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Immunity to Hepatitis B Virus (HBV) Infection Two Decades after Implementation of Universal Infant HBV Vaccination: Association of Detectable Residual Antibodies and Response to a Single HBV Challenge Dose

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Most persons who receive hepatitis B vaccine during infancy will have a level of antibody to hepatitis B surface antigen (anti-HBsAg) of <10 IU/liter 10 to 15 years later; however, most will demonstrate immune memory by an anamnestic response to a vaccine challenge dose. To determine whether there was a difference in anamnestic response among college students vaccinated during infancy, we compared anti-HBsAg levels after a 20-μg dose of Engerix-B in those with a residual anti-HBsAg level of 0 IU/liter and those with levels of 1 to 9 IU/liter. Anti-HBsAg was measured before (baseline) and 2 weeks after a challenge dose; a response was defined as a level of ≥10 IU/liter after the dose among those with <10 IU/liter at the baseline. Of the 153 students who completed the study, 130 (85%) had an anti-HBsAg level of <10 IU/liter at the baseline, 72 had a level of 0 IU/liter, and 58 had levels ranging from 1 to 9 IU/liter. Students with a levels of 1 to 9 IU/liter were more likely to respond to the challenge dose than those with a baseline anti-HBsAg level of 0 IU/liter (83% versus 50%; $P < 0.001$). The presence of any detectable anti-HBsAg among persons vaccinated in the remote past may indicate the persistence of immune memory.

Hepatitis B vaccination initiated at birth is a safe and effective means of preventing perinatal and childhood hepatitis B virus (HBV) infections (1). After primary vaccination, a decrease in levels of antibody to hepatitis B surface antigen (anti-HBsAg) occurs and most persons vaccinated at birth will have anti-HBsAg levels less than the accepted threshold of protection (10 IU/liter) 10 to 15 years after the primary series (2). Persons beginning employment or training in health care professions are typically required to "prove" immunity against HBV infection (3). As universal infant hepatitis B vaccination has been recommended in the United States since 1991, an increasing proportion of persons presenting for health care employment or training received hepatitis B vaccine in the remote past. Among adults "found" to have an anti-HBsAg level of <10 IU/liter many years after the primary series, demonstration of immune memory by an anamnestic response requires one additional vaccine dose and a second quantitative anti-HBsAg test. In settings such as occupational and student health clinics, where such testing occurs frequently and consumes considerable resources (e.g., vaccine, laboratory costs, staff time, patient visits), a more direct means to identify persons who retain hepatitis B vaccine-induced immunity despite having experienced a decline in anti-HBsAg to <10 IU/liter is desirable. To determine the likelihood of response to a single vaccine challenge dose among college-aged students in American Samoa, where a universal hepatitis B vaccination program was implemented in the 1980s, we compared the serologic response to a single hepatitis B vaccine dose among students found to have an anti-HBsAg level of 0 IU/liter versus those with levels of 1 to 9 IU/liter.

MATERIALS AND METHODS

Study participants. The prevalence of HBsAg in American Samoa was 7% in 1985. As a result, the territory initiated a program of universal hepatitis B immunization starting at birth with plasma-derived vaccine in 1986 and with recombinant vaccine in 1989, which resulted in a high degree of vaccination coverage among infants and young children (4). For this study, participants were recruited from students enrolled in American Samoa Community College in 2010. The criteria for enrolment were (i) an age of 18 to 23 years, (i) verbal or written attestation of hepatitis B vaccination during infancy, and (iii) no history of allergy to hepatitis B vaccine. The target study enrollment was a convenience sample of 250 of the approximately 2,000 students enrolled at the college. The Human Subjects Committees of all participating institutions approved the study protocol.

Hepatitis B vaccine challenge dose and laboratory testing. After written informed consent was obtained, information on demographics, height, weight, risk factors for HBV exposure (e.g., sexual, family history of hepatitis B, drug use), and vaccination history (confirmed by vaccination record, if available) were collected from each participant. Blood was drawn for serologic testing immediately before (baseline) and 2 weeks after a challenge dose of hepatitis B vaccine (20 μg of Engerix) was administered by injection into the deltoid muscle with a standard-size needle. Serum specimens were frozen and shipped to the CDC Hepatitis Reference Laboratory for testing. Baseline specimens were tested for antibody to anti-HBsAg and total antibody to hepatitis B core antigen (anti-HBcAg) with the VITROS ECi Immunodiagnostic System (Ortho-Clinical Diagnostics, Inc., Rochester, NY). Specimens positive for anti-HBcAg were tested for HBsAg and HBV DNA. Postchallenge specimens were tested for anti-HBsAg only. A response to the challenge dose was defined as a postchallenge anti-HBsAg level of ≥10 IU/liter among persons with a

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TABLE 1 Responses to a hepatitis B vaccine challenge dose of college students with a reported history[a] of hepatitis B vaccination during infancy

Baseline anti-HBsAg titer (IU/liter) (*n*)	No. (%) with postchallenge anti-HBsAg titer (IU/liter) of:			GMC (IU/liter)
	0	1–9	≥10	
<10 (130)	12 (9)	34 (26)	84 (65)	
0 (72)	12 (17)	24 (33)	36 (50)[b]	7.1[c]
1–9 (58)	0	10 (17)	48 (83)	99.8

[a] Either written documentation or verbal report by student was accepted.
[b] $P < 0.001$, chi-square or Fisher exact test.
[c] $P < 0.001$, *t* test.

TABLE 2 Subanalysis of responses to a hepatitis B vaccine challenge dose of 42 students with documented receipt of hepatitis B vaccine at birth (≤7 days of age) and a total of three doses completed by 12 months of age

Baseline anti-HBsAg titer (IU/liter) (*n*)	No. (%) with postchallenge anti-HBsAg titer (IU/liter) of:			GMC (IU/liter)
	0	1–9	≥10	
<10 (42)	3 (7)	14 (33)	25 (60)	
0 (24)	3 (13)	10 (42)	11 (46)[a]	9.3[b]
1–9 (18)	0	4 (22)	14 (78)	92.5

[a] $P = 0.025$, chi-square or Fisher exact test.
[b] $P = 0.01$, *t* test.

baseline anti-HBsAg level of <10 IU/liter. The results of serologic testing were not available for the investigators or participants until after completion of the study.

Statistical analysis. To examine hepatitis B immunity, we determined the proportion of participants with serologic evidence of hepatitis B immunity and HBV infection. Among students with baseline anti-HBsAg level of <10 IU/liter, we compared the proportion of those who responded to the challenge dose with an anti-HBsAg level of 0 IU/liter versus those with levels of 1 to 9 IU/liter at the baseline. Data were analyzed with PASW Statistics 18 (SPSS, Inc., Somers, NY). The chi-square test and Fisher exact test were used to compare categorical variables, as appropriate. Geometric mean concentrations (GMCs) of anti-HBsAg were calculated, and the *t* test was used to compare differences between GMCs. Students with no detectable anti-HBsAg were assigned a value of 0.05 IU/liter for calculation of GMCs.

RESULTS

Study population. Of 213 students who volunteered to participate, 212 were enrolled and completed a questionnaire, underwent baseline blood work, and received a challenge dose of hepatitis B vaccine. Only 2 (0.9%) of the 212 were positive for anti-HBcAg, and both were negative for HBsAg and HBV DNA. Of the 212 students enrolled, 153 (72%) returned 2 weeks later for the follow-up anti-HBsAg test. The median age of these 153 students was 20.2 years (25 to 75% interquartile range, 19.2 to 22.3), 60% were female, the median body mass index was 33.8 kg/m^2 (25 to 75% interquartile range, 25.2 to 35.1), 60% reported being sexually active, and 1% had a family history of hepatitis B (nonmaternal). The demographic and vaccination history of students who completed the study was not different from that of those who did not complete it. Of the 153 students who completed the study, 52 (34%) provided only a verbal history of hepatitis B vaccination and 101 (66%) provided documentation of hepatitis B vaccination.

Comparison of responses to a hepatitis B vaccine challenge dose among students with <10 IU/liter at the baseline: baseline anti-HBsAg level of "zero" versus "not zero." Of the 153 students who completed the study, 131 (86%) had an anti-HBsAg level of <10 IU/liter at the baseline; 73 had a level of 0 IU/liter, and 58 had levels of 1 to 9 IU/liter (51 of 58 were <5 IU/liter; baseline GMC, 2.0 IU/liter). Thirty-six (49%) of 73 with a level of 0 IU/liter and 48 (83%) of 58 with levels of 1 to 9 IU/liter responded to the challenge dose ($P < 0.001$) (Table 1). Relative to those with a baseline anti-HBsAg level of 0 IU/liter, students with levels of 1 to 9 IU/liter were more likely to respond to the challenge dose (odds ratio, 4.9; 95% confidence interval, 2.2 to 11.2). The anti-HBsAg GMCs after the challenge dose among students whose baseline anti-HBsAg level was 0 IU/liter versus those with baseline anti-HBsAg levels of 1 to 9 IU/liter were 9.8 IU/liter (range, 0 to 560) and 99.8 IU/liter (range, 1 to 960), respectively ($P = 0.001$). Among the 58 students with anti-HBsAg levels at the baseline ranging from 1 to 9 IU/liter, 21 had a level of ≥3 IU/liter; of these 21 students, 20 responded to the challenge dose.

To determine whether these findings would apply to the subset of students with vaccination records, we performed a subanalysis of those with documentation of hepatitis B vaccination initiated at birth and receipt of three doses by 12 months of age. Of the 101 students with written documentation of hepatitis B vaccination, 47 had records that specified a history of three vaccine doses initiated at birth and completed by an age of 12 months. The demographic characteristics of these 47 students were similar to those of the overall cohort of 153 students. Of these 47 students, 43 (91%) had a baseline anti-HBsAg level of <10 IU/liter; 25 had a level of 0 IU/liter, and 18 had levels of 1 to 9 IU/liter (17 of 18 were <5 IU/liter; baseline GMC, 1.7 IU/liter) (Table 2). Eleven (44%) of 25 with a level of 0 IU/liter and 14 (78%) of 18 with levels of 1 to 9 IU/liter responded to the challenge dose ($P = 0.03$). Relative to those with a baseline anti-HBsAg level of 0 IU/liter, students with levels of 1 to 9 IU/liter were more likely to respond to the challenge dose (odds ratio, 4.5; 95% confidence interval, 1.1 to 17.4). The postchallenge anti-HBsAg GMCs of students with 0 IU/liter at the baseline and those with levels of 1 to 9 IU/liter at the baseline were 10.8 (range, 0 to 560) IU/liter and 92.5 (range, 1 to 960) IU/liter, respectively ($P = 0.01$). Of the 18 students with baseline anti-HBsAg ranging from 1 to 9 IU/liter, 4 had a baseline level of ≥3 IU/liter; of these four, all responded to the challenge dose.

Among the students who completed this study, there was no association of age (18 to 20 years versus 21 to 25 years), ethnicity (all were Pacific Islanders), gender, history of sexual activity, or family history of hepatitis B with the response to a vaccine challenge dose.

DISCUSSION

We describe herein the results of a study of hepatitis B immunity and the response to a single challenge dose among college students in a setting where universal hepatitis B vaccination at birth has been recommended for over 20 years. Consistent with studies among similar-age birth dose cohorts in settings of historically intermediate to high hepatitis B endemicity, nearly 90% of the students had a residual anti-HBsAg level of <10 IU/liter approximately 20 years after the primary vaccination series (2). Although we had no previous serologic test results among the participants to determine when HBV exposure may have occurred, only two stu-

dents were anti-HBcAg positive (only one of these two had documented hepatitis B vaccination dates) and both were negative for HBsAg and HBV DNA.

To our knowledge, this is only one of a few studies to have reported a differential response to a challenge dose among persons with an anti-HBsAg level of 0 IU/liter compared to those with levels of 1 to 9 IU/liter at the baseline. A study in Taiwan that was published in 2007 showed a statistically significantly stronger response in persons with any detectable anti-HBsAg than in those without detectable anti-HBsAg at the baseline (5), although these participants had received four doses of plasma vaccine. In our case, students with a baseline anti-HBsAg level not equal to 0 IU/liter (though still <10 IU/liter) were significantly more likely to achieve a postchallenge anti-HBsAg level of ≥10 IU/liter than were those with a baseline level of 0 IU/liter; postchallenge anti-HBsAg GMCs were 10 times higher among those with any detectable anti-HBsAg at the baseline than among those whose baseline anti-HBsAg was 0 IU/liter. As the primary anti-HBsAg responses of these students to vaccination were not known, some with a baseline anti-HBsAg level of 0 IU/liter could have been primary nonresponders to hepatitis B vaccination or perhaps were never vaccinated for hepatitis B at all. For such students, the receipt of a single vaccine dose might not result in an antibody response of ≥10 IU/liter (1).

Although the results of this study are consistent with others conducted in similar populations, the findings should be interpreted with caution. First, the study is in large part ecological, and the sample population represented only a minority of the students enrolled at the college. Of the participating students, a substantial number lacked or had incomplete vaccination records available to document dates of receipt of hepatitis B vaccine. However, hepatitis B vaccine coverage was reportedly high during the late 1980s in American Samoa (4), and the baseline anti-HBsAg levels and responses to a challenge dose of those with levels of <10 IU/liter at the baseline did not differ greatly between those with and those without documentation of hepatitis B vaccination dates, suggesting that some, if not most, with missing or incomplete records likely received the vaccine on schedule, according to guidelines. Lastly, the package insert for the VITROS ECi anti-HBsAg assay used for this study states that anti-HBsAg levels of <5 IU/liter are "negative" and levels of ≥5 and <12 IU/liter are "indeterminate" (6); nonetheless, we found a statistically significant difference in the response to a challenge dose between students with a baseline anti-HBsAg level of 0 IU/liter and those with levels of 1 to 9 IU/liter. Moreover, as shown in Tables 1 and 2, study participants who had levels of 1 to 9 IU/liter at the baseline (most of whom had a baseline anti-HBsAg level of <5 IU/liter) never had a postchallenge anti-HBsAg level of 0 IU/liter. This supports our contention that anti-HBsAg levels of 1 to 4 IU/liter, although considered "negative," in this study appeared truly distinct from a value of 0 IU/liter.

Among adults who received hepatitis B vaccine in the remote past but who have an ongoing risk of HBV exposure in the future, there is value in the ability to identify those who retain immune memory despite having had a decrease in anti-HBsAg to a level of <10 IU/liter. In addition to the protection of those at risk of infection, there is an interest in conserving the resources—additional vaccine doses, serologic tests, and appointments at student or occupational health clinics—often necessary to identify such persons. Future studies with larger sample sizes might compare the performance of this and other anti-HBsAg assays at the lower levels of detection. Also, studies that aim to identify correlates of cellular immunity to hepatitis B among persons vaccinated in the remote past might consider comparing persons with and those without detectable residual anti-HBsAg, rather than examining all persons with levels of <10 IU/liter as a homogeneous group. From a clinical practice standpoint, the cost-effectiveness of a differential approach to the revaccination of persons found to have an anti-HBsAg level of <10 IU/liter could be compared between those with a level of "zero" and those with a level of "not zero" many years after primary immunization. Most importantly, ongoing surveillance or periodic serosurveys are needed to detect breakthrough infections and illness among health care personnel, health professional students, and other vaccinated persons at risk of HBV exposure to ensure the long-term effectiveness of hepatitis B vaccine.

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