The Hepatitis B Vaccine Protects Re-Exposed Health Care Workers, But Does Not Provide Sterilizing Immunity

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BACKGROUND & AIMS: Infection with hepatitis B virus (HBV) can be prevented by vaccination with HB surface (HBs) antigen, which induces HBs-specific antibodies and T cells. However, the duration of vaccine-induced protective immunity is poorly defined for health care workers who were vaccinated as adults. **METHODS:** We investigated the immune mechanisms (antibody and T-cell responses) of long-term protection by the HBV vaccine in 90 health care workers with or without occupational exposure to HBV, 10–28 years after vaccination. **RESULTS:** Fifty-nine of 90 health care workers (65%) had levels of antibodies to HBs antigen above the cut-off (>12 mIU/mL) and 30 of 90 (33%) had HBs-specific T cells that produced interferon-gamma. Titers of antibodies to HBs antigen correlated with numbers of HBs-specific interferon-gamma–producing T cells, but not with time after vaccination. Although occupational exposure to HBV after vaccination did not induce antibodies to the HBV core protein (HBcore), the standard biomarker for HBV infection, $CD4^+$ and $CD8^+$ T cells against HBcore and polymerase antigens were detected. Similar numbers of HBcore- and polymerase-specific $CD4^+$ and $CD8^+$ T cells were detected in health care workers with occupational exposure to HBV and in patients who acquired immunity via HBV infection. Most of the HBcore- and polymerase-specific T cells were $CD45RO^+$ $CCR7^-CD127^-$ effector memory cells in exposed health care workers and in patients with acquired immunity. In contrast, most of the vaccine-induced HBs-specific T cells were $CD45RO^-CCR7^-CD127^-$ terminally differentiated cells. **CONCLUSIONS: HBs antigen vaccine-induced immunity protects against future infection but does not provide sterilizing immunity, as evidenced by HBcore- and polymerase-specific $CD8^+$ T cells in vaccinated health care workers with occupational exposure to HBV. The presence of HBcore- and HBV polymerase-specific T-cell responses is a more sensitive indicator of HBV exposure than detection of HBcore-specific antibodies.**

Keywords: Immunization; Immune Response; T Cell; Virus.

Chronic hepatitis B virus (HBV) infection is a serious health problem, with >360 million people infected worldwide and about 1 million deaths per year due to HBV-related liver disease.[1] Infection with HBV can be prevented by vaccination with HB surface antigen (HBsAg), which induces HBs-specific antibodies and T cells.[2–4] A complete 3-dose course of the vaccine induces HBs-specific antibodies (anti-HBs) in >95% of healthy infants and in >90% of healthy adults, which are considered protected upon HBV exposure.[5,6]

Anti-HBs titers rapidly decline within the first year after vaccination and more slowly thereafter.[7] In representative studies conducted 10–15 years after primary vaccination, 11%–63% of vaccinees displayed anti-HBs titers below the cut-off.[8–10] Breakthrough infections, diagnosed by appearance of antibodies against HBV core protein (HBcore) were infrequent and typically clinically asymptomatic. In addition, booster vaccination of those subjects who had lost anti-HBs responses induced recall responses within 2–4 weeks.[11] Most of these studies focused on vaccinated infants[11,12] in areas where HBV infection is endemic.[8,10] In those studies, HBV exposure resulted in natural boosts of the vaccine-induced humoral immune response with 8.2% of the vaccinees experiencing 4-fold increases in anti-HBs levels between yearly tests.[13] In addition, children who were born to HBsAg and HBeAg-positive mothers and vaccinated after birth were much more likely to exhibit antibodies to HB core antigen by their teenage years if they live in endemic areas.[10]

In contrast, much less is known about the longevity of HBsAg-specific immune responses in persons who have been vaccinated as adults and who reside in nonendemic countries. In this population, anti-HBs titers can wane faster due to absence of natural antigen required to maintain immune memory. Whether and when booster vaccinations are recommended for persons who were vaccinated as adults is controversial. Health care workers are of particular interest in this context because they were among the first to be required to receive the HB vaccine and have the longest follow-up after HBsAg vaccination. Here, we assessed the immunological mechanisms of long-term protection in health care workers who were vaccinated during adulthood and experienced differential levels of occupational re-exposure to HBV. In addition, we compared their immune responses with those of

Abbreviations used in this paper: anti-HBs, antibody to hepatitis B surface antigen; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; HBcore, HBV core protein; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBVpol, HBV polymerase; HDV, hepatitis D virus; IFN, interferon; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PE, phycoerythrin; PHA-M, phytohemagglutinin.

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Table 1. Characteristics of Studied Health Care Workers

	Vaccinees' exposure score			*P* value (comparing exposure scores)	Nonvaccinated controls	Subjects recovered from acute HBV	*P* value (comparing all groups)
	0	1	≥2				
Subjects, n	43	14	33		10	25	
Male, n (%)	13 (30)	2 (14)	10 (30)	NS	3 (30)	14 (56)	NS
Age, *y*, mean ± SD	47.7 ± 10	47.7 ± 9	47.0 ± 11	NS	47.3 ± 12	56.7 ± 9	.004
Time since vaccination, *y*, mean ± SD	17.4 ± 5	18.2 ± 5	18.1 ± 5	NS	NA	NA	
Anti-HBc–positive, n	0	0	0		0	25	

NA, not applicable; NS, not significant.

individuals who acquired natural immunity by recovering from acute HBV infection.

Materials and Methods

Study Cohort

Ninety health care workers were studied for humoral and cellular immune responses 10–28 years after a documented complete course of HBsAg vaccination. Seventy-one health care workers had received recombinant HBs vaccine (Engerix B or Recombivax), and 14 health care workers had received a plasma-derived HBs vaccine (Heptavax). For 5 health care workers the vaccine type was unknown. This immunological analysis was part of a larger recall study of HBsAg vaccinees conducted in the Liver Diseases Branch (ClinicalTrials.gov identifier: NCT01182311). Twenty-five nonvaccinated patients who had recovered from acute hepatitis B more than 10 years ago, and 10 subjects who had never received the HBsAg vaccine and had never been HBV infected were studied for comparison. All subjects gave written informed consent for research testing under a protocol approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board.

A risk-assessment questionnaire was used to determine occupational exposure to HBV. One point was given if a subject was regularly working with HBV-infected patients. Additional points were given for each incident of exposure to HBV-infected blood. Based on the total number of points each vaccinee was assigned an exposure score of 0, 1, or ≥2 (Table 1).

Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were separated from heparin-anticoagulated blood by Ficoll-Histopaque (Mediatech, Manassas, VA) density gradient centrifugation, washed 3 times with phosphate-buffered saline (PBS; Mediatech) as described previously,[14] and cryopreserved in liquid nitrogen until use.

Synthetic Peptides

Two hundred and eighty-two 15-mer peptides were synthesized according to the HBV Galibert sequence[15] (Genbank accession number V01460) with a 10 amino acid overlap (Mimotopes, Clayton, Australia) and resuspended in PBS containing 5% dimethyl sulfoxide (DMSO) to generate 1 HBVcore pool (41 peptides), 2 HBs pools (38 peptides each), and 4 HBVpolymerase (HBVpol) pools (3 HBVpol pools consisting of 41 peptides each and 1 HBVpol pool consisting of 42 peptides). The T-cell response to each of these HBV peptide pools was compared with the response to a pool of 41 overlapping 15-mer peptides of the hepatitis D virus (HDV) large antigen (Mimotopes), which was used as a negative control.

Antibody Assays

Antibodies against HBs and HBcore were quantitated in sera using the VITROS Immunodiagnostics anti-HBs Quantitative Reagent Pack and the VITROS antibody to hepatitis B core antigen assay on the VITROS ECi Immunodiagnostic System (Ortho-Clinical Diagnostics, Raritan, NJ).

Interferon-Gamma Enzyme-Linked Immunospot Assay

Cryopreserved PBMCs were thawed, resuspended in RPMI 1640 containing 5% fetal calf serum (FCS) and 2 mmol/L L-glutamine (Mediatech), and stimulated in quadruplicates of 3×10^5 cells/well with each of the 7 HBV peptide pools, the negative control HDV peptide pool (1 μg/mL of each peptide), 1 μg/mL phytohemagglutinin (PHA-M; Invitrogen, Carlsbad, CA) or DMSO as described.[14] The number of specific spots (ie, the number of spots in the presence of antigen minus the number of spots in the absence of antigen) was determined using an AID ELISpot Reader Version 3.5 (Autoimmun Diagnostika GmbH, Strassberg, Germany). A positive response was defined as >3-fold the DMSO background response.

Intracellular Cytokine Staining

To differentiate between interferon (IFN)-γ–producing CD4 and CD8 T cells, 2×10^6 PBMCs were stimulated with the HBV peptide mixes, the negative control HDV peptide pool (final concentration of 1 μg/mL per peptide, respectively), DMSO, or with 1 μg/mL PHA-M in 300 μL culture medium (RPMI 1640, supplemented with 10% FCS, 2 mmol L-glutamine, 100 g/mL streptomycin, and 100 U/mL penicillin) in the presence of 1 μg/mL anti-CD28 and anti-CD49d antibodies (BD Bioscience, San Diego, CA). After 2 h, 0.3 μL Golgi-Plug (BD Bioscience) were added. After an addditional 16 h, cells were washed and stained with ethidium monoazide, anti–CD19-phycoerythrin (PE)-Cy5 (BD Biosciences), anti–CD14-PE-Cy5 (AbD-Serotec, Raleigh, NC) to exclude dead cells, B cells, and monocytes, and with anti–CD3-Alexa-Fluor700, anti–CD4-PacificBlue, and anti–CD8-AmCyan (all BD Biosciences) to identify T-cell subsets. Cells were washed again, fixed, and permeabilized with the Cytofix/Cytoperm Kit (BD Bioscience), stained with antibodies against IFN-γ for 30 min at 4°C, washed, resuspended in PBS, and immediately analyzed by flow cytometry. A positive response was defined as >3-fold the DMSO background response.

To characterize the memory T-cell phenotype, cells were stained with anti–CD4-Alexa-Fluor700, anti–CD8-V500, anti–CD45RO-APC-H7, CD127-V450, and CCR7-PE-Cy7, in addition to the