



Original Article

Complementary Presence of HBV Humoral and T-cell Response Provides Protective Immunity after Neonatal Immunization

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Abstract

Background and Aims: Hepatitis B vaccination is the most cost effective way to prevent hepatitis B virus (HBV) infection. Hepatitis B vaccine (HepB) efficacy is usually assessed by anti-hepatitis B surface antigen (HBsAg) level, but there are few reports of humoral and cellular immune responses to HepB in children after neonatal vaccination. **Methods:** A group of 100 children with a history of primary hepatitis B immunization were included in this study to evaluate the efficacy of HepB. Blood samples were obtained from 80 children before, and 41 children after, a single HepB booster dose. Children with low anti-HBsAg (HBs) titers of <100 mIU/mL received a booster dose after giving their informed consent. Anti-HBsAg, T-cell response and percentage of B-cell subsets were assayed before and after the booster. **Results:** Of the 80 children, 81.36% had positive T cell and anti-HBsAg responses at baseline. After the booster dose, the anti-HBsAg titer ($p < 0.0001$), positive HBsAg-specific T-cell response ($p = 0.0036$), and spot-forming cells ($p = 0.0003$) increased significantly. Compared with pre-existing anti-HBsAg titer <10 mIU/mL, the anti-HBsAg ($p = 0.0005$) and HBsAg-specific T-cell responses ($p < 0.0001$) increased significantly in preexisting anti-HBsAg titer between 10 and 100 mIU/mL group. Change of the HBV-specific humoral response was the reverse of the T-cell response with age. Peripheral blood lymphocytes, B cells, and subset frequency decreased. **Conclusions:** HBV immunization protection persisted at least 13 years after primary immunization because of the complementary presence of HBV-specific humoral antibodies and a T-cell immune response. One dose of a HepB booster induced protective anti-HBsAg and promoted

an HBsAg-specific T-cell response. In HBV endemic regions, a HepB booster is recommended to children without anti-HBsAg because of effectiveness in HBV prevention.

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Introduction

Hepatitis B vaccination is the most cost effective way to prevent acute or chronic HBV infection and reduce complications of hepatitis B infection. The Chinese government has adopted routine hepatitis B vaccination, and importation of a yeast-derived hepatitis B vaccine (HepB) began in the late 1980s. Routine immunization began in 1992, and HepB was integrated into the Expanded Program of Immunization in 2002.¹ Following the implementation of routine HBV vaccination China has successfully changed from a highly endemic to a moderately endemic country. Serosurveys found that HBsAg seropositivity decreased by 52%, from 9.8% to 4.7% in the general population; by 97%, from 9.7% to 0.3% in children <5 years of age; and by 92.4%, from 10.5% to 0.8% in children <15 years of age from 1992 to 2014.^{2–4} It is estimated that 80 million acute HBV infections and 20 million chronic HBV infections have been prevented since 1992.⁵

The need for a HepB booster in children after neonatal immunization is controversial. Many studies have not identified a need of booster immunization in healthy children. The protection afforded by primary HepB immunization can last 30 years; only 0.7% of vaccinees had HBV breakthrough infections in the 5–20 years after neonatal HBV vaccination.^{6–8} Immune memory for HepB persists in children with waning or undetectable anti-HBsAg concentrations,⁹ but the loss of HepB immune memory has been reported in 25–50% of vaccinees after 15 years of age,^{10,11} and 10.1% had no immune response to a HepB booster after the initial vaccination.¹² A HepB booster has been recommended for at-risk youths who with a history

Keywords: Hepatitis B virus; Hepatitis B vaccine; Booster; Children.

Abbreviations: Anti-HBc, hepatitis B core antibody; Anti-HBsAg, hepatitis B surface antibody; CMIA, chemiluminescent microparticle immunoassay; ELISpot, enzyme-linked immunospot; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HepB, hepatitis B vaccine; IFN- γ , interferon gamma; PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell.

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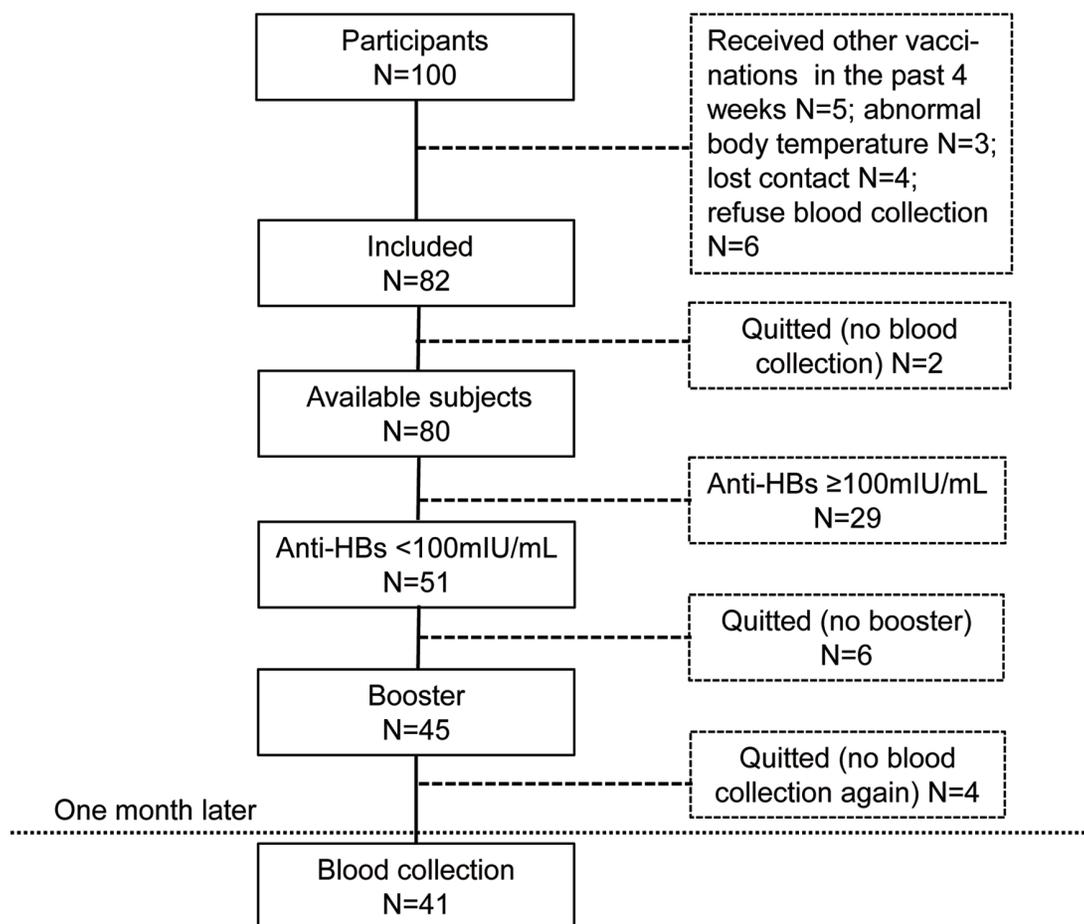


Fig. 1. Flowchart of inclusion and withdrawal of study participants. Blood samples were obtained from 80 eligible children before a dose of HepB booster and 41 children post-booster. N, number; Anti-HBsAg, antibodies to hepatitis B surface antigen.

of primary immunization. We previously reported that anti-HBsAg declined with age in children from 93.7% at 1 year of age to 42.3% at 9 years of age.^{13,14} Whether a protective immune response is elicited in children without anti-HBsAg is not known, and the need for booster doses has not been resolved.

This study investigated the protective humoral and cellular immunity responses following primary immunization and a HepB booster for children who had lost protective antibodies. The efficacy of a HepB booster in children with low baseline anti-HBsAg levels between 10 and 100 mIU/mL was evaluated.

Methods

Design and trial participants

This prospective single-center cohort study was performed at Clinical Research Center of Children’s Hospital of Chongqing Medical University, a general children hospital with patients from all over the country. The study was approved by the institutional ethics review committee of and registered at ClinicalTrials.gov (NCT03867643). All children and their legal guardians provided written informed consent. All procedures were conducted following the ethical principles of the Declaration of Helsinki.

Children born after January 1, 2005 in Chongqing, China

who completed primary vaccination with a series of three doses of HepB containing 10 µg HBsAg each beginning at birth, and not receiving a booster dose were eligible for inclusion. Children with a history of allergy or adverse reaction to the vaccine, immunosuppressive treatment or immunodeficiency, any vaccination in the previous 4 weeks, with an acute disease or anti-infective therapy in the past 4 weeks, fever (axillary temperature $\geq 38^{\circ}\text{C}$) in the previous week, history of blood transfusion, history of infectious diseases (e.g., hepatitis, AIDS, syphilis, gonorrhea, etc.), family history of HBV in three generations of lineal relatives, or abnormalities on physical examination were excluded. Figure 1 is a flowchart of participant selection. A group of 100 children aged 1–13-year were included via the hospital’s official website. Blood samples were obtained from 80 children before the HepB booster, which contained 20 µg HBsAg (Huabei Pharmaceutical Co., Hebei, China), and from 41 children 1 month after the booster.

HBV seromarkers

Blood samples were collected for determination of HBV seromarkers by chemiluminescent microparticle immunoassay (CMIA) with the Architect system (Abbott Laboratories). HBsAg seropositivity was >0.05 IU/mL and anti-HBsAg titers ≥ 10 mIU/mL were considered seroprotective. Sample cutoff values of anti-hepatitis B e antigen ≥ 1.0 and anti-hepatitis B core antigen of ≥ 1.0 were considered positive.

Table 1. Baseline characteristics of the 80 study participants

Variable	Anti-HBsAg <10 mIU/mL, n=21 (%)	10 ≤ anti-HBsAg <100 mIU/mL, n=30 (%)	Anti-HBsAg ≥100 mIU/mL, n=29 (%)	p-value ^a
Sex				
Boys	15 (71.43)	18 (60)	11 (37.93)	0.049*
Girls	6 (28.57)	12 (40)	18 (62.07)	
Age, years				
1–3	2 (9.52)	7 (23.33)	6 (20.69)	0.56
4–6	6 (28.57)	9 (30)	12 (41.38)	
7–9	8 (38.10)	6 (20)	5 (17.24)	
10–13	5 (23.81)	8 (26.67)	6 (20.69)	
Pregnancy				
Normal	17 (81)	30 (100)	27 (93.10)	0.039*
Premature delivery	4 (19)	0 (0)	2 (6.90)	
Birth weight				
Normal	17 (80.96)	27 (90)	26 (89.66)	0.22
Overweight	2 (9.52)	3 (10)	3 (10.34)	
Underweight	2 (9.52)	0 (0)	0 (0)	
History of allergies	0 (0)	0 (0)	3 (10.34)	
History of hepatitis infection	0 (0)	0 (0)	0 (0)	
History of blood transfusion	0 (0)	0 (0)	0 (0)	
History of surgery	0 (0)	0 (0)	0 (0)	
History of radiotherapy and chemotherapy	0 (0)	0 (0)	0 (0)	
History of HBV infection of parents or grandparents	3 (14.29)	8 (26.67)	10 (34.48)	0.28

^aPearson χ^2 or Fisher exact test. *Statistically significant.

Detection of interferon (IFN)- γ -secreting HBsAg-specific T cells

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation, and HBsAg-specific cytokine-secreting T cells were identified with a human IFN- γ ELISpot PLUS assay (Mabtech, Stockholm, Sweden). IFN- γ precoated 96-well plates were preincubated with Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Invitrogen, USA) for 30 minutes at room temperature before adding 5×10^5 PBMCs/well in 200 μ L RPMI 1640. PBMCs were stimulated with 10 μ g/ml of recombinant HBsAg (Bersee, Beijing, China). Wells containing PBMCs and RPMI 1640 with anti-CD3 mAb (Mabtech, Stockholm, Sweden) were positive controls and wells without any stimulant were negative controls. The culture plates were incubated for 48 h at 37°C in a 5% CO₂ atmosphere and were then read with an ELISpot reader (AID, Strassberg, Germany). A response two-fold greater than that of the negative control was considered positive.¹⁵

Assay of B lymphocyte subpopulations

The B-cell phenotypes isolated from peripheral blood samples were evaluated by flow cytometry (FACSCanto II; BD Biosciences, San Jose, Calif). The mAbs were from BD Biosciences, and the B lymphocyte subpopulations included aCD19-ApC, aCD24-pE, aCD27-BV450, aCD38-perCp-Cy5.5,

and aIgD-BV510. The data were analyzed by FACS Diva.

Statistical analysis

The data were analyzed and compared by SPSS version 20.0 (IBM Corp. Armonk, NY, USA); graphs were drawn by Graphpad prism (version 8.0). Continuous variables were compared with the Student *t*-test. Comparisons of categorical variables were performed by χ^2 or Fisher's exact tests. The Spearman rank correlation was used to evaluate the associations between ELISpot results and anti-HBsAg titers. *P*-values <0.05 were considered statistically significant.

Results

Participant baseline characteristics

The characteristics of the 80 available subjects are shown in Table 1 and the characteristics of the 51 participants with prebooster anti-HBsAg titers <100 mIU/mL are shown in Supplementary Table 1. All participants had received a three-dose primary neonatal HBV vaccination and were grouped by anti-HBsAg titer. Twenty-one had baseline titers of <10 mIU/mL, 30 had titers ≥ 10 and <100 mIU/mL, and 29 had titers ≥ 100 mIU/mL. Between-group comparisons of sex, age, weeks of pregnancy week, birth weight, and

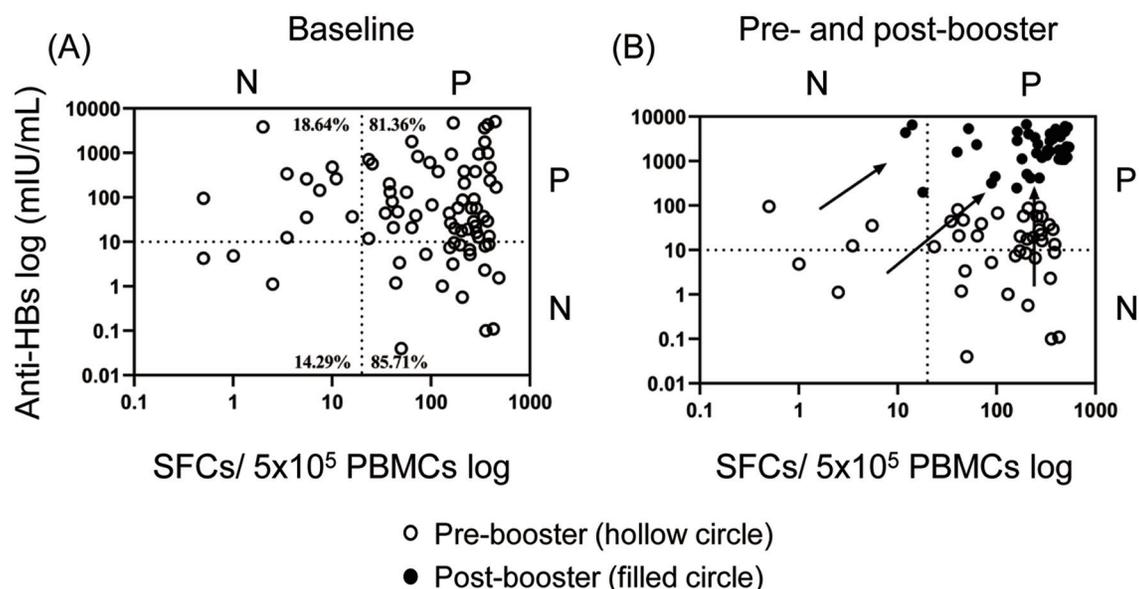


Fig. 2. Distribution of positive and negative humoral and cellular immunity pre- and post-booster. (A) Distribution of positive and negative anti-HBsAg and IFN- γ -secreting HBsAg-specific T cells at baseline ($n=80$). (B) Changes of anti-HBsAg titers and IFN- γ -secreting HBsAg-specific T cells prebooster (open circle) and post-booster (solid circle) ($n=41$). PBMCs, peripheral blood mononuclear cells; SFC, spot-forming cell; P, positive; N, negative; pre-, prebooster; post-, post-booster.

disease history among each group found that boys were at a lower titers than girls ($p=0.049$) and more preterm infants than normal term infants were anti-HBsAg negative at baseline ($p=0.039$).

HBsAg-specific T-cell responses are frequent in antibody-negative participants

The ELISpot assay results of HBsAg-specific T-cell responses in children without anti-HBsAg and the distribution of positive and negative humoral and cellular immunity is shown in Figure 2. Of the antibody-negative participants, 85.71% had positive HBsAg-specific T-cell responses, 18.64% of the antibody-positive subjects had negative responses, and 96.25% of the children had positive HBsAg-specific T cell or anti-HBsAg responses. Of 41 children given a booster dose, all antibody-negative subjects became positive and their HBsAg-specific T-cell responses were enhanced.

Children with high prebooster anti-HBsAg titers had high post-booster humoral responses

Pre- and post-booster anti-HBsAg titers are shown in Figure 3. The titers increased after booster administration to >100 mIU/mL in all children ($p<0.0001$) and to >1,000 mIU/mL in 56.25% those with prebooster titers of 0–10 mIU/mL and 100% of children with prebooster titers from 10–100 mIU/mL ($p=0.0005$). Children with high prebooster anti-HBsAg titers had higher humoral responses than those with low prebooster titers.

Post-booster IFN- γ -secreting HBsAg-specific T-cell response depended on the prebooster anti-HBsAg titer

The pre- and post-booster HBsAg-specific T-cell responses are shown in Figure 4. The ELISpot results indicated significant increases of the percentage ($p=0.0036$) and the

magnitude of response ($p=0.0003$) of spot-forming cells (SFCs) following booster administration. The magnitude of the response in IFN- γ -secreting HBsAg-specific T cells was not associated with the anti-HBsAg titer after neonatal immunization ($p=0.1140$). The post-booster T-cell response showed the same change trend as the humoral response, with a significant increase ($p=0.0004$) in the number of IFN- γ -secreting HBsAg-specific T cells. Compared with children with low anti-HBsAg prebooster titers (0–10 mIU/mL), those with pre-existing titers between 10 and 100 mIU/mL had significantly stronger HBsAg-specific T-cell responses to the booster vaccination. The intensity of T-cell immunoreactivity depended on the pre-existing anti-HBsAg titer.

Association between HepB humoral and T-cell response and age

The association between humoral and T-cell response and age is shown in Figure 5, which shows the changes of anti-HBsAg titers and HBV-specific T-cell response in children of different ages. Post-booster anti-HBsAg titers increased significantly in all four age groups of the 41 children who were vaccinated. The anti-HBsAg titers were higher in 1- to 3-year-old than in 10- to 13-year-old children ($p=0.031$) and pre- and post-booster ELISpot assay results were significantly different in 10- to 13-year-old children ($p=0.0172$). As shown in Figure 5C, differences in prebooster anti-HBsAg titers were the opposite of T-cell values in each age group. The percentage of protective antibodies initially decreased with age and then increased in children 1–13 years of age. The T-cell response increased initially and then decreased. After the booster dose (Fig. 5D) the percentage of protective antibody titers decreased in each age group from 1 to 13 years of age, but the positive T-cell response increased in each age group. The overall positive anti-HBsAg and T-cell response rates in each age group were similar. The results show that the change of the HBV-specific humoral response was associated differences in the T-cell response in the four age groups.

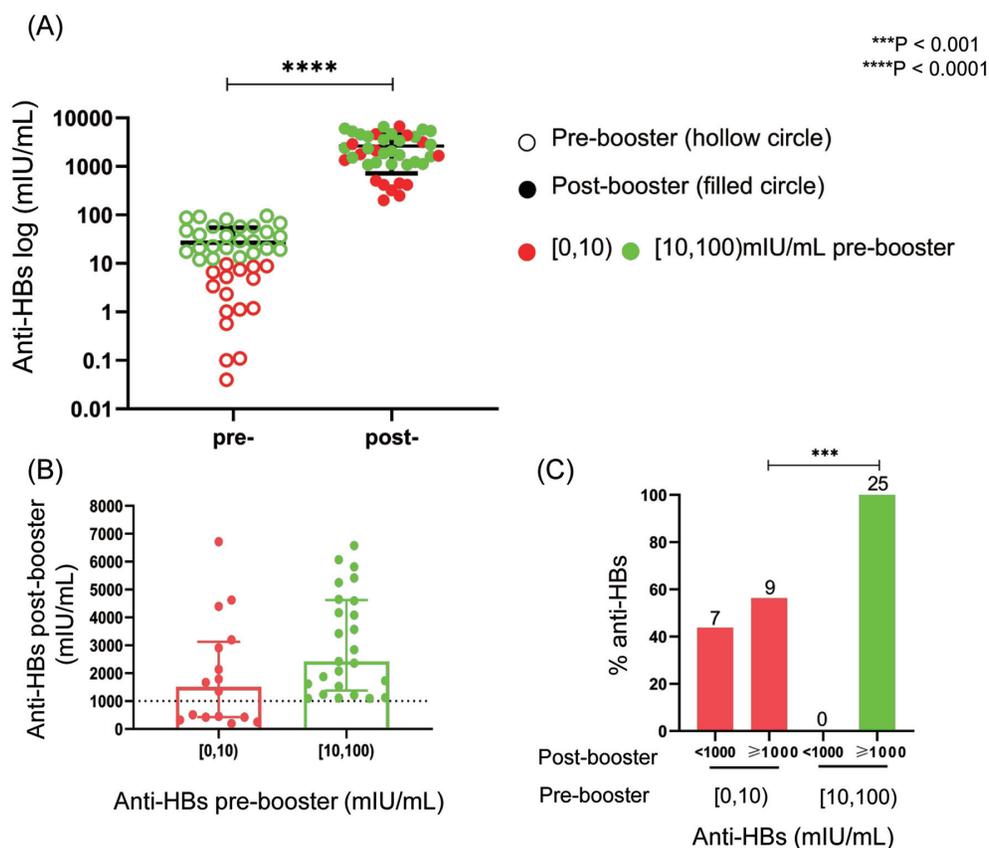


Fig. 3. Humoral response to HepB booster vaccination in children (n=41). Subjects were grouped by prebooster anti-HBsAg titer, 0–10 mIU/mL (red), 10–100 mIU/mL (green). (A) Prebooster (open circle) and post-booster (solid circle) Anti-HBsAg. (B) Post-booster Anti-HBsAg titer in children with different pre-existing anti-HBsAg. Boxes and whiskers are medians and interquartile range. (C) Percentage of children with post-booster anti-HBsAg of <1,000 mIU/mL and $\geq 1,000$ mIU/mL in children with pre-existing anti-HBsAg of 0–10 mIU/mL and 10–100 mIU/mL. Pre-, pre-booster; Post-, post-booster.

Changes of immune B-cell subsets after booster administration

The gating strategy for definition of the B-cell subsets is depicted in Figure 6A. Changes in the immune B-cell subsets before and after booster vaccination (Fig. 6) included a decrease in B-cell frequency in peripheral lymphocytes ($p=0.0002$), and antibody-secreting cells included plasmablasts. Changes in the numbers of CD19⁺ B-cells were similar in participants with low and with high baseline anti-HBsAg titers. One month after booster vaccination, class-switched and unswitched memory B-cell frequencies decreased significantly. In children with low pre-existing anti-HBsAg titers (0–10 mIU/mL), there were a significant rise in naïve B cells ($p=0.0387$) and DN B cells ($p=0.0134$). In children with high baseline anti-HBsAg titers (10–100 mIU/mL), the percentages of both naïve B cells ($p<0.0001$) and DN B cells ($p=0.0013$) increased.

Discussion

The Chinese Center for Disease Control and prevention (CDC) reported that three-dose HBV vaccination coverage before 1 year of age was 83–99.53% between 2001 and 2017.¹⁶ Our previous serosurvey found that 46.03–72.29% of children from 1 to 14 years of age were seroprotected, and that 3.33–25.79% of all age groups had anti-HBsAg titers of <10 mIU/mL,¹³ which was consistent with a CDC sur-

vey of HBV seroprevalence in various age groups in China.⁴

HepB is one of the safest available vaccines. It prevents HBV infection and reduces the occurrence of liver cancer.¹⁷ All the children in this study had completed the three-dose primary vaccination series that begins with a dose at birth. We analyzed the immune response to a HepB booster dose after completing neonatal immunization to determine whether children without detectable anti-HBsAg (i.e., titers <10 mIU/mL) were still protected and whether or not children without anti-HBsAg need a HepB booster vaccination.

This is the first study to show that protective immunity from neonatal immunization exists in children because of the complementary presence of HBV-specific humoral and T-cell immune responses. A detectable T-cell response to HBsAg was found in 85.71% of children with anti-HBsAg titers of <10 mIU/mL. The presence of HBsAg-specific INF- γ in children up to 13 years of age suggests that protection may be long lasting. A study by Wang *et al* reported that most anti-HBsAg negative vaccinees had positive HBsAg-specific immune-cell responses.¹⁸ Leuridan *et al* reported activation of immune cells in vaccinees based on cell proliferative response.¹⁹ Long lasting cellular immunity has also been shown by detection of secretion of cytokines by Th1 and Th2 lymphocytes after stimulation by HBsAg.⁷ The previous results confirm that T cell immunity persists regardless of anti-HBsAg, which is consistent with our results. HBsAg-specific T-cell responses initially increased and then a decrease with age, which was the reverse of changes in anti-HBsAg titers. In neonates, adaptive immune responses to pathogens are relatively weak and narrowly focused,

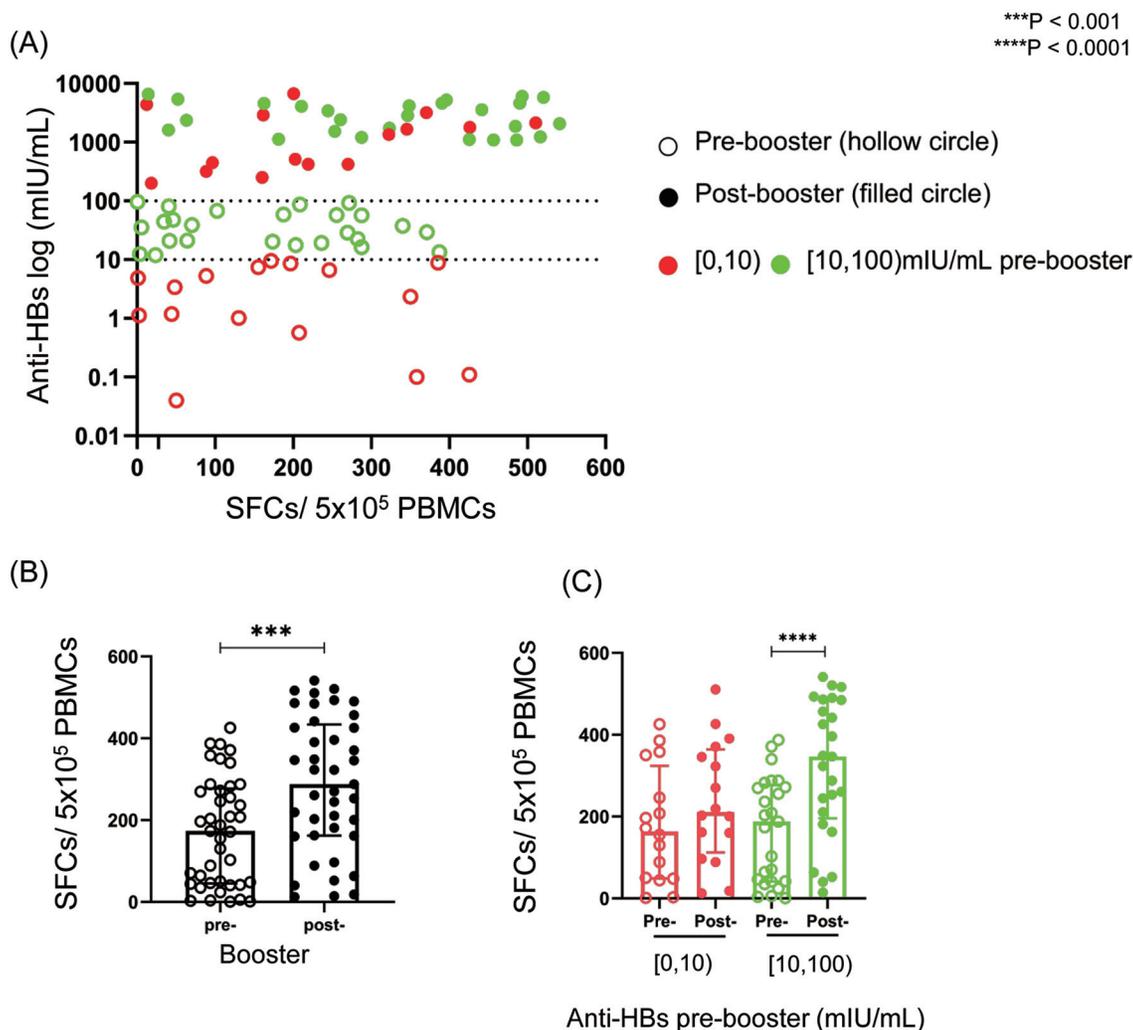


Fig. 4. T-cell response to HepB booster vaccination (n=41). Subjects were grouped by prebooster anti-HBsAg titer, 0–10 mIU/mL (red) or 10–100 mIU/mL (green). (A) Correlation of anti-HBsAg titer and IFN- γ -secreting HBsAg-specific T cells prebooster (open circle) and post-booster (solid circle). (B) IFN- γ -secreting HBsAg-specific T cells were significantly increased post- compared with prebooster ($p=0.0004$). (C) Post-booster HBsAg-specific T-cell responses were significantly stronger in children with higher pre-existing anti-HBsAg titers ($p<0.0001$). SFC, spot-forming cells; PBMCs, peripheral blood mononuclear cells; Pre-, pre-booster; Post-, post-booster.

causing T-cell hyporesponsiveness.²⁰ In younger children, HBV-specific T cells are lacking and fail to produce adequate amounts of IFN- γ , but the response gradually improves with age.²¹ Before and after HepB booster, the direction of change in anti-HBsAg titer in this study was opposite that of the HBsAg-specific T-cell response in each age group. In vaccine development, determining the balance between humoral and cellular responses is the key challenge.²² The complementary existence of anti-HBsAg and T-cell responses is important for the persistence of protection following vaccination. There is no need to worry about the decline in anti-HBsAg in populations. It is precisely because of the dynamic balance that screening for HBsAg-specific T-cell immunity is not recommended for the general population. Routine screening for anti-HBsAg in vaccinees is sufficient to evaluate the protection afforded by HepB.

One dose of HepB booster was effective in children without anti-HBsAg. All those given a HepB booster dose produced protective anti-HBsAg and an enhanced HBV-specific T-cell response 4 weeks after the vaccination. All children with anti-HBsAg <10mIU/ml produced anti-HBsAg with ti-

ters >100 mIU/mL, demonstrated an anamnestic response to the booster dose,²³ even when detectable anti-HBsAg were absent at the time of exposure. We found that humoral and T-cell responses to the HepB booster depended on the pre-existing anti-HBsAg titer. Only 56.25% of children with pre-booster anti-HBsAg <10mIU/ml had anti-HBsAg $\geq 1,000$ mIU/mL 4 weeks post-vaccination. Those with prebooster anti-HBsAg <10mIU/ml were less likely to produce high titers of anti-HBsAg compared with children who had anti-HBsAg titers from 10 to 100 mIU/ml. Equally, the intensity of the T-cell booster response also depended on the prebooster anti-HBsAg titer. After the booster, the numbers of IFN- γ -secreting HBsAg-specific T cells in children with prebooster anti-HBsAg titers of 10–100 mIU/ml were significantly increased compared with children with anti-HBsAg titers <10mIU/ml. That has been previously reported.²⁴ Establishment of immune memory by routine vaccination against HBV at birth is key for the effectiveness of the HepB booster and for long-term immunity.

Although immune memory for HepB is persistent in children, a booster is recommended for children without an-

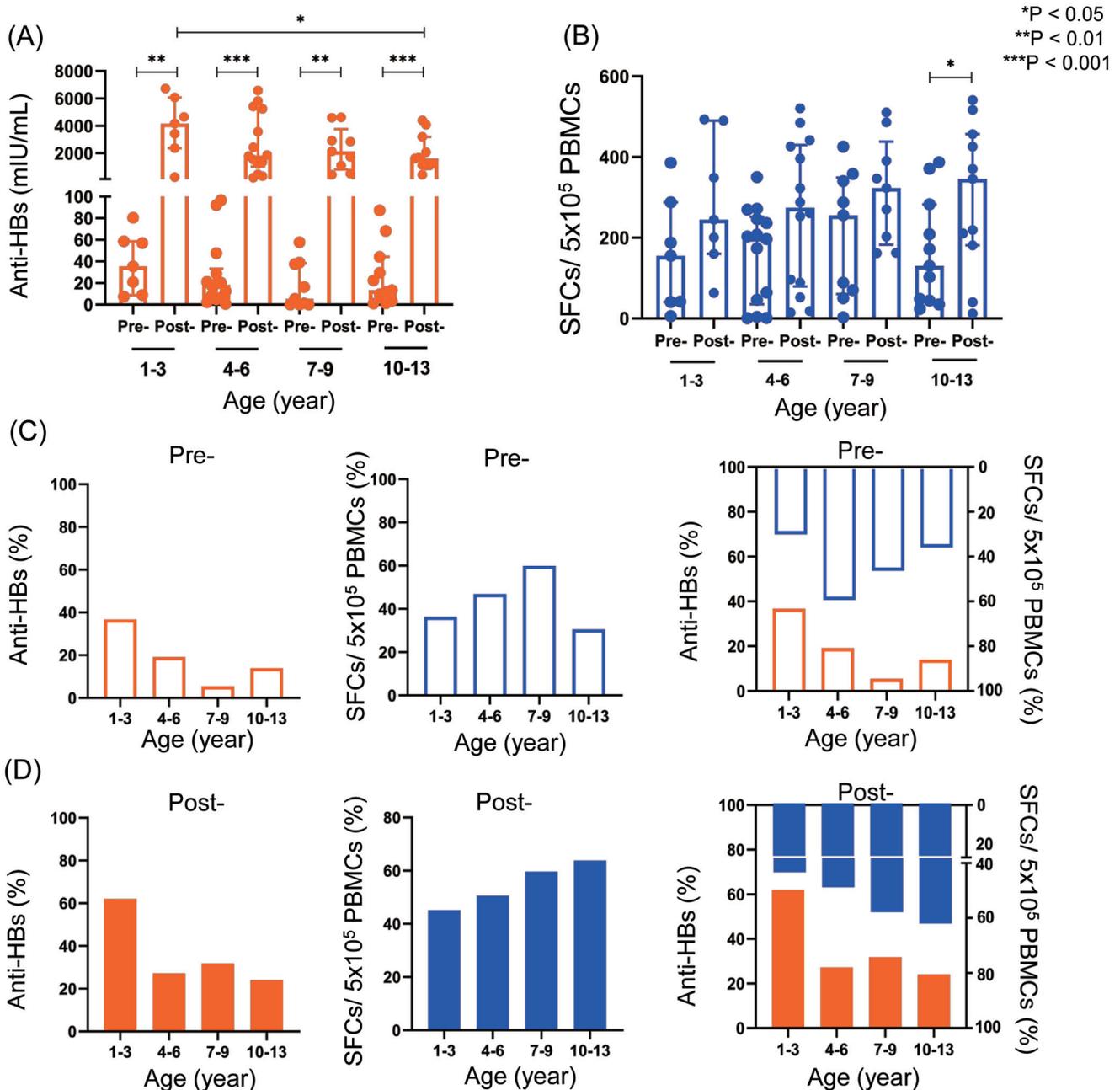


Fig. 5. Age-related humoral (orange) and T-cell responses (blue) before and after booster vaccination. Vaccinees ($n=41$) were stratified to groups 1–3, 4–6, 7–9, and 10–13 years of age. (A) Pre- and post-booster anti-HBsAg titers. (B) Pre- and post-booster IFN- γ -secreting HBsAg-specific T cells. (C, D) Age-related prebooster (open column) and post-booster (solid column) anti-HBsAg seropositivity and HBsAg-specific T-cell response. Y-axis: the median of each value divided by the maximum in children prebooster or post-booster. SFC, spot-forming cells; PBMCs, peripheral blood mononuclear cells; Pre-, pre-booster; Post-, post-booster.

ti-HBsAg in HBV endemic regions. The available evidence does not provide a compelling basis for recommending a booster dose of HepB.^{6,25} Moreover, chronic HBV infection is on the decrease after primary immunization even in children without detectable (<10 mIU/mL) anti-HBsAg.^{6,26} More attention should be paid to children over 10 years of age. According to our previous study, the prevalence of HBsAg and anti-HBc increased from 0.46% to 1.40% between 11 and 16 years of age compared with 5.69% to 7.8% between 1 and 10-years of age.¹⁴ That suggests that the risk of exposure to HBV is increased in children who are

older than 10 years of age. A HepB booster should be given at that age to reduce the risk of breakthrough infection. In this study, a significant number of vaccinees with low anti-HBsAg following neonatal vaccination had large increases of anti-HBsAg titer within 4 weeks of a single booster dose. HepB has been continuously improved since its launch in 1986. The safety of HepB has been confirmed and vaccination coverage in China has continuously improved.¹ Sero-surveys show that the prevalence of HBV has significantly decreased,^{2–4,27} and that the change is closely associated with hepatitis B vaccination. Some individuals with anti-

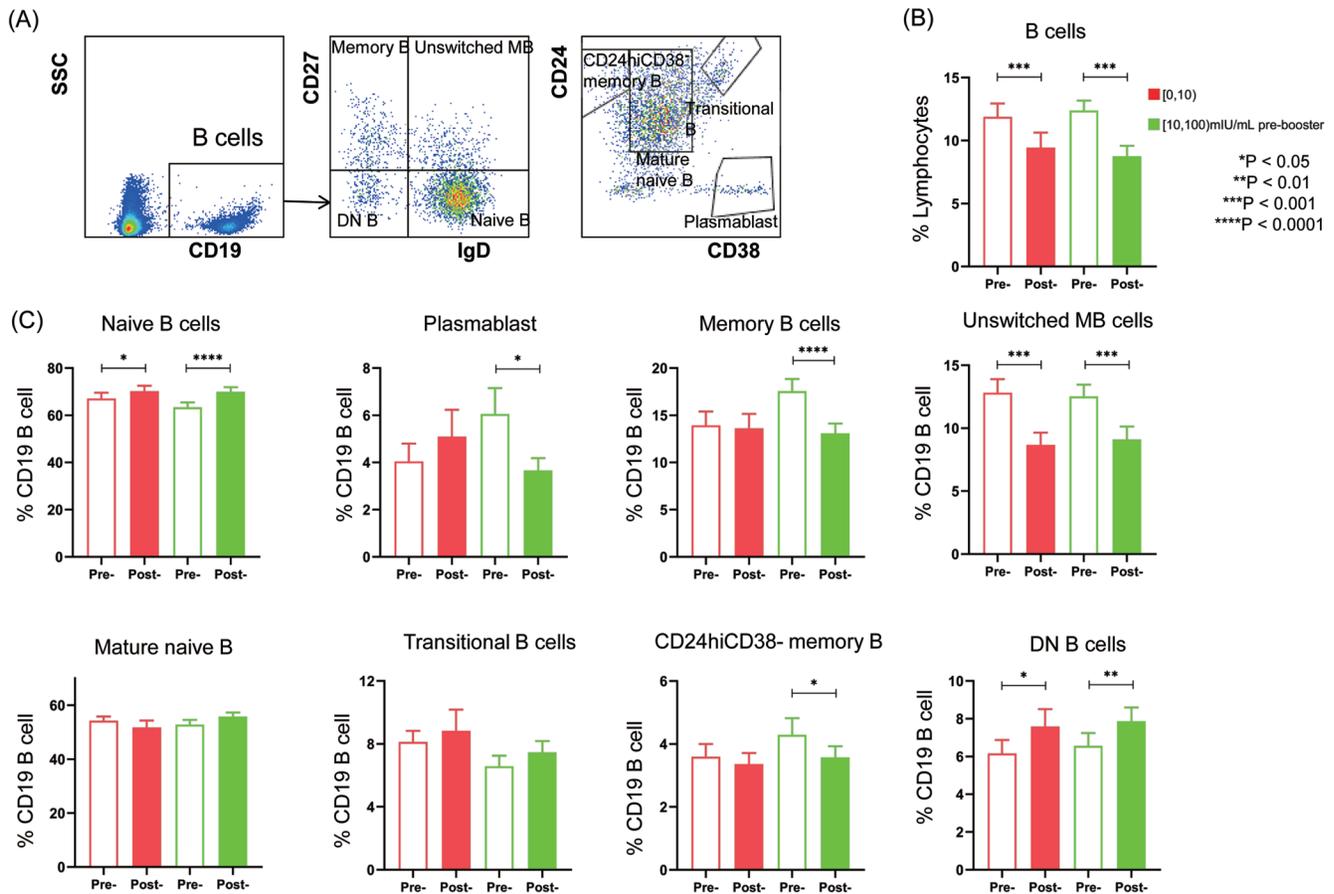


Fig. 6. Peripheral immune B-cell subsets in participants (n=41) before (open column) and 1 month after hepatitis B booster vaccination (solid column). Children were grouped by prebooster anti-HBsAg, 0–10 mIU/mL (red) and 10–100 mIU/mL (green). (A) Stepwise gating of B-cell for identification of memory B (CD19⁺ CD27⁺), CD24^{hi} CD38⁻ memory B (CD19⁺ CD24⁺⁺ CD38⁻), unswitched memory B (CD19⁺ CD27⁺ IgD⁺), double negative B (CD19⁺ CD27⁻ IgD⁻), mature naive B (CD24^{int} CD38^{int}); naive B (CD19⁺ CD27⁺ IgD⁺), transitional B (CD19⁺ CD24⁺⁺ CD38⁺⁺) cell subsets and plasmablasts (CD19⁺ CD24⁻ CD38⁺⁺). (B) Among peripheral lymphocytes, B-cell frequency decreased in two groups ($p = 0.0002$). (C) In children with low pre-existing anti-HBsAg titers, naive B cells ($p = 0.0387$) and DN B cells ($p = 0.0134$) increased and unswitched MB cells decreased ($p = 0.0005$). In children with high anti-HBsAg titers naive B cells ($p < 0.0001$) and DN B cells ($p = 0.0013$) increased in subjects, and plasmablasts ($p = 0.0456$), memory B cells ($p = 0.0001$), unswitched MB cells ($p = 0.0002$), and CD24^{hi}CD38⁻ memory B cells ($p = 0.0137$) decreased. DN B, double negative B cell; MB, memory B cell; Pre-, prebooster; Post-, post-booster. *significant difference.

HBsAg <10mIU/ml and at high risk of HBV exposure will require only one HepB booster to achieve seroprotective anti-HBsAg titers.

In addition to a T-cell response, B cells respond to HepB by generating a protective anti-HBsAg titer. Our results found that total B cells, including some antibody-secreting cells, significantly decreased after booster vaccination. Decreases in plasmablasts, memory B cells, and unswitched memory B cells were observed in children with pre-existing anti-HBsAg titers of 10–100 mIU/mL. Immunization is known to be followed by rapid activation of circulating memory cells to terminally differentiate into low-affinity plasma cells or to form germinal centers, which mediate further proliferation and selection for antigen binding later.^{28,29} In this study, there were declines in memory B cells, unswitched memory B cells and plasmablasts at 4 weeks post-booster. But the children did show a rise in anti-HBsAg in the peripheral blood, so they may have produced high affinity antibody-secreting cells at before blood collection.³⁰

The main limitation of this study is the limited sample size. This study was a clinical trial and it was difficult to include a large numbers of children in each age group due to children's particularity. In addition, evaluation on the efficacy of one dose of HepB booster may be insufficient in

this study. We were unable to assess the expression of other more activation markers or cytokines. Further study is warranted to evaluate more biomarkers in cellular response to HepB. More doses and long-term follow-up may also be required in the follow-up study.

In conclusion, this study had comprehensively analyzed humoral and cellular immune response to HepB booster in children after neonatal vaccination. Protection from primary HBV immunization persists at least 13 years after primary immunization on account of the complementary presence of HBV-specific humoral and T-cellular immune response. In addition, we demonstrated that one dose of HepB booster is efficient enough to produce protective anti-HBsAg and enhance HBsAg-specific T-cell responses. As an effective way, HepB booster immunization could be recommended to children without anti-HBsAg in the endemic areas to prevent HBV infection.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

YZ, HX, AH and YH were responsible for the study concept and design, YH, YY, TW and ZL collected participant samples, YH, YY, TW and ZL performed study procedures, YH and YZ performed the statistical analysis and drafted the manuscript.

Data sharing statement

The datasets in this study are available from the corresponding author upon reasonable request.

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