for 10 days and declined thereafter [32]. In this regard, we might have underestimated the response of cellular immunity against the HBV antigen. However, in Bauer et al.'s study, effector T cell responses were indeed detectable by IFN- γ ELISPOT assay 28 days after a booster.

In conclusion, the present large-scale study provides evidence that an anamnestic anti-HBs response was absent in 10.1% of 15–18-year-old individuals in a country that used to have high endemicity. This was coupled with a lack of T cell cytokine responses. Our findings suggest that the protective effects provided by neonatal HB immunization with plasma-derived HB vaccines starts to diminish during adolescence. The clinical significance of having such a large fraction of adolescents lose their immunity against HB remains speculative. A recent large-scale epidemiologic study has confirmed the efficacy of neonatal HB vaccination for up to 20 years [9]. However, for individuals who have become naive to HBV infection, the real threat may emerge when the vaccinated subjects begin to engage in high-risk behaviors for HBV transmission in areas of endemicity. One may argue that the chance of becoming a chronic carrier of HBV is small during adolescence or adulthood [34]. Boosters for certain high-risk groups or for individuals living in the areas of high endemicity may be a reasonable alternative. Continuous surveillance of HBV infection in adolescents and young adults will be needed to understand the clinical significance of this decay of immune memory.

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