Complementary Presence of HBV-Specific Humoral and T-Cellular Immune Response Provide the Long-Lasting Immune Protection After Neonatal Immunization

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Research Article

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Abstract

Background: Hepatitis B vaccination is the most cost-effective way to prevent HBV infection. Currently, hepatitis B vaccine (HepB) efficacy was usually assessed by anti-HBs level, but there were little comprehensive analyses of humoral and cellular immune response to HepB in children after neonatal immunization.

Methods: A total of 145 children with primary hepatitis B immunization history were involved in this study to evaluate the efficacy of HepB. Blood samples were obtained from 80 eligible children before one dose of HepB booster and 41 children post-booster. Children with anti-HBs at a low level (<10mIU/mL and [10,100) mIU/mL) were received one dose of HepB booster after informed consent. Subjects were be measured anti-HBs, HBsAg-specific T cell responses and frequency of B cell subsets before and after booster.

Results: Among 80 subjects, 81.36% of children showed both T cell and anti-HBs responses positive at baseline. After one dose of booster, anti-HBs titer (P<0.0001), positive rate of HBsAg-specific T cell response (P=0.0036) and magnitude of SFCs (P=0.0003) increased significantly. Comparing preexisting anti-HBs titer <10mIU/mL with anti-HBs titer [10,100) mIU/mL, anti-HBs response (P=0.0005) and HBsAg-specific T lymphocyte response (P<0.0001) increased significantly. The change tendency of HBV specific humoral response is complementary to T cellular response with age.

Conclusion: Protection from primary HBV immunization persists long on account of the complementary presence of HBV-specific humoral and T-cellular immune response. One dose of HepB booster is efficient enough to produce protective anti-HBs and enhance HBsAg-specific T cell response. In the HBV endemic areas, HepB booster immunization is still the most economical and effective way to prevent HBV infection, especially in children without anti-HBs.

Highlights

1. This is a comprehensive study to evaluate children with the protection acquired by primary immunization from the aspect of humoral and cellular immunity.
2. This is the first study to show that because of the complementary presence of HBV-specific humoral and T-cellular immune response, protective immunity from neonatal immunization in children persists long.
3. One dose of HepB booster is efficient enough to produce protective anti-HBs and enhance HBsAg-specific T cell response.
4. In the HBV endemic areas, HepB booster immunization is still the most economical and effective way to prevent HBV infection, especially in children without anti-HBs.

Introduction

Hepatitis B vaccination is the most cost-effective way to prevent acute or chronic HBV infection and reduce complications related to hepatitis B infection now. The Chinese government give priority to routine Hepatitis B vaccination for health care. In the late 1980s, the yeast-derived Hepatitis B vaccine (HepB) was imported by Chinese government. In 1992, HepB was introduced into routine immunization management, and in 2002 HepB was integrated into China's Expanded Program on Immunization (EPI) [1]. Since the announcement of Chinese HBV vaccination program in 1992, China had successfully transformed from HBV highly endemic country to a moderately endemic country. According to the serosurveys in China, from 1992 to 2014, the positive rate of HBsAg decreased 52% (from 9.8–4.7%) in the population, 97% (from 9.7–0.3%) in children < 5 years old and 92.4% (from 10.5–0.8%) in children < 15 years old respectively[2–4]. It is estimated that 80 million HBV infections and 20 million chronic HBV infections have been prevented since 1992[5].

At present, the need for HepB booster in children after neonatal immunization is still controversial. On the one hand, many previous studies have identified no need for a booster dose to healthy children. The protection afforded by primary immunization with HepB can lasts 30 years[6, 7] and only 0.7% of vaccinees had HBV breakthrough infection 5–20 years after neonatal HBV vaccination[8]. Immune memory for HepB exist persistently in children even with waning or undetectable concentrations of anti-HBs[9]. On the other hand, 25%-50% of HBV vaccinees have lost their immune memory to the HepB after 15 years old[10, 11]. About 10.1% had no immune response to HepB booster after the initial vaccination[12]. These results recommended for at-risk youths who had primary immunization to receive HepB booster. Our previous study has identified protective antibody (anti-HBs) in children declined with age, from 93.7% in 1-year-old to 42.3% in 9-year-old[13, 14]. Whether there is protective immune response in children without anti-HBs is little known, and the need for booster doses is in debate.

In this study, we aimed to carry out a comprehensive study to evaluate the protection acquired by primary immunization from the aspect of humoral and cellular immunity. Based on the analyses, we further assess HepB booster for children who have lost protective antibodies and pioneeringly investigate the efficacy of HepB booster in children with low anti-HBs at a level of [10,100) mIU/mL.

Participants And Methods

Design and trial participants

This prospective single-center cohort study performed in Clinical Research Centre of Children's Hospital of Chongqing Medical University (CHCMU). CHCMU is a general children hospital with patients from all over the country. Healthy children were recruited based on the inclusion & exclusion criteria. The study was approved by the Ethics Review Committee of Children's Hospital of Chongqing Medical University and registered in ClinicalTrials.gov (NCT03867643). All children and their legal guardians provided written informed consent.

Inclusion criteria: (1) Born after Jan. 1st, 2005 in Chongqing, China; (2) Completion of the full primary immunization of HepB after birth; (3) No HBV booster vaccine history. Exclusion criteria: (1) History of

allergy or adverse reaction of vaccine; (2) History of immunosuppressive treatment or immunodeficiency; (3) Any kind of vaccination in the past four weeks; (4) Any acute disease or anti-infective therapy in the past four weeks; (5) Fever history in the past one week (axillary temperature ≥ 38 ℃); (6) Blood transfusion history; (7) History of infectious diseases (hepatitis, AIDS, syphilis, gonorrhea, etc.); (8) The family history of HBV in three generations of lineal relatives; (9) Abnormal physical examination.

The flowchart of inclusion of participants in this study was shown in Figure 1. In this study, 145 subjects aged 1-13 years (“0 year” means <12 months) were recruited through our program of our hospital’s official website. Participants not accord with the criteria and those who refused to participate were excluded. Blood samples were obtained from 80 eligible children before a dose of hepatitis B vaccine booster (20μg of HBsAg, Huabei Pharmaceutical Co., Ltd) and 41 children one month after booster.

HBV seromarkers measure

Blood sample was collected for HBV seromarkers testing by chemiluminescent microparticle immunoassay (CMIA) method by the Architect i system (Abbott Laboratories). The concentration of HBsAg was >0.05 IU/mL was regarded as positive. Anti-HBs-positive and seroprotection was regarded as anti-HBs titer ≥10 mIU/mL. Sample value/cut-off values (S/CO) were quantitative indicators of anti-HBe and anti-HBc. Anti-HBe S/CO ≤ 1.0 and anti-HBc S/CO ≥1.0 were defined as positivity.

Interferon γ (IFN-γ)-secreting HBsAg-specific T lymphocytes detection

PBMCs were extracted using density gradient centrifugation. Enzyme-linked immunospot (ELISpot) assays were performed for quantification of HBsAg-specific cytokine-secreting T cells by using the human IFN-γ ELISpot PLUS (ALP) (Mabtech, Sweden). IFN-γ pre-coated 96-well plates were preincubated with RPMI1640 (Gibco®, Invitrogen, USA) for half an hour at room temperature were then added in two replicates at 5×10^5 PBMCs/well in 200uL RPMI1640. PBMCs were stimulated with 10 g/ml of recombinant HBsAg (Bersee, China). Positive controls are wells containing PBMCs and RPMI1640 with anti-CD3 mAb (Mabtech, Sweden). Negative controls are wells without any stimulant. Incubations of plates were conducted for 48 hours at 37 degrees Celsius in 5 percent carbon dioxide and then developed for counting spots in ELISpot reader (Biosys, Germany). A positive result was regarded as a measurable response, 2 folds the negative control level as appropriate[15].

Analysis of frequency of B lymphocytes sub-populations

Peripheral blood was collected for use in flow cytometry to evaluate the phenotypes of immune B-cells. The following mAbs were used to define B-cell subsets: anti-CD19 (APC), anti-human CD24 (PE), anti-CD27 (BV450), anti-CD38 (PerCP-Cy5.5), and anti-IgD (BV510; all from BD Biosciences). Cells were then examined using a FACSCanto II flow cytometer (BD Biosciences) and raw data were analyzed using FACS Diva software. The gating strategy for definition of the B-cell subsets is depicted in Supplementary Figure.

Statistical analysis

The data were analyzed and compared by SPSS (version 20.0). Graphs were made by GraphPad Prism (version 8.0). Continuous variables were compared by the Student t test. For categorical variables, comparisons were performed by the Chi-squared test or Fisher exact tests as appropriate. In addition, Spearman rank correlation was used to evaluate association between ELISpot results and the anti-HBs titer. *P* values smaller than 0.05 were assumed to be statistical difference.

Results

Baseline information of participants

Demographic and characteristics of 80 subjects were presented in Table. All subjects had received 3-dose of neonatal HBV vaccination. We defined groups on the basis of anti-HBs level, with anti-HBs<10mIU/mL group comprising 21 individuals, 10≤ anti-HBs<100 mIU/mL group comprising 30 children, 29 children in anti-HBs ≥100 mIU/mL group. We compared gender, age, pregnancy week, birth weight and disease history among each group. Compared to girls, boys were at a lower anti-HBs level (*P*=0.049). Preterm infants showed a higher rate of anti-HBs negative than normal baby (*P*=0.039).

HBsAg-specific T cell responses are widespread in antibody-negative subjects

To make sure whether there is protective immune response in children without anti-HBs, we use ELISpot assay to measure HBsAg-specific T cell responses. Distribution of positive and negative humoral and cellular immunity was shown in Figure 2. Among the antibody-negative subjects, 85.71% of children showed HBsAg-specific T cell responses positive, while 18.64% antibody-positive subjects showed negative results. Totally, 96.25% of children showed HBsAg-specific T cell or anti-HBs responses positive. In 41 children received one dose of booster, all antibody-negative subjects became positive and HBsAg-specific T cell responses were enhanced.

Higher levels of anti-HBs pre-booster achieved higher humoral response post-booster

In order to explore the detailed changes in humoral response to Hepatitis B vaccine, we analyzed the anti-HBs level before and after booster in children (Figure 3). After booster, anti-HBs titer significantly increased (*P*<0.0001). In this study, all individuals received a hepatitis B vaccine booster produced anti-HBs with titers over 100 mIU/mL. We further found that 56.25% and 100% of children with anti-HBs pre-booster of [0, 10) mIU/mL and [10,100) mIU/mL produced anti-HBs with titers over 1000 mIU/mL respectively (*P*=0.0005). Higher levels of anti-HBs pre-booster achieved higher humoral response post-booster according to the increasing anti-HBs titers pre-booster with increasing post-booster.

The magnitude of HBsAg-specific IFN-γ-producing T cells post-booster depended on the pre-booster anti-HBs titer

We analyzed the HBsAg-specific T lymphocyte responses pre-booster and post-booster (Figure 4). ELISpot detection indicated that the positive rate (P=0.0036) and magnitude of SFCs (P=0.0003) increased significantly post-booster, while the magnitude of the HBsAg-specific IFN-γ-producing T cells did not related to the anti-HBs titer after neonatal vaccination (P=0.1140). T lymphocyte responses post-booster showed the same change trend as humoral response. After booster, the quantity of HBsAg-specific IFN-γ–producing T cells significantly increased (P=0.0004). Compared to children with low anti-HBs titer pre-booster, HBsAg-specific T lymphocyte responses increased significantly post-booster in children with higher preexisting anti-HBs titer (P<0.0001). The intensity of T-cellular immunoreactivity post-booster also depended on the pre-booster anti-HBs titer.

A complementary presence between humoral and T-cellular response to HBV vaccine with age

To explore the relation of humoral and T-cellular response with age, we analyzed the changes of anti-HBs level and HBsAg-specific IFN-γ–producing T cells in children pre- and post-booster in different age groups (Figure 5). Among the 41 vaccinees, post-booster anti-HBs titers increased significantly compared to pre-booster in four age groups. 1- to 3-year-old group showed higher post-booster anti-HBs titer than 10- to 13-year-old group (P=0.031). Only 10- to 13-year-old group showed significantly difference in HBsAg-specific IFN-γ–producing T cells pre- and post-booster (P=0.0172). At baseline, changes in anti-HBs titers are opposed to HBsAg-specific T Cell responses in each group. After booster, children had the same trend. These results revealed that the change tendency of HBV specific humoral response is complementary to T cellular response with age.

Change of immune B-cell subsets after booster in neonatal vaccines

We also analyzed the changes of immune B-cell subsets in participants pre- and post-booster (Supplementary Figure). Among the peripheral lymphocytes, B-cell frequency decreased significantly in two groups (P=0.0002). Antibody-secreting cells were differentiated by B-cell, such as plasmablasts. Among the $CD19^+$ B-cell subsets, the change trend was similar in two preexisting anti-HBs titer subgroups. One month after booster, unswitched memory B cells and class-switched memory B cells decreased significantly. In children with low preexisting anti-HBs titer group ([0, 10) mIU/mL), Naive B cells (P=0.0387) and DN B cells (P=0.0134) were significantly increased. In high preexisting anti-HBs titer group ([10,100) mIU/mL), Naive B cells (P<0.0001) and DN B cells (P=0.0013) were significantly increased.

Discussion

The Chinese Centre for Disease Control and Prevention (CDC) reported that the coverage of three-dose vaccination before age 1 year was 83-99.53% between 2001 and 2017[16]. According to our previous serosurvey, the proportion of seroprotection in 1–14 years old children was 46.03–72.29%, and the rate of anti-HBs < 10 mIU/ml in children ranged from 3.33–25.79% in all age groups[13]. The data were comparable with the CDC survey data of the HBV seroprevalence among different age groups in China[4].

Hepatitis B vaccine is one of the safest immunizations to prevent HBV infection and reduce the future risk of liver cancer[17]. In this study, all children had completed three-birth-dose HepB. We analyzed their immune response to HepB booster after neonatal immunization. We mainly focused on two questions: whether there is protective immune response in children without anti-HBs (titer < 10mIU/mL); whether or not need a dose of HepB booster in children.

This is the first study to show that protective immunity from neonatal immunization in children persists long on account of the complementary presence of HBV-specific humoral and T-cellular immune response. A detectable T-cell response to HBsAg (85.71%) was also found in anti-HBs negative children (< 10mIU/mL) when measured HBsAg-specific INF-γ, which suggest that protection lasts long. In the study group of Wang RX et al, most anti-HBs negative vaccinees showed positivity in HBsAg specific immune cells response[18]. Leuridan et al demonstrated the activation of immune cells in vaccinees based on cell proliferative response[19]. Long-lasting cellular immunity was also proved by detection of the secretion of cytokines by Th1 and Th2 lymphocytes after HBsAg stimulation[7]. These results confirmed T cell immunity persists long regardless of anti-HBs, which is consistent with our views. In addition, HBsAg-specific T Cell responses showed a trend for an initial increase and then a decrease with age while anti-HBs titers were opposite. After birth, children can mount an immune response against pathogens, but adaptive immune responses are relatively weak and narrowly focused in neonates resulting in T cell hypo-responsiveness[20]. In younger children, HBV-specific T cells are defective and not able to secrete enough IFN-γ but gradually improves with age[21]. Before and after HepB booster, the tendency of changes in anti-HBs titer is opposed to HBsAg-specific T Cell response in each age group of children. In vaccine development, determining the balance between humoral and cellular response is the key challenge[22]. The complementary existence between protective antibody responses and T-cell responses is important to the persistence of protection from vaccine. So, there is no need to worry about the decline in anti-HBs in population. It is precisely because of this dynamic balance that screening for HBsAg-specific T Cell immunity is not recommended for the general population. The routine screening for anti-HBs in vaccinees is sufficient to evaluate the protection afforded by HepB.

One dose of HepB booster is efficient enough. All individuals who had received an HepB booster demonstrated protective anti-HBs and enhanced HBsAg-specific T cell responses at 4 weeks post-booster. We found that humoral and T cellular immune response to Hepatitis B vaccine booster depended on the pre-booster anti-HBs titer. Only 56.25% of children with pre-booster anti-HBs < 10mIU/ml demonstrated anti-HBs ≥ 1000mIU/mL at 4 weeks post-vaccination. Those with pre-booster anti-HBs < 10mIU/ml were less likely to produce more anti-HBs compared to anti-HBs= [10,100) mIU/ml. Equally, the intensity of T-cellular immunoreactivity post-booster also depended on the pre-booster anti-HBs titer. After booster, the quantity of HBsAg-specific IFN-γ–producing T cells in children with pre-booster anti-HBs= [10,100) mIU/ml significantly increased compared to anti-HBs < 10mIU/ml. This phenomenon has been observed in other previous studies[23]. Therefore, a series of 3-dose of HepB at 0, 1 and 6 months and the long-term immunity obtained by vaccine are of seminal importance.

Although immune memory for HepB exist persistently in children, HepB booster is recommended to children without anti-HBs in the endemic areas due to the increasing risk of exposure to HBV. Currently, although the available evidence does not provide a compelling basis for recommending a booster dose of Hepatitis B vaccine[6, 24] and chronic HBV infection rarely occurs after primary immunization even when anti-HBs is lower than 10 mIU/mL[6, 25]. However, according to our previous study, the prevalence of HBsAg and anti-HBc began to rise significantly in the 11–16-year-old children compared with 1-10-year-old children (from 0.46–1.40% and from 5.69–7.8% respectively)[14]. This may suggest that the risk of exposure to hepatitis B virus is increased in > 10 years old children, and more attention should be paid to this age group, and HepB booster should be conducted to reduce the risk of breakthrough infection. Some individuals with anti-HBs < 10mIU/ml who are at high risk of HBV exposure will require only one HepB booster to achieve protective anti-HBs. Hepatitis B vaccine has been continuously optimized since it was launched in 1986. The immunization strategy of HepB in China has been continuously improved and its safety has been confirmed[1]. Furthermore, serosurvey shows that the prevalence of HBV is significantly reduced[2–4, 26], which is closely related to hepatitis B vaccination, which further proves that vaccination against hepatitis B is an economic and effective means to prevent and control hepatitis B[27].

In addition to T cellular immune response, B cells also participate in HBV vaccine response by generating a protective level of anti-HBs. Our results demonstrated that total B cells including some antibody-secreting cells decreased dramatically after booster. Furthermore, reductions in plasmablasts, memory B cells and unswitched memory B cells were observed in individuals, especially in children with pre-booster anti-HBs= [10,100) mIU/ml. After vaccination, there is rapid activation of circulating memory cells to terminally differentiate into low-affinity plasma or formation of germinal centers, which mediate further proliferation and selection for antigen binding later[28, 29]. In this study, there was a decline in memory B cells, unswitched memory B cells and plasmablasts appearing in the peripheral blood post-booster. But subjects did show an increase in anti-HBs in the blood, so they must have produced antibody-secreting cells at some time before blood collection which may be high-affinity[30].

In conclusion, this study had comprehensively analyzed humoral and cellular immune response to Hepatitis B vaccine booster in children before and after neonatal immunization. Protection from primary HBV immunization persists long 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-HBs and enhance HBsAg-specific T cell responses. As the most economical and effective way, HepB booster immunization could be recommended to children without anti-HBs in the endemic areas to prevent HBV infection.

Abbreviations

Anti-HBc: Hepatitis B core antibody; Anti-HBe: Hepatitis B e antibody; Anti-HBs: Hepatitis B surface antibody; CHCMU: Children's Hospital of Chongqing Medical University; CMIA: chemiluminescent microparticle immunoassay; ELISpot: Enzyme-linked immunospot; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HepB: Hepatitis B vaccine; IFN-γ: Interferon γ; PBMCs: peripheral blood

mononuclear cells; Pre-: pre-booster; Post-: post-booster; S/CO: Sample value/cut-off values; SFCs: spot-forming cells.

Declarations

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Conflicts of interest: We declare no competing interests.

Ethical Approval: This study was approved by the Ethics Review Committee of Children's Hospital of Chongqing Medical University.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Authors' contributions

Study concept and design: Yao Zhao, Yunmei Huang.

Sample collection: Yunmei Huang, Yuting Yang, Tingting Wu, Zhiyu Li.

Performing experiments: Yunmei Huang, Yuting Yang, Tingting Wu, Zhiyu Li.

Statistical analysis: Yunmei Huang, Yao Zhao.

Drafting the manuscript: Yunmei Huang, Yao Zhao.

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Tables

Table. Baseline demographic data and characteristics of the study groups.

Variable	Anti-HBs<10mIU/mL N =21 (%)	10≤ anti-HBs <100 mIU/mL N =30 (%)	Anti-HBs ≥100 mIU/mL N =29 (%)	*P* value[a]
Gender				
Boys	15 (71.4)	18 (60)	11 (37.9)	0.049*
Girls	6 (28.6)	12 (40)	18 (62.1)	
Age				
1-3y	2 (9.5)	7 (23.3)	6 (20.7)	0.56
4-6y	6 (28.6)	9 (30)	12 (41.4)	
7-9y	8 (38.1)	6 (20)	5 (17.2)	
10-13y	5 (23.8)	8 (26.7)	6 (20.7)	
Pregnancy week				
Normal	17 (81)	30 (100)	27 (93.1)	0.039*
Premature delivery	4 (19)	0 (0)	2 (6.9)	
Birth weight				
Normal	17 (81)	27 (90)	26 (89.7)	0.22
Overweight	2 (9.5)	3 (10)	3 (10.3)	
Underweight	2 (9.5)	0 (0)	0 (0)	
Past history of allergies	0 (0)	0 (0)	3(10.3)	
Past history of hepatitis infection	0 (0)	0 (0)	0 (0)	
Past history of blood transfusion	0 (0)	0 (0)	0 (0)	
Past history of operations	0 (0)	0 (0)	0 (0)	
Past history of radiotherapy and chemotherapy	0 (0)	0 (0)	0 (0)	
History of HBV infection in their parents or grandparents	3 (14.3)	8 (26.7)	10 (34.5)	0.28

[a] By the Pearson $\chi 2$ or Fisher exact tests, as appropriate. * Statistically significant.

Figures

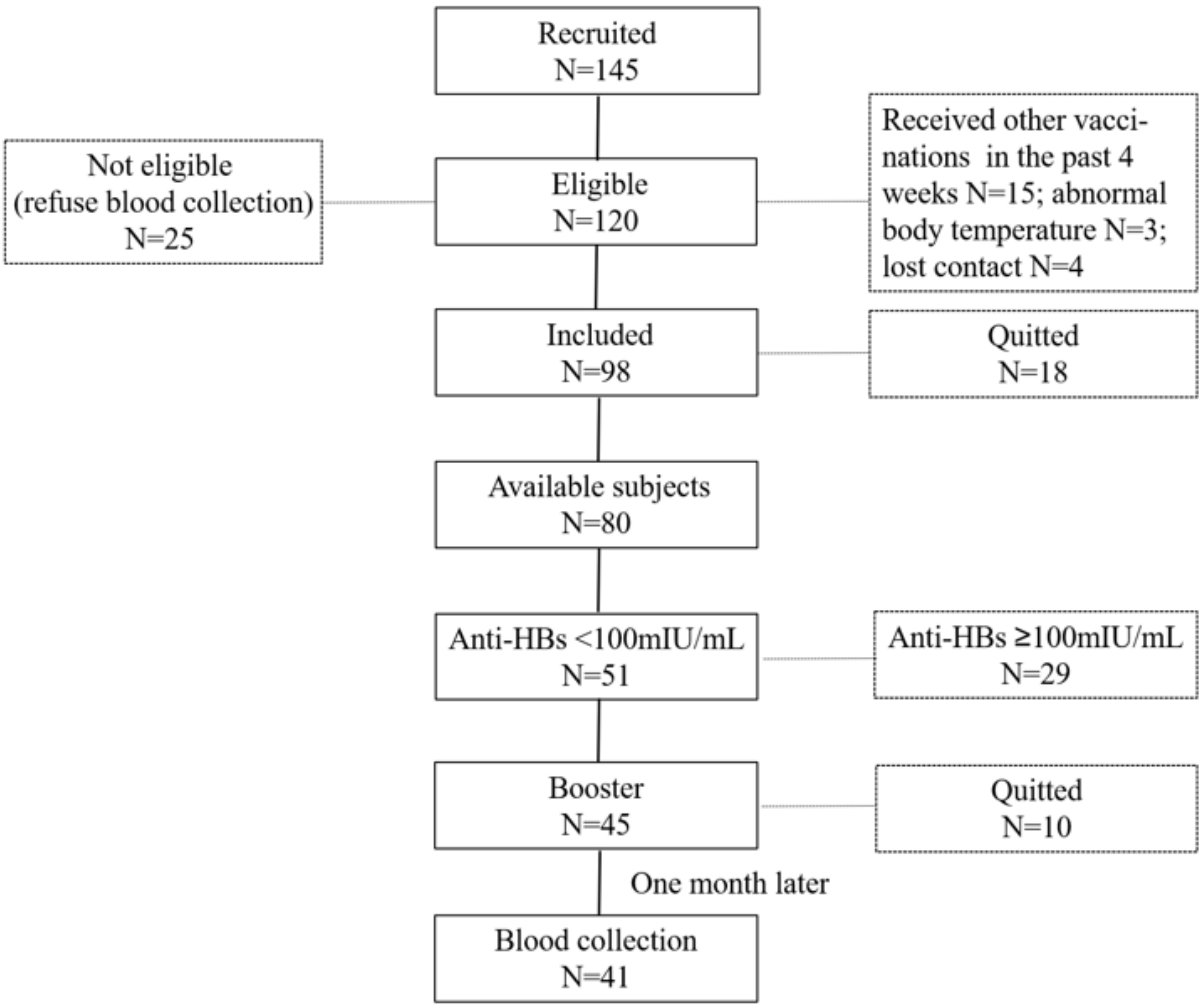

Figure 1

Flowchart of inclusion of participants in this study. Children not accord with the criteria were excluded. Blood samples were obtained from 80 eligible children before a dose of hepatitis B vaccine booster and 41 children post-booster. Abbreviations: N: number; Anti-HBs: antibodies to hepatitis B surface antigen.

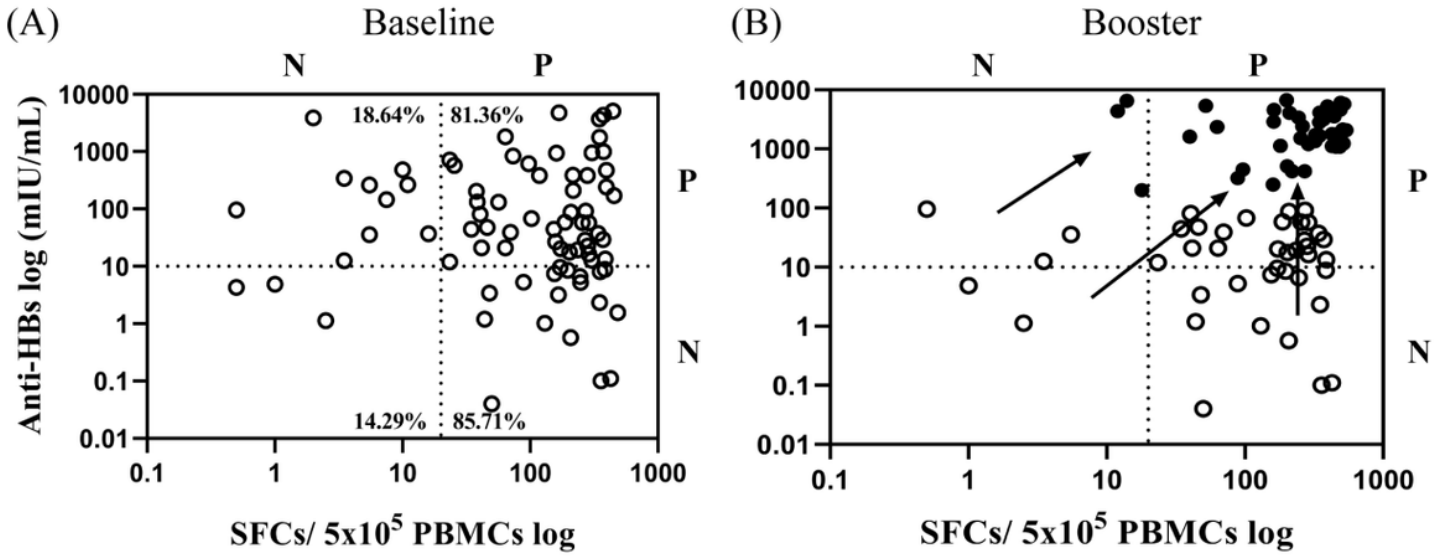

Figure 2

Distribution of positive and negative humoral and cellular immunity pre- and post-booster. (A) Distribution of positive and negative anti-HBs and HBsAg-specific IFN-γ–producing T cells at baseline (n=80). HBsAg-specific T cell responses are detected in antibody-negative subjects. (B) Changes of anti-HBs titers and HBsAg-specific IFN-γ–producing T cells pre-booster (hollow circle) and post-booster (filled circle) (n=41). Abbreviations: SFC: spot-forming cells; PBMCs, peripheral blood mononuclear cells; P: positive; N: negative; Pre-: pre-booster; Post-: post-booster.

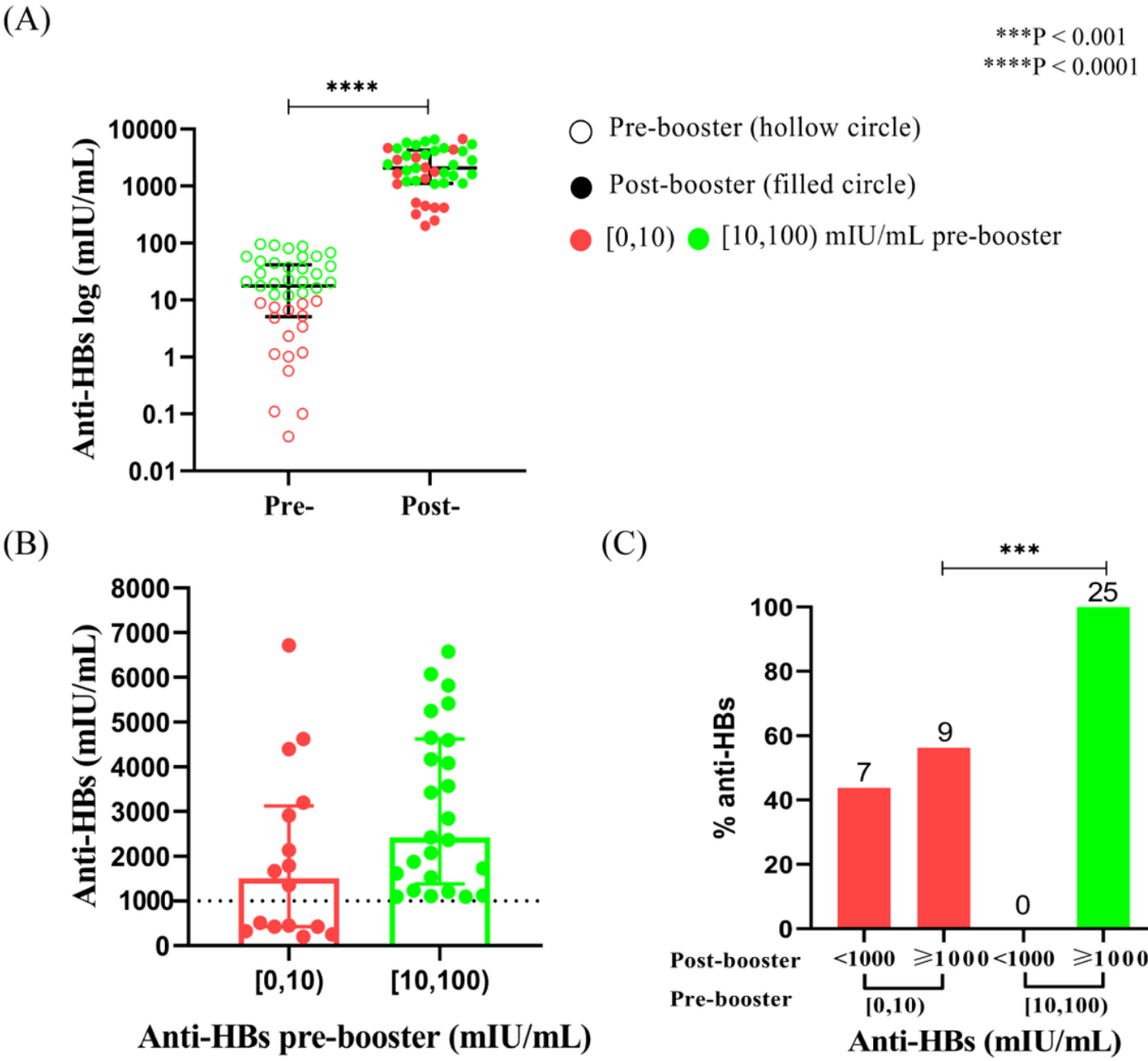

Figure 3

Humoral response to Hepatitis B vaccine booster in children (n=41). Subjects were grouped by different titers of anti-HBs pre-booster ([0,10) mIU/mL (red), [10,100) mIU/mL (green)). (A) Comparison of anti-HBs pre-booster (hollow circle) and post-booster (filled circle). (B) Anti-HBs titers post-booster in children with different preexisting anti-HBs. Boxes and whiskers represent median and interquartile range. (C) Percentage of children with anti-HBs post-booster of <1000 mIU/mL and ≥1000 mIU/mL in two preexisting anti-HBs groups ([0,10) mIU/mL and [10,100) mIU/mL). The intensity of humoral immunoreactivity depended on the pre-booster anti-HBs titer. Abbreviations: Pre-: pre-booster; Post-: post-booster.

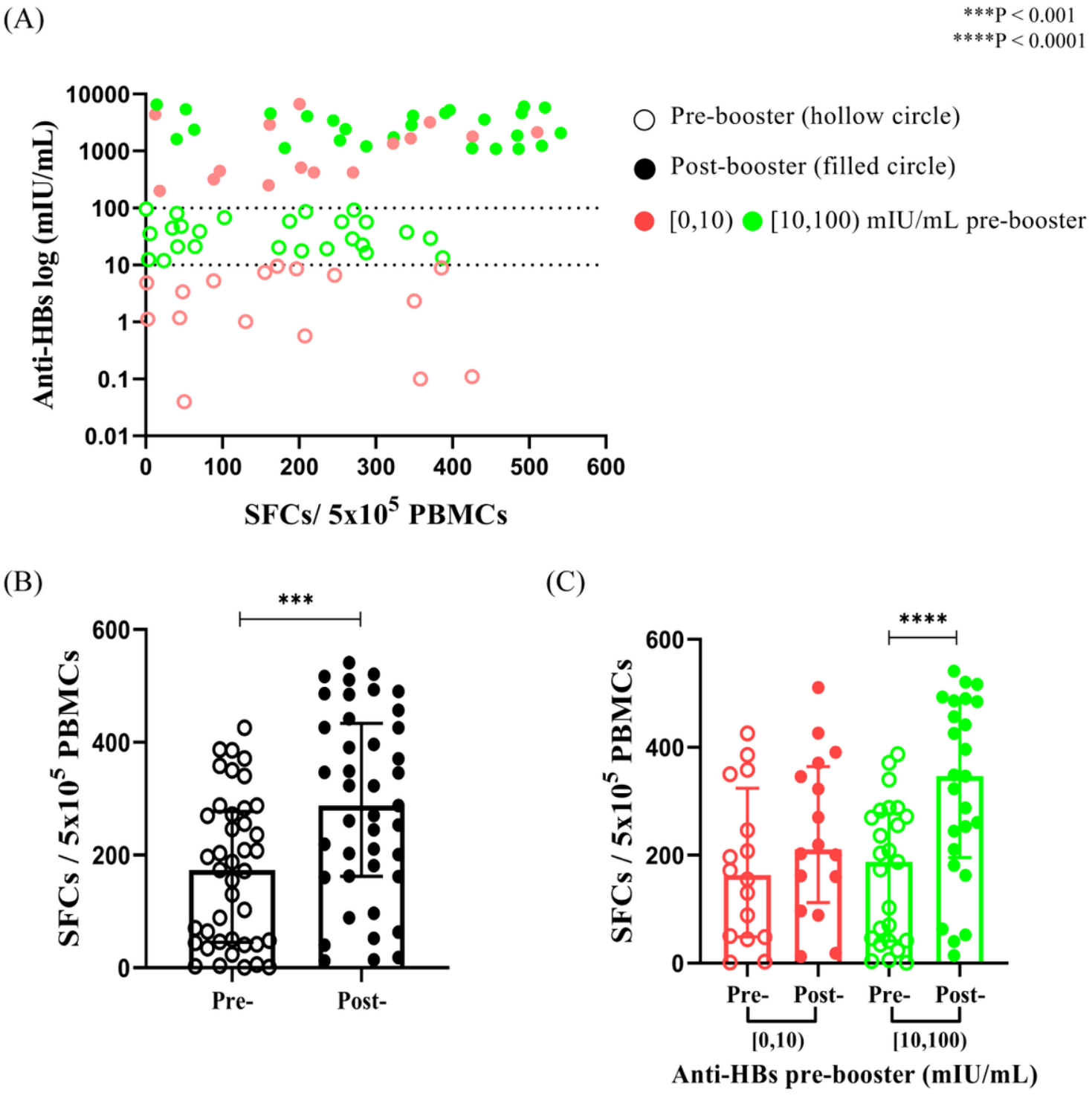

Figure 4

T cellular response to Hepatitis B vaccine booster in children (n=41). Subjects were grouped by different titers of anti-HBs pre-booster ([0,10) mIU/mL (red), [10,100) mIU/mL (green)). (A) The correlation of anti-HBs titers and HBsAg-specific IFN-γ–producing T cells pre-booster (hollow circle) and post-booster (filled circle). (B) HBsAg-specific IFN-γ–producing T cells post-booster increased significantly compared to pre-booster (P=0.0004). (C) HBsAg-specific T lymphocyte responses increased significantly post-booster in

children with higher preexisting anti-HBs titer (P<0.0001). Abbreviations: SFC: spot-forming cells; PBMCs, peripheral blood mononuclear cells; Pre-: pre-booster; Post-: post-booster.

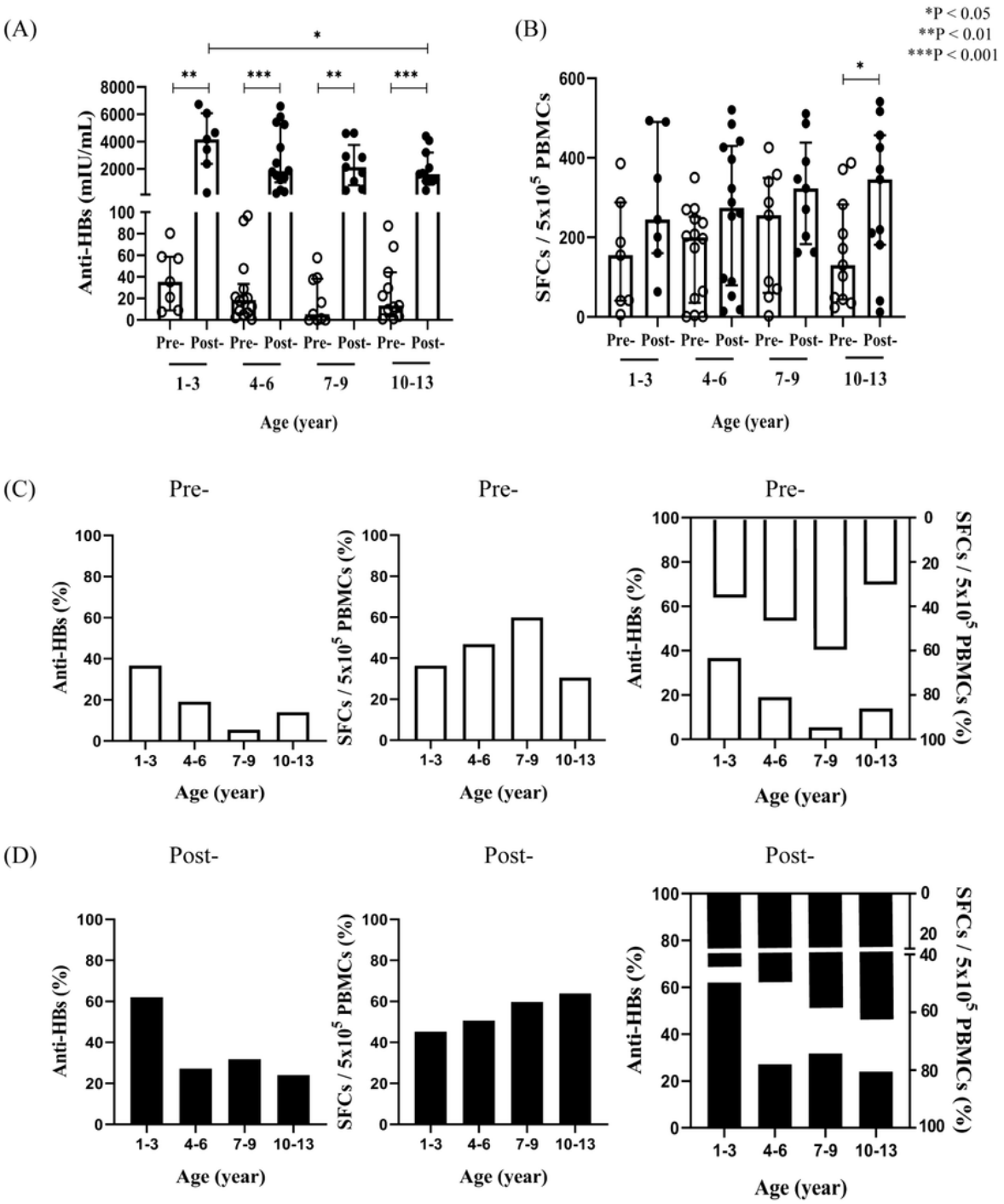

Figure 5

Changes of age related humoral and T-cellular response in children pre-booster and post-booster. Vaccinees (n=41) were stratified in four age groups: 1- to 3-year-old, 4- to 6-year-old, 7- to 9-year-old and 10- to 13-year-old. (A) Comparison of anti-HBs level pre-booster and post-booster in four age groups. (B)

Comparison of HBsAg-specific IFN-γ–producing T cells pre-booster and post-booster in four age groups. (C, D) Changes of age related anti-HBs level and HBsAg-specific IFN-γ–producing T cells in children pre-booster and post-booster. Y-axis: the median of each value divided by the maximum in children pre-booster (hollow column) or post-booster (filled column). Hepatitis B specific humoral and T cellular immunity are in dynamic balance with age. Abbreviations: SFC: spot-forming cells; PBMCs, peripheral blood mononuclear cells; Pre-: pre-booster; Post-: post-booster.

Supplementary Files

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- supplementaryfigure.docx