Antibody Levels and Protection after Hepatitis B Vaccine: Results of a 22-Year Follow-Up Study and Response to a Booster Dose

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Background. The duration of protection in children and adults (including health care workers) resulting from the hepatitis B vaccine primary series is unknown.

Methods. To determine the protection afforded by hepatitis B vaccine, Alaska Native persons who had received plasma-derived hepatitis B vaccine when they were 6 months of age were tested for antibody to hepatitis B surface antigen (anti-HBs) 22 years later. Those with levels <10 mIU/mL received 1 dose of recombinant hepatitis B vaccine and were evaluated on the basis of anti-HBs measurements at 10–14 days, 30–60 days, and 1 year.

Results. Of 493 participants, 60% (298) had an anti-HBs level ≥10 mIU/mL. A booster dose was administered to 164 persons, and 77% responded with an anti-HBs level ≥10 mIU/mL at 10–14 days, reaching 81% by 60 days. Response to a booster dose was positively correlated with younger age, peak anti-HBs response after primary vaccination, and the presence of detectable anti-HBs before boosting. Considering persons with an anti-HBs level ≥10 mIU/mL at 22 years and those who responded to the booster dose, protection was demonstrated in 87% of the participants. No new acute or chronic hepatitis B virus infections were identified.

Conclusions. The protection afforded by primary immunization with plasma-derived hepatitis B vaccine during childhood and adulthood lasts at least 22 years. Booster doses are not needed.

Hepatitis B vaccine (both plasma derived and recombinant) has been shown to be highly efficacious in preventing infection with hepatitis B virus (HBV). Immunization with this vaccine starting at birth has dramatically reduced the subsequent development of chronic hepatitis B in young children from perinatal or early childhood exposure to HBV [1, 2]. In US health care workers, the incidence of acute HBV infection fell substantially after Occupational Safety and Health Administration requirements for vaccination were implemented in the mid-1990s. However, the duration of protection conferred by hepatitis B vaccination is incompletely understood [3, 4]. Studies of infants immunized starting at birth suggest that some loss of immune memory occurs with aging [5–7]. Less is known about the persistence of antibody to hepatitis B surface antigen (anti-HBs) and immune memory after vaccination of older children and adults.

In 1981, immediately after licensure of hepatitis B vaccine in the United States, we immunized a cohort of 1578 Alaska Native adults and children 6 months or older with 3 doses of plasma-derived hepatitis B vaccine [8]. We performed yearly serologic testing for 11 years and again at 15 years [4, 9, 10]. Fifteen years after their
first dose of vaccine, 66% of participants had an anti-HBs level ≥10 mIU/mL, the level thought to provide protection. No participants had clinical signs or symptoms of acute symptomatic hepatitis, and none developed a chronic HBV infection.

Twenty-two years after the primary vaccination series, we revisited a subset of this cohort to (1) determine the proportion that had an anti-HBs level ≥10 mIU/mL and (2) evaluate HBV immune memory by administering a booster dose of hepatitis B vaccine to those with an anti-HBs level <10 mIU/mL.

METHODS

Patients. Beginning in October 2003, we enrolled participants from 7 of the 15 villages in the 1981 immunogenicity study who had a documented response to a primary hepatitis B vaccination series. Persons from those 7 villages who had moved to Anchorage or to the largest town in the region (Bethel, Alaska) were also invited to participate. Persons in the 7 villages did not differ from those in the other 8 villages in terms of sex, age, initial response to the HBV vaccination series, or anti-HBc level at the 15-year follow-up time point.

Persons who had received an additional dose of vaccine in the intervening years were excluded. Persons in Anchorage were recruited beginning in 2005.

Laboratory testing. Serologic specimens from participants were tested for anti-HBs (ETI-AB-AUK PLUS and ABAU-STD-SET; DiaSorin) at the Arctic Investigations Program in Anchorage. The lower limit of detection of this assay was defined as an anti-HBs value ≥2 mIU/mL. Antibody to hepatitis B core antigen (anti-HBc) was determined at the Alaska Native Medical Center (Corzyme; Abbott Laboratories), and positive specimens were further tested for hepatitis B surface antigen (HBsAg) (Auszyme Monoclonal; Abbott Laboratories) and for HBV DNA by nested polymerase chain reaction using primers specific for the S gene, as described elsewhere [11]. An HBV infection was defined as a positive anti-HBc or HBsAg result, as described elsewhere [4, 9, 10].

Persons with an anti-HBs level ≥10 mIU/mL were considered to be immune. Those with an anti-HBs level <10 mIU/mL were offered an intramuscular booster dose of 10 µg of hepatitis B vaccine (Recombivax HB; Merck). Anti-HBs concentration was determined at 10–14 days, 30–60 days, and 1 year after boosting. A positive booster dose response was defined as an anti-HBs level rising to ≥10 mIU/mL at either 10–14 or 30–60 days. Vaccine recipients were given a 3-day diary card to record adverse events.

The present study was approved by the Centers for Disease Control and Prevention Institutional Review Board, the Alaska Area Institutional Review Board, and 3 Alaska Native tribal health boards: the Yukon-Kuskokwim Health Corporation, the Alaska Native Tribal Health Consortium, and the Southcentral Foundation. All participants provided written informed consent.

Hospital and village clinic records were reviewed for any evidence of an illness in persons identified to be anti-HBc positive who were not already identified to be so in previous surveys.

Statistical analysis. Qualitative anti-HBs levels are the proportion of participants with ≥10 mIU/mL and were compared by the likelihood ratio χ² test for categorical covariates and by the Cochran-Armitage test for trend for ordinal and continuous covariates. Quantitative anti-HBs levels are log transformed; undetectable concentrations were assigned a value of 1.0 mIU/mL before transformation. Anti-HBs levels (log transformed) were compared between groups by the t test, the paired t test, or analysis of variance, as appropriate. Exact confidence intervals (CIs) for the incidence of new HBV infections were calculated as derived by Garwood [12]. We compared the time since loss of anti-HBs (time when the anti-HBs level declined to <10 mIU/mL) between responders and nonresponders to a booster dose of hepatitis B vaccine by the Wilcoxon rank sum test. The estimated time of anti-HBs loss was calculated as the chronological midpoint between the last anti-HBs level ≥10 mIU/mL and the first anti-HBs level <10 mIU/mL.

We used logistic regression to test multivariate associations with protective immunity at 22 years and with response to the booster dose. The best-fit regression model was determined by nonautomatic backward selection following the strategy of Hosmer and Lemeshow [13]. All 2-way interactions were evaluated at P < .05. For protective immunity at 22 years, we evaluated age at primary vaccination series, sex, and anti-HBs level after primary series. For response to a booster dose, we evaluated age at booster dose, sex, anti-HBs level after primary series, and preboost anti-HBs level. Age was treated as a categorical variable in all statistical models. We used the Spearman rank order statistic to assess collinearity among predictor variables. Because the time since loss of anti-HBs was highly correlated with anti-HBs level after the primary series and with preboost anti-HBs level, it was not considered in the model. All statistical analyses were conducted using SAS software (version 9.0; SAS), and all P values are 2-sided. Statistical analysis was performed by 2 of the authors (D.B. and L.B.).

RESULTS

Anti-HBs levels. Of 698 persons eligible, 493 (71%) participated (Figure 1). There were 455 participants residing in the 7 villages plus Bethel, and there were 38 in Anchorage. The age, sex, and anti-HBs level after the primary vaccination series were similar for participating persons (n = 493) and eligible nonparticipating persons (n = 205) (data not shown).

The age, sex, and anti-HBs level on study enrollment are
shown in Table 1. At the 22-year follow-up time point, the GMC of anti-HBs among the 493 participants was 21.5 mIU/mL; 298 (60%) had an anti-HBs level $\geq 10$ mIU/mL. Among the 195 persons with an anti-HBs level $< 10$ mIU, 138 (71%) had detectable antibody (anti-HBs level between 2 and 9.9 mIU/mL). In a multivariate logistic regression model, initial anti-

HBs level ($P < .001$), age class ($P < .001$), and sex ($P = .04$) were associated with an anti-HBs level $\geq 10$ mIU/mL (Table 1).

**Breakthrough HBV infections.** Five participants (1%) had a serum specimen positive for anti-HBc; none were positive for HBsAg. All 5 were positive for anti-HBc on $\geq 3$ previous consecutive evaluations; none were ever positive for HBV DNA.
Table 1. Level of Antibody to Hepatitis B Surface Antigen (Anti-HBs) 22 Years after Hepatitis B Vaccination, by Sex, Age, and Postvaccination Anti-HBs Level after the Primary Series—Alaska, 2003–2004

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of cohort</th>
<th>Anti-HBs GMC, mIU/mL</th>
<th>Participants with ≥10 mIU/mL, proportion (%)</th>
<th>Univariate P</th>
<th>Multivariate P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>280 (57)</td>
<td>19.2</td>
<td>160/280 (57)</td>
<td>.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.04</td>
</tr>
<tr>
<td>Male</td>
<td>213 (43)</td>
<td>25.0</td>
<td>138/213 (66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age at primary vaccination (age at 22-year follow-up)</strong></td>
<td></td>
<td></td>
<td></td>
<td>.008&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>0.5–4 (22–26) years</td>
<td>71 (14)</td>
<td>14.1</td>
<td>40/71 (56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–19 (27–41) years</td>
<td>268 (54)</td>
<td>29.1</td>
<td>179/268 (67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–49 (42–71) years</td>
<td>119 (24)</td>
<td>17.1</td>
<td>64/119 (54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50 (&gt;72) years</td>
<td>35 (7)</td>
<td>11.1</td>
<td>15/35 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anti-HBs level after primary series</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>10–99 mIU/mL</td>
<td>56 (11)</td>
<td>3.4</td>
<td>8/57 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100–999 mIU/mL</td>
<td>168 (34)</td>
<td>11.1</td>
<td>64/168 (38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000–4999 mIU/mL</td>
<td>157 (32)</td>
<td>33.4</td>
<td>120/157 (76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5000 mIU/mL</td>
<td>111 (23)</td>
<td>239.9</td>
<td>106/111 (96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>493 (100)</td>
<td>21.5</td>
<td>298/493 (60)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** GMC, geometric mean concentration.

* Logistic regression analysis.
* Likelihood ratio <sup>x</sup><sup>2</sup> test.
* For age 5–19 years versus all others, <sup>P</sup> = .002; for ≥50 years of age versus all others, <sup>P</sup> = .03.
* Cochran-Armitage test for trend.

Three persons previously positive for anti-HBc were no longer positive at this follow-up time point. The cumulative incidence of HBV infections over the course of 22 years of follow-up was 0.74 (95% CI, 0.31–1.45) cases per 1000 persons per year.

**Response to a booster dose of hepatitis B vaccine.** Of 195 eligible persons with a baseline anti-HBs level <10 mIU/mL, 165 (85%) received a booster dose of hepatitis B vaccine (Figure 1). The median age of the 164 persons who provided a serum specimen after the booster dose was 37 years (range, 22–83 years); 99 (60%) were female.

Responses to the booster dose are shown in Table 2. Of the 155 tested at 10–14 days and 30–60 days, 120 (77%) responded with an anti-HBs level ≥10 mIU/mL. If 13 persons who did not respond or were not tested at 10–14 days but who responded at 30–60 days are included, then 133 (81%) of 164 had a protective anti-HBs level measured 10–60 days after the booster dose. The GMC for the group measured 30–60 days after the booster dose was significantly lower than that measured 6 months after the primary vaccination series administered in 1981 (60.6 vs 280.9 mIU/mL; <sup>P</sup> = .001) [8]. Of the 155 participants tested 1 year after the booster dose, 63 (41%) had an anti-HBs level ≥10 mIU/mL; the GMC had fallen to 8.0 mIU/mL.

We evaluated results from the 143 persons who had serum drawn at all 3 follow-up time points (Table 3). At 10–14 days, there was a difference in the proportion with an anti-HBs level ≥10 mIU/mL by age (<sup>P</sup> = .01) (Table 3). This difference was accounted for primarily by persons ≥60 years of age, because this age group had a lower percentage of participants with an anti-HBs level ≥10 mIU/mL (<sup>P</sup> = .004) and a significantly lower anti-HBs GMC at 10–14 days (<sup>P</sup> < .001) and at 30–60 days (<sup>P</sup> = .006) compared with all other groups combined (Table 3). The response to the booster dose at all time points was positively correlated with the participants’ response to the primary vaccination series (Table 3).

Those participants who had a preboost anti-HBs level 2–9.9 mIU/mL had a significantly higher probability of achieving a booster response than did those with no measurable anti-HBs.
level (P < .001 at all 3 follow-up time points). Persons with a longer time since the loss of a protective anti-HBs level were less likely to respond to a booster dose than were those with a more recent loss (Table 3). The proportion of participants responding to the booster dose (as evidenced by an anti-HBs level ≥10 mIU/mL) did not differ by village and ranged from 76% (13/17) to 86% (19/22). At the time of the original vaccine project in 1981, the prevalence of persons positive for HBsAg in these villages ranged from 1.2% to 8.6%; at the time of the 22-year follow-up, the prevalence had fallen to a range of 0.3%–5.3%.

In a multivariate model, we found that the response to a booster dose was significantly associated with anti-HBs level after the primary vaccination series (P < .001) and age at the time of the booster dose (P = .02). The predicted probabilities of responding to a booster dose 22 years after the primary series are displayed in Figure 2. There was no difference in the proportion who responded between those <30 years of age and those 30–39 years of age; therefore, we combined these 2 age classes. Similarly, there was no difference between participants with an anti-HBs level of 500–999 and ≥1000 mIU/mL after the primary series, so we also combined these 2 groups. Participants aged 40–59 years and those who had an anti-HBs level ≥500 mIU/mL after the primary series were most likely to respond to the booster dose (Figure 2). No serious adverse events to the booster dose were reported.

Of the 493 study participants, 431 (87% [95% CI, 84.5%–90.3%]) demonstrated long-term protection from hepatitis B vaccination, as defined by an anti-HBs level ≥10 mIU/mL at the screening visit or by a response to a booster dose of ≥10 mIU/mL (Figure 1). Excluding persons who had a level <10 mIU/mL at the initial screening visit but who did not receive a booster dose (n = 31), a total of 93% (95% CI, 91.0%–95.6%) of the cohort was protected. By applying the results of the multivariate logistic regression model to these 31 persons stratified by age and primary response level, we estimate that 25 (80%) would have responded to the booster dose if they had participated. Combining the observed response in 431 of 462 participants with the predicted response in 25 of 31 participants, we estimate that 92.5% of the cohort was protected.

### Table 3. Level of Antibody to Hepatitis B Surface Antigen (Anti-HBs) after a Booster Dose of Hepatitis B Vaccine in 145 Persons with an Anti-HBs Level <10 mIU/mL 22 Years after a Primary Hepatitis B Vaccination Series Who Had Anti-HBs Levels Measured at All 3 Follow-up Time Points—Alaska, 2003–2004

<table>
<thead>
<tr>
<th>Time since booster dose</th>
<th>Characteristic</th>
<th>GMC, mIU/mL</th>
<th>% with &gt;10 mIU/mL</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–14 days</td>
<td>Sex</td>
<td>GMC, mIU/mL</td>
<td>% with &gt;10 mIU/mL</td>
<td>P*</td>
</tr>
<tr>
<td>30–60 days (visit 2)</td>
<td></td>
<td>GMC, mIU/mL</td>
<td>% with &gt;10 mIU/mL</td>
<td>P*</td>
</tr>
<tr>
<td>1 year</td>
<td></td>
<td>GMC, mIU/mL</td>
<td>% with &gt;10 mIU/mL</td>
<td>P*</td>
</tr>
</tbody>
</table>

NOTE. GMC, geometric mean concentration.

*P values are for comparison of the percentages of participants with an anti-HBs level >10 mIU/mL.
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Participants who were vaccinated during older childhood or adolescence are similar in age to children who were first vaccinated during catch-up hepatitis B vaccination programs in the United States and elsewhere [2]. Our study suggests that children vaccinated through these catch-up programs are likely to be protected from HBV infection for decades.

Among participants with an anti-HBs level <10 mIU/mL, we found that the probability of boosting was positively associated with the presence of detectable anti-HBs (2–9.9 mIU/mL) and was negatively associated with a documented anti-HBs level <10 mIU/mL for >7 years. This suggests that the ability to mount an anamnestic response to a booster dose of hepatitis B vaccine may wane in those who do not have detectable anti-HBs for prolonged periods of time. No difference was found in the proportion of participants who had a booster response by village, despite a wide variation in the prevalence of persons positive for HBsAg.

While the protective level of anti-HBs after primary vaccination needed to prevent HBV infection has been shown to be 10 mIU/mL in controlled clinical trials [16, 17], the level of antibody necessary to provide long-term protection against HBV infection is unknown. It is likely that humoral antibody concentration might not be the best indicator of long-term protection, because persons might be protected against chronic infection by other immune mechanisms, such as cellular immunity. Because HBV infection has such a long incubation period (average, 60–90 days), there might be time for residual cellular immunity to prevent clinical illness and chronic infection. The appearance of anti-HBc in a small minority in this cohort might indicate such breakthrough infections in persons who retain sufficient cellular immunity to prevent symptomatic or chronic infection. Assays to measure correlates of protection many years after vaccination are needed.

Previous studies of anti-HBs persistence after hepatitis B vaccination of children and adults from regions of both high and low endemicity have shown excellent protection 4–10 years after immunization, similar to the results through the first 10 years of follow-up of this Alaska cohort [4, 18–21]. The few published studies following subjects after initial hepatitis B vaccination beyond 10 years did not evaluate response to a booster dose but found persistence of anti-HBs up to 18 years [6, 22, 23], similar to our study.

Follow-up studies of children vaccinated against hepatitis B beginning at birth have demonstrated the presence of protective anti-HBs levels at 14 years of age in <10% of those who received a 3-dose schedule and in only 37% of those who received a 4-dose schedule. This is a more rapid decline in anti-HBs level compared with that observed in studies of persons vaccinated as older children or adults [3, 5–7, 24]. In addition, 29%–50% of adolescents immunized at birth did not demonstrate an

DISCUSSION

We report here findings from the largest and longest follow-up study to date of children and adults who responded to primary vaccination with plasma-derived hepatitis B vaccine. After 22 years, an estimated 92.5% of primary responders had evidence of continued protection (anti-HBs level ≥10 mIU/mL or humoral immune memory defined by response to a booster dose). Furthermore, no participants became chronically infected, had laboratory evidence of acute hepatitis B, or had detectable HBV DNA.

When this cohort was recruited in 1981, HBV infection was hyperendemic among Alaska Native people in this region, with a prevalence of HBsAg of 8.2% (range, 0–27.5% per village) [8]. Since then, the introduction of hepatitis B vaccination as a routine childhood immunization coupled with a large-scale catch-up program involving all age groups has virtually eliminated new infections in Alaska. The incidence of acute symptomatic hepatitis B has fallen from 200 cases per 100,000 population in 1981 to 0 cases per 100,000 population since the prevalence of HBsAg in the proportion of participants who had a booster response by village, despite a wide variation in the prevalence of persons positive for HBsAg.

Figure 2. Predicted percentage of persons responding to a hepatitis B vaccine booster dose (antibody to hepatitis B surface antigen [anti-HBs] level >10 mIU/mL, by age and anti-HBs level after the primary vaccination series—Alaska, 2003–2004. Error bars indicate 95% confidence intervals from logistic regression.
anamnestic response to a booster dose [24, 25]. A difference in the duration of immunity among those vaccinated beginning at birth compared with older children and adults could be a consequence of a less-mature immune system at the time of primary immunization. Further evaluation of long-term protection among cohorts vaccinated beginning at birth should be a high priority [2].

A potential limitation of the generalizability of our findings deserves mention. Participants in our study received the primary hepatitis B vaccination series with the plasma-derived vaccine, which is no longer used in the United States. Antibody response and vaccine effectiveness in this and other populations that received the plasma-derived vaccine are similar to those demonstrated in populations vaccinated with recombinant vaccines beginning in the late 1980s [2]. For these reasons, we believe our findings are applicable to persons who received either vaccine type. However, the validity of extending our findings to persons immunized with recombinant hepatitis B vaccines needs to be confirmed by long-term studies similar to ours.

In conclusion, we believe the results of our study are directly relevant to health care workers, including those vaccinated with plasma-derived vaccine in the early 1980s, and to children and adolescents who were vaccinated during catch-up hepatitis B vaccination programs in the 1990s [2]. In light of the strong evidence we present here, hepatitis B vaccine booster doses are not currently indicated. Our unique longitudinal data are crucial for the ongoing evaluation of public health vaccination strategies and programs. For these reasons, we plan additional follow-up of this cohort 30 years after the primary vaccination series.

Acknowledgments

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References