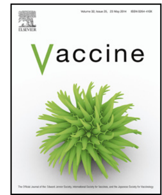
Vaccine 34 (2016) 636–642

Contents lists available at ScienceDirect

Vaccine

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



Characteristics of immune memory 10–15 years after primary hepatitis B vaccination

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ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form 8 November 2015
Accepted 11 December 2015
Available online 21 December 2015

Keywords:
Hepatitis B vaccination
Immune memory
Kinetics
Anti-HBs titre
Cellular immunity
ELISpot

ABSTRACT

Background and aims: The definition of immune memory after hepatitis B vaccination is still under debate. Therefore, we analysed hepatitis B surface antigen (HBsAg)-specific memory in more detail by investigating the kinetics of humoral and cellular responses after hepatitis B booster vaccination.

Methods: The anti-HBs kinetics of 23 individuals with anti-HBs titres below 10 IU/l, who had been vaccinated 10–15 years ago, was monitored at day 0, 3, 7, 14 and 28 after booster vaccination. HBsAg-specific IFNγ- and IL5-secreting cells in enriched $CD4^+$ fraction were measured at day 0, 7 and 28 post-booster by enzyme-linked immunospot assay (ELISpot).

Results: 22 of 23 subjects showed similar anti-HBs kinetic curves, including 3 of 4 subjects who did not reach anti-HBs titres of 10 IU/l. The steep anti-HBs increase started between day 3 and 7 and peaked around day 14. A plateau or only minimal changes were visible between day 14 and 28. 17.4% of subjects showed pre-booster cellular responses, and this rate had increased to 47.8% and 56.5% after 7 and 28 days, respectively. The kinetic patterns of T cell responses differed considerably among subjects. A dominance of Th2 responses (IL5 secretion) over Th1 responses (IFNγ secretion) could be observed.

Conclusions: The presence of B cell memory could be shown by a typical anamnestic anti-HBs response curve after a booster dose in all but one individual. In contrast, T cell responses to booster vaccination, which occurred in approximately 50% of participants, were rather heterogeneous.

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1. Introduction

Protection after hepatitis B vaccination is based on two immune mechanisms: specific antibodies against hepatitis B surface antigen (HBsAg) confer protection against infection with the hepatitis B virus (HBV), whereas HBsAg-specific B and T cell-mediated immune memory protects against HBV-disease after the disappearance of antibodies [1]. HBsAg-specific immune memory is usually shown by an anamnestic response to a booster dose of hepatitis B vaccine. A typical anamnestic response is characterised by a rapid 10–100-fold increase in specific antibodies, which starts 5–8 days after the re-exposure to the antigen and peaks after about 14 days [2–4]. HBsAg-specific memory has been shown to persist for at least 15–17 years after immunisation [5–7]. Long-term follow-up studies have shown that clinical HBV-disease or HBsAg-carrier status rarely occur among successfully vaccinated individuals, even in the case of anti-HBs titres <10 IU/l [8]. These observations led to the conclusion that protection against clinically significant breakthrough infection and chronic carriage is long-term rendering booster doses unnecessary [1,9–11]. However, over the past years, a number of studies have reported about waning immunity over time by showing a loss of the ability to respond to booster vaccination [12–16]. A recent meta-analysis has shown that, 20 years after infant vaccination, about 40% of individuals who had lost protecting antibodies did not respond to booster vaccination [17].

However, one problem in evaluating studies reporting on waning immunity is the lack of a clear definition of immune memory.

Abbreviations: ELISpot, enzyme-linked immunospot assay; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFNγ, interferon γ; IL5, interleukin 5; PBMC, peripheral blood mononuclear cells; SFC, spot forming cells; Th1, T helper 1; Th2, T helper 2.

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http://dx.doi.org/10.1016/j.vaccine.2015.12.033
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Anti-HBs antibodies can be reliably measured by standardised tests, but no standardised method is yet available for measuring specific immune memory. In studies in which immune memory was shown by means of the increase in anti-HBs after booster vaccination, anti-HBs titres were mostly measured only once and at rather different time points, ranging from 10 days to 2 months post-booster [7,18–21]. In addition, in some studies anamnestic response was defined as an increase in anti-HBs to ≥10 IU/l [21–25], in other studies as 4-fold increase in anti-HBs [5], and sometimes both criteria were applied [13,15,20,26]. Because of the differing definitions, it seems to be impossible to differentiate in all cases between anamnestic responses due to the presence of immune memory and primary responses after loss of immune memory.

In contrast to B cell memory, T cell memory has only been investigated in a few studies. In most of these few studies specific T cells were analysed after HBV booster vaccination using ELISpot assays with unfractionated PBMCs (peripheral blood mononuclear cells). However, this method is prone to failure because of the possible activation of nonspecific bystander cells. Moreover, HBsAg-specific T cell memory has been rather heterogeneously analysed in these studies and measured at different time points after booster vaccination [12,27–29].

In the present study we aimed to define optimal criteria for assessing HBsAg-specific immune memory. To assess B cell memory, we studied the kinetics of anti-HBs titres at day 0, 3, 7, 14 and 28 post-booster in subjects who had been vaccinated 10–15 years before and in whom anti-HBs had fallen below the critical value of 10 IU/l. In order to analyse the T cell memory as well, we enriched $CD4^+$ T cells and identified HBsAg-specific T helper 1 (Th1) and T helper 2 (Th2) cell responses in this fraction by measuring IFNγ- and IL5-secreting cells at day 0, 7 and 28 post-booster.

2. Methods

2.1. Study cohort

77 adolescents (33 male, 44 female) aged 14–18 years were included in this study. All subjects had obtained a full course of 3 vaccinations against hepatitis B 10–15 years ago. The interval between the 1st and the 2nd dose was 1–3 months (mean 1.4 months) and between the 2nd and the 3rd dose 6–20 months (mean 11.7 months). 66 subjects had received monovalent vaccines (63 Engerix B®, 3 GenHBvax®), 1 subject the hepatitis A/B combination vaccine Twinrix® and 1 the hexavalent vaccine Hexavac®. In 3 subjects, Engerix B® and GenHBvax® were mixed and in 6 subjects, the brand of at least one vaccine was unknown.

Anti-HBs and anti-HBc were determined using commercial enzyme immunoassays on the Architect System (Abbott, Sligo, Ireland). Subjects with anti-HBs values <10 IU/l were offered a single booster dose of monovalent Hepatitis B vaccine (Engerix B®, GSK, Rixensart, Belgium). The anti-HBs titre of revaccinated subjects was determined at day 0, 3, 7, 14 and 28 post-booster. Additionally, cellular immune responses were analysed by ELISpot at day 0, 7 and 28. Healthy non-vaccinated volunteers were studied as controls.

Written informed consent was obtained from each subject and at least one parent before enrolment in this study. The study protocol had been approved by the Ethics Committee of the University Regensburg before the study start (vote number 10-101-0166).

2.2. Isolation of $CD4^+$ T cells

PBMCs were isolated from heparinised blood on density gradients (Pancoll human, PAN Biotech, Aidenbach, Germany) and stored in liquid nitrogen until used. $CD4^+$ T cells were isolated from PBMCs by negative selection using magnetic cell sorting ($CD4^+$ T cell isolation kit, Miltenyi Biotech, Bergisch-Gladbach, Germany). Purity of the enriched $CD4^+$ fraction was assessed by flow cytometry using anti-CD3-VioBlue and anti-CD4(VIT4)-PerCP (Miltenyi) and usually exceeded 85%. Fewer than 3% of $CD3^+CD4^+$ cells were normally found in the CD4-free cell fraction. Propidium iodide (Miltenyi) was used for exclusion of dead cells.

Flow cytometric analyses were done with FACS Canto II (BD Biosciences, San Diego, CA). Data were analysed with FlowJo software Version X.0.7 (Tree Star, Ashland, OR, USA).

2.3. Detection of IFNγ- and IL5-secreting cells by ELISpot

ELISpot assays were conducted using the FluoroSpot kit for Human IFNγ/IL-5 (Mabtech, Nacka Strand, Sweden) according to the manufacturer's instructions [30,31]. A mixture of 54 peptides (15-mer with 11 amino acid overlap, purity ≥90%, JPT, Berlin, Germany), which covered the entire sequence of the small HBsAg [32], was used for 44 h-stimulation in a final concentration of 2 μg/ml per peptide. Routinely, each sample was analysed in 5 replicates. In 8% (22 of 276 cases), fewer replicates were tested because of the insufficient amount of PBMCs available (2 replicates in 1%, 3 replicates in 1%, 4 replicates in 6%). Cells stimulated with 5 μg/ml phytohaemagglutinin (Biochrom, Berlin, Germany) were tested in duplicates as a positive control. Anti-CD28 was added to each well at a final concentration of 0.1 μg/ml. Each well contained 0.16% of dimethyl sulfoxide. Spots were counted with an AID ELISpot Reader (Advanced Imaging Devices, Straßberg, Germany).

2.4. Definition of positive cellular responses

Spot forming cells (SFC) were determined in parallel for every sample in the following four setups:

(A) 2×10^5 cells of $CD4^+$ fraction and 1×10^5 cells of CD4-free fraction (needed for antigen presentation) without HBsAg peptides,
(B) 2×10^5 cells of $CD4^+$ fraction and 1×10^5 cells of CD4-free fraction with HBsAg peptides,
(C) 1×10^5 cells of CD4-free fraction without HBsAg peptides, and
(D) 1×10^5 cells of CD4-free fraction with HBsAg peptides.

The number of HBsAg-induced SFC/well was calculated for further data analysis as follows: (mean B − mean A) − (mean D − mean C).

Cellular immune responses in revaccinated subjects were defined as positive (HBsAg-specific) when:

(a) the number of SFC in setup B was ≥2-fold higher than the number of SFC in setup A and
(b) the number of HBsAg-induced SFC calculated as mentioned above was ≥4.0 for IL5-secretion and ≥5.2 for IFNγ-secretion (≥mean + 2 SD of the number of SFC measured in 9 non-vaccinated subjects).

2.5. Statistical analysis

Statistical analysis was done with GraphPad Prism 6.02 (GraphPad Software, LA Jolla, CA). Two-sided p values of <0.05 were considered significant. Anti-HBs values <0.1 IU/l were set to 0.1 IU/l for further data analysis.

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