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 level 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.

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 enrollment were (i) an age of 18 to 23 years, (ii) 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
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/m² (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).

**Table 1: Responses to a hepatitis B vaccine challenge dose of college students with a reported history of hepatitis B vaccination during infancy**

<table>
<thead>
<tr>
<th>Baseline anti-HBsAg titer (IU/liter) (n)</th>
<th>No. (%) with postchallenge anti-HBsAg titer (IU/liter) of:</th>
<th>GMC (IU/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (24)</td>
<td>0 (0)</td>
<td>0.05</td>
</tr>
<tr>
<td>1–9 (18)</td>
<td>10 (42)</td>
<td>7.1</td>
</tr>
<tr>
<td>≥10 (9)</td>
<td>3 (13)</td>
<td>25 (60)</td>
</tr>
</tbody>
</table>

*P < 0.001, chi-square or Fisher exact test. *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**

<table>
<thead>
<tr>
<th>Baseline anti-HBsAg titer (IU/liter) (n)</th>
<th>No. (%) with postchallenge anti-HBsAg titer (IU/liter) of:</th>
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*P = 0.025, chi-square or Fisher exact test. *P = 0.01, t test.

**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-
Residual Anti-HBsAg and Response to Vaccine Challenge

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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REFERENCES