Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Monitoring the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children: Protective anti-HBs levels and cellular immune responses

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ARTICLE INFO

Article history:
Received 2 November 2017
Received in revised form 2 March 2018
Accepted 15 March 2018
Available online xxxx

Keywords:
Hepatitis B virus
Yeast-derived recombinant vaccine
Immunogenicity
Lymphocyte subsets
Vaccine failure

ABSTRACT

Vaccination against hepatitis B virus (HBV) is recommended worldwide. The aim of this study was to assess the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children in the context of protective anti-HBs levels and cellular immune responses. Using a random questionnaire survey, 1695 pre-school children were recruited as research subjects during January 2015 to June 2017. Blood samples were obtained to measure HBV serological markers as well as peripheral immunocytes. The children were divided into non-, low- and hyper- responsive groups (NR, LR, and HR) based on the vaccination efficacy. Additionally, the effect of revaccination on the NR group was evaluated at 1 month after completion of the vaccination course. Among a total of 1695 children, 1591 (93.86%) were infants who were followed while undergoing their primary course of hepatitis B vaccination at the 0-1-6 month schedule, and 1249 (79.30%) of them developed antibodies against HBsAg (anti-HBs) titers greater than 10 IU/L. The results of immunocyte studies indicated that the $CD8^+$ T cells, $CD4^+CD45RO^+$ T cells, $CD8^+CD45RA^+$ T cells, and T follicular helper (Tfh) cells increased significantly in NR compared with HR. However, lymphocytes, $CD4^+$ T cells, and $CD4^+CD45RA^+$ T cells in NR were lower than that in HR. 96 of the non-response cases showed seroprotection after revaccination among 103 cases. Therefore, most of the preschool children who received hepatitis B vaccine in infancy achieved significant seroprotection. Seroconversion rates of individuals revaccinated after initial vaccination failure were significantly higher than those after primary vaccination. Different vaccination efficacy groups showed significant changes in circulating immunocytes, which might be a factor affecting the recombinant HBV vaccine's immune effectiveness.

1. Introduction

Chronic hepatitis B virus (HBV) infections, and subsequent development of liver cirrhosis or primary hepatocellular carcinoma (HCC), are serious threats to public health worldwide [1–3]. Recently studies have revealed that viral hepatitis has surpassed HIV-AIDS, tuberculosis, and malaria to become the most significant cause of death across the world [4,5]. As an area of the world in which hepatitis B has achieved epidemic proportions, China has approximately 120 million HBV carriers, among which children aged 5 or less account for 1.0% [6,7]. The age at which infection occurs is a critical factor affecting the outcome of HBV infection, and more than 90% of patients who are infected perinatally will

Abbreviations: HBV, hepatitis B virus; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBc, antibodies against hepatitis B c antigen; anti-HBe, antibodies against HBeAg; Anti-HBs, antibodies against HBsAg; PBMC, peripheral blood mononuclear cells; Tfh, T follicular helper cell; FCM, flow cytometry; NR, non- or poor-responder; LR, low-responder; HR, hyper-responder.

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https://doi.org/10.1016/j.vaccine.2018.03.044
0264-410X/

develop chronic infections [8–10]. Therefore, effective prevention of transmission in the population during the early stages would significantly contribute to the control of HBV prevalence.

Hepatitis B vaccine, particularly the recombinant hepatitis B vaccine, have been widely available for more than two decades. The coverage rate, safety, immunogenicity, and protective effects of these vaccines have been well established [7,11–13]. However, one problem regarding use of these treatments has been the significant regional variations observed with respect to coverage and immune effects of hepatitis B vaccination. Another problem that is more difficult to solve is vaccination failure, in which there is no or poor response to immunization. In this circumstance, the antibodies formed against the hepatitis B surface antigen have a titer less than 10 IU/L (anti-HBs < 10 IU/L) [14,15]. Individuals with vaccination failure are not effectively protected from HBV and are at high risk for becoming infected with this virus in areas where it is endemic.

Presently, the non- or poor-response mechanisms after vaccination remain unclear. Studies have revealed that for effective T and B cell reactivity, the $CD45RO^+$ and $CD45RA^+$ T cell populations play an important role in the immune response of vaccinees [16–18]. Previous studies have also shown that T follicular helper (Tfh) cells can efficiently induce B cells to produce antibodies which constitute the defense against pathogens and prevent invasion [19,20].

In this study, we investigated the coverage and seroprotection rates of recombinant hepatitis B vaccination in preschool children in Zhejiang Province. The effectiveness of revaccination of children who had primary vaccination failure was also addressed, and changes of lymphocyte subsets among the different vaccination efficacy groups were further analyzed. We sought to elucidate the immune system factors associated with preschool children's responses to HBV vaccine, which serve as the basis for hepatitis B prevention and revaccination for these children in our region of China.

2. Materials and methods

2.1. Enrollment of study subjects

We enrolled 1695 pre-school children (from birth to 8 years of age) from Lin'an district in northwestern Zhejiang Province as the subjects of this study between January 2015 and June 2017, and administered 10 μg/0.5 ml/dose of yeast-derived recombinant hepatitis B vaccine for the primary vaccination (Dalian Hissen Biological Products, Dalian, China) and revaccination (Shenzhen Kangtai Biological Products, Shenzhen, China), respectively. The study protocol, conforming to the guidelines of the Declaration of Helsinki, was approved by the Ethics Review Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University. The parents or guardians of all subjects gave written informed consent in accordance with the Declaration of Helsinki.

2.2. Study design

Face-to-face interviews were conducted with each child's parents or guardians to administer the questionnaire survey. The questionnaire was designed to obtain basic information (such as age, sex, and whether breastfeeding was done), immunization history of HBV vaccine and family history of hepatitis B for the enrolled infants or children. Informed consent was obtained during the field survey, and 1695 different individual serum samples were separated for HBV serological marker analysis between January 2015 and September 2016. At the same time, 813 (included in the above 1695 cases) different individual peripheral anticoagulant blood samples were prepared for lymphocyte subgroup detection.

Additionally, in order to learn about the effect of revaccination to primary HB vaccine non-responder, 103 subjects (included in the above 1695 cases) with initial vaccination failure were revaccinated. The effect of revaccination was evaluated at 1 month after completion of the revaccination course between July 2016 and June 2017. All specimen were transported to a specified collection site (First Affiliated Hospital of Zhejiang University) in a timely manner for processing.

2.3. Assessment of serological HBV indicators

Hepatitis B surface antigen (HBsAg), anti-HBs antibody, hepatitis B e antigen (HBeAg), antibodies against HBeAg (anti-HBe), and antibodies against hepatitis B c antigen (anti-HBc) were detected using a commercial chemiluminescent microparticle immunoassay (CMIA) kit (for the Architect-i4000 system, Abbott Laboratories, Lake Bluff, IL, USA). Results for HBV markers meeting the following criteria were deemed positive (+): HBsAg $\geq$ 0.05 IU/mL, anti-HBs $\geq$ 10 IU/L, HBeAg (s/co) $\geq$ 1.000, anti-HBe (s/co) < 1.000, and anti-HBc (s/co) $\geq$ 1.000 [21].

The effects of hepatitis B vaccination were defined as follows: negative for HBV serological markers and anti-HBs < 10 IU/L was regarded as non- or poorly responsive to vaccination (NR). Only-anti-HBs-positive and 10 IU/L $\leq$ anti-HBs $\leq$ 100 IU/L was regarded as having a low-response to vaccination (LR). Similarly, only-anti-HBs-positive and anti-HBs > 100 IU/L was regarded as hyper-responsive to vaccination (HR) [14,15,21]. Individuals with anti-HBs concentrations of 10 IU/L or more were regarded as protected.

2.4. Peripheral blood lymphocyte subgroups measurement

The fresh anticoagulated blood from each individual was stained with CD3-PerCP-Cy5.5, CD4-APC, and CD8-APC-Cy7 mouse anti-human fluorescence monoclonal antibody (all from BD Biosciences, Franklin Lakes, NJ, USA) for 15 min at room temperature. An isotype control was conducted at the same time. A Coulter QPREP specimen processing instrument (Beckman Coulter Co., Brea, CA, USA) was used to lyse red blood cells and fix cells. An aliquot of more than 10^4 cells was counted by a flow cytometer (Coulter Cytomics FC 500, Beckman Coulter). Percentages of the $CD3^+$, $CD4^+$, and $CD8^+$ T cells in the total lymphocyte population were each recorded. Similarly, B lymphocyte subsets and T follicular helper cells (Tfh) in peripheral blood were detected through CD19-APC-Cy7 and CXCR5-FITC fluorescence antibody tests (all from BD Biosciences).

$CD45RA^+$ and $CD45RO^+$ T cells were detected in the PBMC preparations by taking an aliquot of 100 μl and staining it with 20 μl of anti-CD45RO-PE, anti-CD45RA-FITC, anti-CD4-APC, and anti-CD8-APC-Cy7 mouse anti-human fluorescence monoclonal antibody (all from BD Biosciences). The PBMCs and antibodies were mixed thoroughly and incubated in the dark for 15 min. After washing, 500 μl of phosphate-buffered saline was added and the frequency of $CD45RO^+$ and $CD45RA^+$ was gated on the $CD3^+$, $CD4^+$, and $CD8^+$ cells. A total of 10^4 cells were surveyed for each sample. The results were acquired and analyzed by EXPO32 (Beckman Coulter) Software.

2.5. Statistical analysis

GraphPad Prism was used for statistical analysis. Measurement data were presented as means ± standard error of the mean (SEM) or percentages. Analysis and comparison among groups was performed by the non-parametric Mann-Whitney-U tests, one-way ANOVA, or χ^2 analysis. A P value < 0.05 was considered to be statistically significant, with two levels of significance designated by asterisks: *, $P < 0.05$; and **, $P < 0.01$.

Please cite this article in press as: Li X et al. Monitoring the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children: Protective anti-HBs levels and cellular immune responses. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.03.044