Vaccine 29 (2011) 7835–7841

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Presence of immune memory and immunity to hepatitis B virus in adults after neonatal hepatitis B vaccination

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ARTICLE INFO

Article history:
Received 10 April 2011
Received in revised form 11 July 2011
Accepted 20 July 2011
Available online 2 August 2011

Keywords:
Hepatitis B
Neonatal hepatitis B vaccination
Immune memory
Cellular immunity
Booster

ABSTRACT

Neonatal vaccination against hepatitis B virus (HBV) infection was launched in the 1980s in Qidong, China, where HBV and hepatocellular carcinoma were highly prevalent. Presence of immune memory and immunity against HBV in adults needs to be clarified. From a cohort of 806 who received plasma-derived Hep-B-Vax as neonates and were consecutively followed at ages 5, 10, and 20 years, 402 twenty-four-year-old adults were recruited for booster test. Among them 4 (1%) were found to be HBsAg(+), 27 (6.7%) were HBsAg(−)anti-HBc(+), 121 (30.2%) were HBsAg(−)anti-HBc(−)anti-HBs(+), and 252 (62.4%) were HBsAg(−)anti-HBc(−)anti-HBs(−). Of them, 141 subjects with HBsAg(−)anti-HBc(−) were boosted with 10-μg recombinant HBV vaccine on day-0 and 1-month. The conversion rates of anti-HBs ≥10 mIU/ml on D10–12 and 1-month post-booster were 71.4% and 87.3% respectively in the vaccinees who were anti-HBs(+) at age 5, higher than in those who were anti-HBs(−) at age 5, 57.5% and 80.0% respectively, but no statistically significant. After the second dose of booster, all subjects with anti-HBs(+) at age 5 had anti-HBs >500 mIU/ml. However, 6/40 subjects, with anti-HBs(−) at age 5, had anti-HBs <10 mIU/ml, geometric mean concentration was 3.6 (95% CI 2.0–7.7). Of the subjects received booster, 44 subjects were determined the presence of T cell immunity on D10–12, 41 had HBsAg-specific T cells detectable, including 7/10 subjects whose anti-HBs were <10 mIU/ml 10–12 days post-booster. Among 27 HBsAg(−)anti-HBc(+) subjects, 19 had detectable serum HBV-DNA, and an "a" epitope mutation was found in 1/5 HBV isolates. One subject who was anti-HBc(+) at age 20 converted into HBsAg(+) 4 years later. The adults received neonatal HBV vaccination had immune memory and immunity against HBV infection. However, 31.9% of neonatal HBV vaccinees who responded weakly at an early age might be susceptible to HBV infection after childhood.

1. Introduction

Hepatitis B virus (HBV) infection is the leading cause of illness and death in China. Each year, an estimated 263,000 people die from HBV-related liver cancer or cirrhosis, accounting for 37–50% of HBV-related deaths worldwide [1]. Universal hepatitis B (HB) vaccination is the best cost-effective and preventive strategy for controlling HBV [2,3]. HB vaccination of neonates and infants has proven to be highly effective in inducing protective antibodies to HBV surface antigen (anti-HBs) and in reducing the prevalence of HBV surface antigens (HBsAg) among children [2,3]. After HB vaccines were included in the Expanded Program on Immunization (EPI) in China, the HBsAg-positive rate of children decreased significantly from 10% to 1–2% [4].

Qidong was one of the areas in China with a high prevalence of HBV infection and hepatocellular carcinoma [5]. Neonatal vaccination against HBV infection was launched in the 1980s [2]. Although this national vaccination program has been very successful, clinical serological surveys conducted in different areas worldwide, including China, showed that the anti-HBs wane over time. Serum levels of anti-HBs ≥10 mIU/ml are considered to be protective against HBV infection [3]. However, the HB vaccine was considered to induce protective immunity, which was present even after the period when the anti-HBs had waned [6,7]. There was also some evidence that a natural boosting effect may occur from exposure to individuals with chronic HBV, and that this is a mechanism for long-term

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doi:10.1016/j.vaccine.2011.07.098

protection against the disease in areas where there is high HBV endemicity [8]. However, recent studies conducted in college students in Taiwan suggest that HBV breakthrough infections might occur in young adults, who received their primary immunization as children, who initially had low responses to the HB vaccine [9,10]. These reports provoked concerns about the long-term protection of neonatal HB vaccination against HBV infection in adults and its preventive effect on HBV-related primary hepatocellular carcinoma (HCC) [3,10]. Therefore, there is concern about what could happen if these vaccinated subjects begin to engage in behaviors that would put them at high-risk for HBV transmission in endemic areas. Booster immunizations for certain high-risk groups or individuals living in the endemic areas have been suggested by some researchers [9].

Currently, the HBsAg is still as high as 7.2% based on the seroepidemiological survey on HBV infection conducted in 2006 among the overall population [4]. The uncertainties about whether the immune memory and immunity, including humoral and cellular immunity, among young adults should be clarified. In this study, a total of 141 young adults confirmed to have received neonatal plasma-derived Hep-B Vax (manufactured and donated to the project by Merck & Co. through the WHO) were sampled from a long-term, consecutively followed cohort, and boosted with recombinant HB vaccine. Our results demonstrate that the neonatal HB vaccinees had immune memory as well as immunity against HBV infection. Natural boosters might play an important role in maintaining the immune memory. However, 31.9% of the neonatal HB vaccinees responded weakly after vaccination and might be susceptible to HBV infection after childhood.

2. Subjects and methods

2.1. Subjects

The study was conducted from September of 2009 to June of 2010. Based on the records in our database, 806 subjects and their parents were informed by letters to participate in the study, who were born in 1985 and were vaccinated with 5 μg of plasma-derived Hep-B Vax after birth and at 1 and 6 months, and were consecutively followed at ages 5, 10, and 20 years, respectively [2], after vaccination. At their age 24, 402 subjects were unable to be followed, a total of 404 subjects were recruited for booster test (Figs. 1 and 2), their serum samples were obtained. After general physical examination, the following subjects were excluded: 3 pregnant, 1 hyperthyroidism, 4 HBsAg(+), 27 HBsAg(−)anti-HBc(+), and 9 who reportedly received HBV boosters after age 20. Totally 141 subjects, 74 males and 67 females, participated in the booster test, the other 219 ones refused to attend the study. HBsAg and anti-HBc were measured twice within a 2-month interval. Signed informed consent was obtained from all participants.

2.2. Protocol for the booster and follow-up

Study protocol was approved by the Board Committee of Good Clinical Practice in the Cancer Institute/Hospital of CAMS, Beijing (protocol #09-59/354). The participants received 10 μg Vecon recombinant Hepatitis B vaccine via intramuscular injection, a yeast product of Shenzhen Kangtai Biological Products Co., Ltd (Shenzhen, China). This recombinant HB vaccine had been reported to induce well anti-HBs response in infants [11,12]. Study flow chart is shown in Fig. 1. All subjects were followed up to 6–7 months after the booster. At 10–12 days after the booster, 44 subjects were randomly sampled and the specific T cell responses to recombinant HBsAg and purified HBeAg were determined using IFN-γ ELISPOT assays.

2.3. Laboratory examinations

HBsAg and anti-HBc was determined using the ELISA kits from Shanghai Kehua Bioengineering Co., Ltd (Shanghai, China). The lower limit of HBsAg detection is 0.5 ng/ml. Anti-HBs were quantified using reagents from Roche Diagnostic GmbH in Cobas e 601 (D-69298 Mannheim, Germany). The range of this assay is 2–1000 mIU/ml. During follow-up at ages 5, 10, and 20 years, HBsAg, anti-HBc, and anti-HBs were determined using the reagents from Abbott (IL, USA). Anti-HBs <10 mIU/ml were considered to be negative [anti-HBs(−)].

2.4. HBV DNA extraction, amplification, and S gene sequencing

HBV DNA was extracted from 200 μl serum using QIAamp MiniElute Virus kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions from 4 subject who were HBsAg(+) and 27 ones who were anti-HBc(+) but HBsAg(−). Strict precautions were taken to avoid possible contamination [1]. Three negative controls using 200 μl of fetal bovine serum were included. Serum HBV-DNA was quantified by TaqMan real-time PCR. The S gene in HBV-DNA was amplified and sequenced as described previously [13].

2.5. Determination of HBsAg- and HBeAg-specific T cells in peripheral blood mononuclear cells (PBMCs) by ELISPOT assays

PBMCs were isolated by Ficoll density gradient separation according to standard laboratory protocols. HBsAg-specific and HBeAg-specific IFN-γ-producing T cells in the PBMCs were determined by ELISPOT assays using the reagents from BD Pharmingen (San Diego, CA, USA) according to the manufacturer's instructions. Briefly, membrane-bottomed 96-well plates (MAHA, Millipore) were coated with monoclonal antibodies against IFN-γ (5 μg/ml, 100 μl/well) in PBS (pH 7.2) overnight at 4 °C. After washing and blocking, 5×10^5/well freshly isolated PBMCs in RPMI 1640 containing 5% autologous human serum were added. In each well, 50 ng/well of recombinant HBsAg (Dalian Hissen Bio-pharm Inc, China) or 50 ng/well of purified HBeAg (kindly provided by Dr. Zhu AG at Beijing Hepatitis Research Institute) were added and incubated for 46–48 h at 37 °C. The spots were analyzed using CTL (Cellular Technology Ltd) ImmunoSpot®V5.0 Professional software in an ImmunoSpot® Versa Analyzer (Beijing, China Office).

2.6. Statistics

SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA) was used for data analysis. Differences in frequency between groups were tested by chi-square test. Differences in antibody concentration, and spot-forming cells (SPF) in response to HBsAg or HBeAg were examined using *t*-tests. All *P* values were two-tailed and $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Persistent immunity against chronic HBV infection present among 24-year-old adults who received neonatal HBV vaccinations

The cohort originally consisted of 904 neonates born in 1985, and was established during the 1980s as previously reported after neonatal vaccination with Hep-B Vax [2,14,15]. To verify the presence of immunity against chronic HBV infection after vaccination, we determined their serum HBsAg and anti-HBs periodically. At their age 5, a total of 806 subjects who were HBsAg(−) were then determined the presence of anti-HBs. Among them, 549 (68.1%)