

# Antibody Levels and Protection after Hepatitis B Vaccination: Results of a 15-Year Follow-up

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**Background:** The duration of protection afforded by hepatitis B vaccination is unknown.

**Objective:** To determine antibody persistence and protection from hepatitis B virus (HBV) infection.

**Design:** Prospective cohort study.

**Setting:** 15 villages in southwest Alaska.

**Participants:** 1578 Alaska Natives vaccinated at age 6 months or older.

**Intervention:** During 1981–1982, participants received 3 doses of plasma-derived hepatitis B vaccine. This cohort was followed annually over the first 11 years, and 841 (53%) persons were tested at 15 years.

**Measurements:** Antibody to hepatitis B surface antigen (anti-HBs), markers of HBV infection, and testing to identify HBV variants.

**Results:** Levels of anti-HBs in the cohort decreased from a geometric mean concentration of 822 mIU/mL after vaccination to 27

mIU/mL at 15 years. Initial anti-HBs level, older age at vaccination, and male sex were associated with persistence of higher anti-HBs levels at 15 years when analyzed by a longitudinal linear mixed model. After adjustment for initial anti-HBs level and sex, those vaccinated at age 6 months to 4 years had the lowest anti-HBs level at 15 years. Asymptomatic breakthrough infections were detected in 16 participants and occurred more frequently in persons who did not respond to vaccination than those who responded ( $P = 0.01$ ). Among infected persons with viremia, 2 were infected with wild-type HBV and 4 had HBV surface glycoprotein variants, generally accompanied by wild-type HBV.

**Limitations:** The loss of participants to follow-up at 15 years was 47%. However, characteristics of persons tested were similar to those of persons lost to follow-up.

**Conclusions:** Hepatitis B vaccination strongly protected against infection for at least 15 years in all age groups. Antibody levels decreased the most among persons immunized at 4 years of age or younger.

*Ann Intern Med.* 2005;142:333-341.

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Universal vaccination of infants with hepatitis B vaccine is included in the immunization programs of most countries and has been shown to be effective in reducing the rate of chronic hepatitis B virus (HBV) infection (1, 2). Protection has been demonstrated in persons and populations vaccinated for 5 to 10 years, and rates of asymptomatic breakthrough HBV infection have been extremely low (3–9). However, the duration of protection afforded by hepatitis B vaccination beyond 10 years and the possible need for booster doses of this vaccine are unknown.

Alaska Natives have a high prevalence of chronic HBV infection, primarily acquired during early childhood (10). Between November 1981 and May 1982, Alaska Natives residing in 15 villages in southwest Alaska were enrolled in a cohort study to ascertain the immunogenicity and long-term effectiveness of hepatitis B vaccination (11–14). We report data on the persistence of antibodies to hepatitis B surface antigen (anti-HBs), incidence of HBV infection, and the genetic characteristics of the HBV isolates in persons with breakthrough infections 15 years after initial vaccination of this cohort.

## METHODS

### Participants and Data Collection

A total of 1578 Alaska Natives who were serologically negative for hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) were vac-

nated on a 0-, 1-, and 6-month schedule with 3 doses of plasma-derived hepatitis B vaccine (Heptavax, Merck & Co., Inc., West Point, Pennsylvania) beginning in 1981 (11). Persons younger than 20 years of age received the 10- $\mu$ g dose, and adults received the 20- $\mu$ g dose. Of the 1578 persons vaccinated, 1436 (91%) were tested for an anti-HBs response 6 months after the last vaccine dose.

From 1982 to 1992, serum specimens were obtained annually and once again during 1996 from as many of the 1578 consenting participants as possible. The Institutional Review Boards of the Alaska Area Native Health Service, the Indian Health Service, the Centers for Disease Control and Prevention, and the Yukon-Kuskokwim Health Corporation and the Norton Sound Regional Alaska Native health boards approved this study. All participants 18 years of age and older and parents of children younger than 18 years of age had provided signed informed consent to par-

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**Context**

Although administration of hepatitis B vaccine for infants is routine practice in many countries, we do not know whether the protection that this vaccine offers lasts beyond 10 years. Such information is essential to develop policies about booster vaccination.

**Contribution**

Of 841 Alaska Natives who received 3 doses of hepatitis B vaccination during 1981–1982 and were followed for 15 years, 84% had protective levels of antibody to hepatitis B surface antigen that indicated continued protection. The greatest decline in antibody levels occurred in people who received vaccine before 4 years of age. Definite asymptomatic breakthrough infections occurred in 16 participants.

**Cautions**

Only about half of the initial cohort of 1578 was available for testing at 15 years.

—The Editors

participate in the study; children older than 7 years of age gave verbal assent. The number of HBsAg-positive persons in each village was obtained from a registry used to follow patients with chronic HBV infection (15).

**Serologic Testing**

All serum specimens were tested for HBsAg, anti-HBs, and anti-HBc by radioimmunoassay using commercial test kits (Abbott Laboratories, Abbott Park, Illinois). At the initial testing of the cohort for anti-HBs, results were reported in sample ratio units. However, subsequent anti-HBs results were reported in milli international units (mIU) per mL using a World Health Organization reference standard (12–14). To ensure comparability of results over time, all serum specimens from each participant with sufficient volume (99.9% of all specimens collected during the study) were retested to determine anti-HBs levels in mIU/mL.

**Detection of HBV DNA and Nucleic Acid Sequencing**

Hepatitis B virus DNA was extracted from serum specimens (50  $\mu$ L) of participants with serologic markers of HBV infection by using commercially available reagents (MasterPure Complete DNA and RNA Purification Kit, Epicentre Technologies, Madison, Wisconsin), as described previously (16, 17). The HBsAg genomic region was then amplified by dilution cloning polymerase chain reaction by using previously described primers and methods to identify circulating variants of HBsAg (16, 17). The polymerase chain reaction products were purified and the nucleic acid sequence of the amplified region were determined by using prism dye or dRhodamine terminator cycle reactions (Applied Biosystems, Foster City, California) and automated

sequencing (ABI Model 373 or 377, Applied Biosystems) (18). Sequence data were further analyzed by Sequence Navigator (ABI) and GCG software (19).

**Definitions**

The initial anti-HBs level was measured 6 months after the third dose of vaccine and 1 year after the first dose of vaccine. Participants with an initial anti-HBs level of at least 10 mIU/mL were considered vaccine responders. An anti-HBs level of 2 mIU/mL or greater was considered a positive result on subsequent specimens. A booster response was defined as a 2-fold or greater increase in anti-HBs levels between serologic test results.

A definite HBV infection in a participant was defined as 1) 2 or more consecutive serum specimens positive for anti-HBc more than 1 year after the initial vaccine dose, 2) a single positive anti-HBc result with a positive HBV DNA result, or 3) any HBsAg-positive test result. A “possible” HBV infection was defined as a single positive or 2 non-consecutive positive anti-HBc results. Participants who developed anti-HBc were interviewed for history of icterus or other clinical signs or symptoms compatible with acute hepatitis, and village and hospital medical records were reviewed for evidence of an illness compatible with viral hepatitis.

**Statistical Analysis**

Among persons who inadvertently received additional doses of hepatitis B vaccine during the follow-up period, anti-HBs results after the additional vaccine dose or doses were excluded from the analyses. Results for anti-HBs among persons with definite HBV infections were excluded from analyses after anti-HBc appeared.

The primary outcomes in this study were the cumulative number of persons with a definite HBV infection during all follow-up years and the anti-HBs levels at the 15-year follow-up. The definitions of age classes (0 to 4 years, 5 to 19 years, 20 to 49 years,  $\geq 50$  years) were similar to those in a previously published analysis of this cohort (14). Although these data have been presented previously (11–14), we have provided the proportion of persons initially responding to vaccination. Quantitative anti-HBs levels are presented as geometric mean concentrations (GMCs). In bivariate analyses, analysis of variance was used to test the 15-year anti-HBs concentrations (log-transformed). Incidence rates of definite HBV infection were compared by using the Fisher exact test.

We analyzed factors associated with anti-HBs levels over the 15 years after the first vaccine dose by using a linear mixed model (PROC MIXED in SAS version 9.1, SAS Institute, Inc., Cary, North Carolina) (19). We chose a longitudinal mixed linear model because it makes inferences by using information from the entire cohort collected at all follow-up time points. Levels of anti-HBs were log-transformed before analysis, and concentrations of 0 mIU/mL were assigned a value of 1.0 mIU/mL. Factors considered in the model were time (entered as a continu-

ous covariate; linear and quadratic term were considered), age class at initial vaccination, sex, the log of the initial anti-HBs level, presence of an HBsAg-infected person in the household at the start of the study, and the proportion of village residents with chronic HBV infection at the end of follow-up, along with interaction terms involving time. Significance of 1 factor alone, such as age or sex, is called a main effect and is not of primary interest for this presentation. Of primary interest are the interaction terms between time and other factors. A significant interaction of time with another variable, such as sex, indicates that the decline in anti-HBs level differed between males and females. We obtained parameter estimates by using restricted maximum likelihood. We used an unstructured covariance matrix to account for dependence of observations across time within individuals. Backward elimination of statistically nonsignificant terms yielded a final model of main effects and time interaction terms. If the time interaction term was statistically significant, the main effect term remained in the model regardless of statistical significance. We used the Wald chi-square statistic to test covariates. Contrast tests were 2-sided, and an  $\alpha$  level of 0.05 was required. We used residual plots to evaluate model fit.

A secondary outcome was a boost in anti-HBs level at the 11- or 15-year follow-up among persons without additional doses of vaccine. We used the chi-square test or the Cochran–Mantel–Haenszel test to compare age and sex of persons with a booster response to those of persons without a booster response at both follow-up years. All *P* values were exact where appropriate and were 2-sided; results were considered statistically significant at the 0.05 level. We conducted analyses by using StatXact4 (Cytel Software Corp., Cambridge, Massachusetts) and SAS software, version 9.1 (SAS Institute, Inc.).

### Missing Data

Throughout the entire study, anti-HBs determinations were observed for 68% of all potential observational time points (Appendix Table 1, available at [www.annals.org](http://www.annals.org)). The 15 remote rural Alaskan study villages are accessible only by airplane. During each year, study personnel flew into each village for 1 to 2 days and attempted to recruit all available participants. Persons not available or out of the village for the day were considered missed for that time point. Persons who moved out of 1 of the 15 original villages were considered lost to follow-up unless they relocated to Anchorage, Alaska, or the regional hub city, Bethel. Primary analysis used a linear mixed model, which assumed that the missing data were missing at random (dependent on observed data but independent of the anti-HBs level at the time of the missed observation). Appendix Table 1 shows results on the extent of missing data.

Characteristics of the 783 persons tested at 15 years and their respective anti-HBs levels were compared with those of persons not participating (Appendix Table 2, available at [www.annals.org](http://www.annals.org)). In univariable analysis, we

used the chi-square test and the Wilcoxon rank-sum test to compare categorical and continuous covariates, respectively, between persons participating and not participating in the 15-year follow-up study. We used logistic regression to test for differences in initial anti-HBs levels between participants and nonparticipants after adjustment for age class and sex. For each person, we calculated the proportion of follow-up years (before dropout) with intermittent observations for comparisons, and we compared these proportions among groups by use of the Kruskal–Wallis test.

Sex and age class at time of initial vaccination significantly differed between the groups (Appendix Table 2). The groups did not differ for median initial anti-HBs level or the percentage of persons initially responding to the vaccine. After adjustment for the differences in sex and age class, median initial anti-HBs level and the percentage of persons initially responding to the vaccine did not differ. Characteristics of persons with more intermittent missing values were similar to those of persons not participating in the 15-year follow-up. Persons with more intermittent missing values differed by age class and sex but not by initial anti-HBs level (before and after adjustment for sex and age class).

### Role of the Funding Source

The Centers for Disease Control and Prevention and the Indian Health Service funded this study. Merck & Co., Inc., Vaccine Division, provided hepatitis B vaccine and partially supported travel to the villages during the first 10 years of follow-up. However, Merck had no role in the design, conduct, or analysis of the study or in the preparation or review of the manuscript.

## RESULTS

Since the last report on this cohort (14), 967 (61%) persons were tested at 11 years (November 1992) and 841 (53%) persons were tested at 15 years (November 1996) after their first dose of vaccine (Appendix Table 1). Sixteen persons had evidence of definite HBV infection, of whom 10 were tested at the 15-year follow-up. Seventy-eight persons had received an additional dose of vaccine, of whom 48 were drawn at the 15-year follow-up. Levels of anti-HBs were used in analysis from 783 (93%) of the 841 persons tested at 15 years (Appendix Table 1).

### Anti-HBs Levels over Time

The GMC of the 1436 persons tested 6 months after their third dose was 822 mIU/mL, and 1351 (94%) were considered vaccine responders. Among participants 6 months to 19 years of age, 99% (990 of 1003) responded to the vaccine series compared with 91% (263 of 289) of those 20 to 49 years of age and 68% (98 of 144) of those 50 years of age and older; the GMCs for these 3 age groups were 1748, 242, and 50 mIU/mL, respectively.

Fifteen years after their first dose of vaccine, 661 (84%) of the 783 participants tested had anti-HBs levels of

**Table 1. The Predicted Geometric Mean Concentrations of Antibody to Hepatitis B Surface Antigen 15 Years after Initial Hepatitis B Vaccination from Linear Mixed Model\***

Sex and Age Class	Predicted Anti-HBs Level, mIU/mL		
	Initial Anti-HBs Level		
	100 mIU/mL	1000 mIU/mL	10 000 mIU/mL
<b>Female</b>			
0–4 y	1.8	11.6	72.9
5–19 y	4.3	27.0	169.9
≥20 y	8.4	52.9	332.9
<b>Male</b>			
0–4 y	2.6	16.6	104.1
5–19 y	6.1	38.6	242.8
≥20 y	12.0	75.6	475.5

\* Anti-HBs = antibody to hepatitis B surface antigen.

2 mIU/mL or greater, a level considered positive, and 517 of 783 (66%) had anti-HBs levels of 10 mIU/mL or greater. Of those, 144 (18%) had anti-HBs levels between 2 and 9.9 mIU/mL, 299 (38%) had levels between 10 and

99.9 mIU/mL, and 218 (28%) had levels greater than 100 mIU/mL. The GMC in this group was 27 mIU/mL. The GMC at 15 years differed by age group at initial vaccination ( $P < 0.001$ ). The GMC in persons vaccinated at age 5 to 19 years was 42 mIU/mL 15 years later compared with 23 mIU/mL in those vaccinated at age 6 months to 4 years, 19 mIU/mL for those vaccinated at age 20 to 49 years, and 9 mIU/mL for those vaccinated at 50 years of age or older. Of the 677 participants who responded to hepatitis B vaccine with an initial anti-HBs level of 10 mIU/mL or greater, 88% (599) had detectable HBs at 15 years; 127 (19%) had anti-HBs levels between 2 and 9.9 mIU/mL, 270 (40%) had levels between 10 and 99.9 mIU/mL, and 202 (30%) had levels greater than 100 mIU/mL at 15 years. The overall GMC among initial responders was 32 mIU/mL at 15 years and was 23 mIU/mL, 46 mIU/mL, 24 mIU/mL, and 15 mIU/mL for those vaccinated at age 6 months to 4 years, 5 to 19 years, 20 to 49 years, and 50 years of age or older, respectively.

Levels of anti-HBs 15 years after the first vaccine dose varied by level of the initial anti-HBs response ( $P < 0.001$ , controlling for age class). Persons with an initial anti-HBs

**Table 2. Antibody Concentrations and Markers of Hepatitis B Virus Infection in 24 Study Participants with Evidence of Breakthrough Hepatitis B during 15 Years after Hepatitis B Immunization\***

Age at First Vaccine Dose, y	Sex	Time from First Dose to Anti-HBc Positivity, y	Anti-HBs Level, mIU/mL			HBV DNA Status	HBV Conversion Status†
			Highest before Infection	1 y before Infection	At Time of First Anti-HBc-Positive Result		
22	Female	1	22	NA	214‡	Positive	Definite
54	Female	2	5	5	604	Positive	Definite
44	Female	4	505	173	176	Negative	Definite
45	Female	4	8	1	3026	Positive	Definite
11	Female	5	518	30	21	Positive	Definite
1 <sup>8/12</sup>	Male	5	608	54	183	Negative	Definite
47	Male	5	37	0	209§	Positive	Definite
25	Male	5	181	18	16	Negative	Definite
46	Female	6	44	0	1424	Negative	Definite
46	Female	7	2	NA	229	Negative	Definite
1 <sup>4/12</sup>	Female	7	1011	11	540	Negative	Definite
16	Male	8	23	NA	132	Negative	Definite
11 <sup>1/12</sup>	Female	8	456	2	333	Negative	Definite
6	Female	8	1817	142	210	Negative	Definite
42	Female	9	0	0	0	Negative	Definite
1 <sup>2/12</sup>	Male	11	12	0	29¶	Positive	Definite
17	Male	5	86	9	5809	Negative	Possible
59	Male	5	7	NA	406	Negative	Possible
4	Female	6	4474	292	1692	Negative	Possible
1 <sup>5/12</sup>	Female	6	11	4	3	Negative	Possible
49	Female	7	6284	NA	3939**	Negative	Possible
1 <sup>8/12</sup>	Male	9	4850	4850	1417	Negative	Possible
9	Male	11	18 456	951	889	Negative	Possible
65	Female	15	2	0	0	Negative	Possible

\* Anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen; HBV = hepatitis B virus; NA = specimen not available.

† Definite: Conversion from anti-HBc negativity to anti-HBc positivity on at least 2 consecutive specimens or anti-HBc-positive specimen with coincident positivity for HBV DNA; possible: anti-HBc positivity on 1 specimen with coincident boost in anti-HBs level, 2 nonconsecutive anti-HBc-positive specimens, or single hepatitis B surface antigen-positive result.

‡ Level in standard ratio units.

§ Hepatitis B surface antigen-positive 5 years after anti-HBc conversion date.

¶ Hepatitis B surface antigen-positive at time of first anti-HBc-positive result.

|| Received a fourth dose of vaccine 4 years after the first vaccine dose.

\*\* No anti-HBc-positive result, single hepatitis B surface antigen-positive result; participant received fourth dose of HBV vaccine 1 year before hepatitis B surface antigen-positive result.

**Table 3. Number of Persons with Definite Hepatitis B Virus Breakthrough Infections and Rate per 1000 Persons per Year by Group at Initial Vaccination, Sex, and Initial Response to 3 Doses of Plasma-Derived Hepatitis B Virus Vaccine\***

Characteristic	Person-Years at Risk (Number of Persons)	Definite HBV Breakthrough Infections		P Value
		Number	Rate (per 1000 persons/year)	
<b>Initial responder to vaccine</b>				
Yes	16 368 (1351)	10	0.61	0.01†
No	947 (85)	4	4.22	
Unknown	1682 (142)	2	1.19	
<b>Age at first vaccine dose</b>				
0–4 y	3172 (246)	4	1.26	0.02‡
5–19 y	9936 (842)	3	0.30	
20–49 y	4119 (335)	8	1.94	
≥50 y	1770 (155)	1	0.56	
<b>Sex</b>				
Male	9059 (805)	5	0.55	>0.2
Female	9938 (773)	11	1.11	
<b>All participants</b>	<b>18 997 (1578)</b>	<b>16</b>	<b>0.84</b>	<b>NA</b>

\* HBV = hepatitis B virus; NA = not applicable.

† P value compares rate in persons who initially responded to the vaccine to the rate in persons who did not respond to the vaccine.

‡ P value comparing rate for all 4 age groups.

level of 4000 mIU/mL after vaccination had a GMC of 231 mIU/mL 15 years later, compared with 47 mIU/mL, 13 mIU/mL, and 3 mIU/mL among those whose initial anti-HBs levels were 1000 to 4000, 250 to 1000, and 10 to 250 mIU/mL, respectively.

The anti-HBs GMC was 975 mIU/mL for women and 722 mIU/mL for men after initial vaccination but had decreased to 23 mIU/mL for women compared with 32 mIU/mL for men 15 years after the first hepatitis B vaccine dose ( $P < 0.01$ , linear model controlling for age class and initial anti-HBs level).

At 11 and 15 years, 62 participants without documentation of additional vaccine doses had a booster response. A booster response was associated with age at the time of first vaccination for those 19 years of age and younger (relative risk, 1.94;  $P = 0.01$ ) versus those 20 years of age or older. Among those older than 20 years of age, female sex was associated with a booster response (relative risk, 3.29;  $P = 0.01$ ;  $P = 0.02$  for interaction between sex and age group).

Factors related to the persistence of anti-HBs (log-transformed) over the 15-year follow-up period were determined in a mixed linear model (Appendix Table 3, available at [www.annals.org](http://www.annals.org)). Persistence of anti-HBs levels were not affected by the presence of an HBsAg-positive member in the household at the start of the study or by the proportion of persons in the village who were HBsAg-positive at the end of follow-up (main effect or in interaction with time). After adjustment for sex and initial anti-HBs level, those vaccinated at 20 to 49 years of age and those vaccinated at 50 years of age or older did not significantly differ (main effect and interaction with time). Persons with higher initial responses to the vaccine had the greatest declines over time in anti-HBs level ( $P < 0.001$ , interaction

term between initial anti-HBs level and time). Females had a greater anti-HBs decline than males ( $P < 0.001$ , interaction term between sex and time). The 15-year predicted GMC among women who were 20 years of age or older at the time of vaccination and who initially responded to the vaccine to an anti-HBs level of 1000 mIU/mL was 53 mIU/mL compared with 76 mIU/mL for males (Table 1). The greatest anti-HBs decline occurred among those first vaccinated at 0 to 4 years of age, followed by those vaccinated at age 5 to 19 years of age and 20 years of age or older, respectively ( $P < 0.001$ , interaction term between age class and time). Among females 0 to 4 years of age at the time of vaccination who initially responded to the vaccine with an anti-HBs level of 1000 mIU/mL, the predicted anti-HBs GMC at 15 years was 12 mIU/mL, compared with 27 mIU/mL among persons vaccinated at age 5 to 19 years and 53 mIU/mL among persons vaccinated at 20 years of age or older (Table 1). When we compared the predicted level from the model to the observed anti-HBs level, there were a number of outliers not well fit by the model. These were related to increases in anti-HBs levels during follow-up thought to be due to natural boosting. The characteristics of these increases have been described previously (20), and we did not remove these observations.

### Breakthrough HBV Infections

During the 15 years after vaccination, 16 participants were found to have a definite breakthrough infection and 8 were found to have a possible breakthrough infection; none had clinical signs or symptoms of hepatitis (Table 2). The incidence of new HBV infections during the 15-year follow-up period was 0.84 (95% CI, 0.48 to 1.37) infection per 1000 persons per year when only the definite

**Table 4. Sequential Results of Antibody to Hepatitis B Core Antigen and Hepatitis B Virus DNA among Persons with a Definite Hepatitis B Virus Breakthrough Infection after Vaccination\***

Age at First Vaccine Dose, y	Years of Anti-HBc Negativity	Years of Anti-HBc Positivity	HBV DNA		Sequence/Dilution PCR†
			Result	Years	
22	1981	1982, 1984, 1986	Negative	1982	WT 127Leu, 140 Ser
			Positive	1984	
			Positive	1986	
54	1982	1983–1997	Positive	1982	140 Ser WT
			Positive	1983	
			Negative	1984	
			Negative	1990	
			Negative	1997	
47	1981–1985	1986–1992	Negative	1982	145R+ WT  140 Ser
			Negative	1983	
			Negative	1984	
			Negative	1985	
			Positive	1986	
			Negative	1987	
			Negative	1988	
			Negative	1989	
			Negative	1990	
			Positive‡	1991	
1 2/12	1982–1991 1996–1997	1992–1993	Positive	1988	118A  118A 118A 118A 118A
			Negative	1989	
			Positive	1990	
			Positive	1991	
			Positive	1992	
			Positive	1993	
			Negative	1996	
Negative	1997				
45	1982–1984 1986–1992	1985	Negative	1982	WT
			Negative	1983	
			Negative	1984	
			Positive	1985	
			Negative	1986	
			Negative	1987	
			Negative	1990	
			Negative	1992	
11	1982–1985 1987 1989–1996	1986, 1988	Negative	1982	WT
			Negative	1984	
			Negative	1986	
			Negative	1987	
			Positive	1988	
			Negative	1989	
			Negative	1992	
Negative	1996				

\* Anti-HBc = antibody to hepatitis B core antigen; HBV = hepatitis B virus; PCR = polymerase chain reaction; WT = wild-type virus.

† Listed by codon position in surface antigen and amino acid change.

‡ 1991 specimen was positive for hepatitis B surface antigen.

breakthrough infections were considered; when we considered all infection categories, including persons who did not initially respond to vaccination, the incidence was 1.27 (CI, 0.81 to 1.89) infections per 1000 persons per year. Of the 24 possible and definite new HBV infections, 10 occurred during the first 5 years after the first dose of hepatitis B vaccine, 11 between 6 and 10 years, and 3 between 11 and 15 years. **Table 3** shows the incidence rates by age at initial vaccination, sex, and initial response to the vaccine. Persons who did not initially respond to the vaccine were more likely to have an HBV breakthrough

infection than persons who initially responded to the vaccine ( $P = 0.01$ ). When we included possible infections in the analysis, the  $P$  values for age class, sex, and initial response to vaccine were 0.01, greater than 0.2, and 0.001, respectively.

Hepatitis B virus DNA was found in serum specimens of 6 of the 16 persons with a definite breakthrough infection, and in 5 of these 6 persons on multiple occasions after the first appearance of anti-HBc (**Table 4**). Wild-type HBsAg was identified in 2 persons, while 3 were infected with both wild-type HBsAg and HBsAg variants. Among

the HBsAg variants, mutations were identified at positions 118, 127, 140, and 145.

## DISCUSSION

Our study, conducted in a large cohort that included persons of both sexes initially vaccinated over a wide range of ages, showed that protection induced by hepatitis B vaccine remains robust for at least 15 years, as evidenced by the low number of breakthrough HBV infections. At 15 years, 84% of the cohort tested still had anti-HBs present, including 88% of those who had responded to the initial vaccine series. To fully utilize the large amount of data available to us and account for persons at each follow-up year who were missing, we used a mixed linear effects model to analyze the anti-HBs results. We found that persistence of anti-HBs among vaccinated persons in this cohort was related to higher peak antibody level at 6 months after completion of the vaccine series, older age at vaccination, and male sex. When we examined the interaction term between age class and time, we found that the age group with the greatest decline in anti-HBs level was that first vaccinated at 0 to 4 years of age, followed by those vaccinated at age 5 to 19 years and 20 years of age or older, respectively.

Studies that follow children administered hepatitis B vaccines during infancy and early childhood in countries with a high endemicity of chronic HBV infection have shown that from 4 to 10 years after vaccination, more than 50% of participants had measurable anti-HBs levels of at least 10 mIU/L or sample ratio units (2, 21–25). However, since these children resided in households with HBsAg-positive persons, the high rates of antibody persistence could be secondary to “natural” boosting from inapparent exposure to infected persons. Natural boosting was found in 5.4% of participants in our cohort and has been described elsewhere (6, 9, 20).

One limitation of our study, which is common to all long-term follow-up studies, was the loss to follow-up over the 15 years: 47% of participants were not available for testing at 15 years. Because of the short duration of each village visit for the study, we believe that most missing participants were temporarily or permanently out of the village or unavailable to come to the clinic. Results presented in **Appendix Tables 1, 2, and 3** demonstrate that although missing values appear related to 2 important covariates—sex and age at the time of initial vaccination—it does not appear related to the participants’ previous levels of anti-HBs. The absence of a detected association between missing observations and previous anti-HBs levels supports the assumption made in the use of a linear mixed model that data are missing at random (independent of the anti-HBs level at the time of the missed data point). We believe the design and conduct of the study support this assumption, as does the lack of dependence between missing observations and previous anti-HBs levels. However, there is

no formal way to test this assumption since we could not obtain anti-HBs results in persons who were missing at 15 years.

Studies in health care workers have found that 3 and 13 years after initial immunization, anti-HBs levels of 10 sample ratio units or greater by radioimmunoassay were present in 52% to 76% of participants, respectively (26–28), but in these studies, administering a booster dose of hepatitis B vaccine has universally resulted in an anamnestic response (27, 28). Thus, immune memory against HBV infection lasts longer than persistence of anti-HBs. Our study would support these studies and suggests that protection for health care workers who responded to this vaccine should last at least 15 years.

Long-term follow-up data for adults who continue to be regularly exposed to HBV infection are limited. In a large cohort of men who have sex with men, 85% of responders still had detectable anti-HBs and 60% had levels of 10 mIU/mL or greater 7 years after receiving plasma-derived hepatitis B vaccine (6). Although many participants subsequently died of HIV infection during the follow-up period and breakthrough infections were common, the vaccine was 100% effective in preventing the chronic carrier state or acute symptomatic hepatitis at 9-year follow-up (29). In a study in villagers from Zambia, 74% of adults had detectable anti-HBs levels 9 years after initial immunization (23).

Before the introduction of hepatitis B vaccination in the Alaska Native population, the incidence of HBV infection was 50 per 1000 persons per year (11). After vaccination, breakthrough HBV infections were few and asymptomatic and did not increase throughout the follow-up period even in the face of a declining proportion of persons with detectable anti-HBs. The rate of breakthrough infections was higher in those who initially did not respond to vaccination than in those who responded. Only 6 persons with breakthrough infections were found to be HBV DNA positive, and only 1 remained positive over at least a 3-year period. The significance of HBV infections that are anti-HBc positive, HBV DNA positive, and HBsAg negative is unclear (30, 31), and up to 50% of persons who recovered from acute HBV infection have been found to have low-level persistence of HBV DNA (32, 33). During the study period, more than 70% of HBeAg-positive carriers in this population spontaneously seroconverted to anti-HBe, resulting in lower circulating levels of HBV DNA in chronically infected individuals (15). Thus, it appears that another unanticipated long-term benefit in immunized populations, in addition to the elimination of new HBeAg-positive infections, would be a decrease in infectivity among persons chronically infected.

Variants of HBsAg have been reported infrequently in immunized groups and among infants who received post-exposure hepatitis B vaccine and hepatitis B immune globulin (34, 35). We found HBsAg variants in 4 persons with breakthrough HBV infections (**Table 4**). The HBV vari-

ants coexisted with wild-type HBV in 3 patients, and infections were transient in all but 1 patient. The clinical and epidemiologic significance of these variants is unclear since all infected persons were asymptomatic and infection resolved in most. The presence of HBsAg variants in these persons with asymptomatic breakthrough infections probably reflects the distribution of variants among the Alaska Native population with chronic infection and is unlikely to represent selection by anti-HBs, as observed in exposed neonates receiving hepatitis B immune globulin (34). Breakthrough infections with HBsAg variants have been reported from other immunized populations but appear to be extremely uncommon (36, 37). Furthermore, a study in chimpanzees has shown that commercial hepatitis B vaccine protects against 1 of these variants (38).

In conclusion, in this population, hepatitis B vaccine completely protected against serious sequelae caused by HBV during the 15 years of follow-up. Since antibody levels 15 years after vaccination appeared to wane more rapidly in persons immunized when they were younger than 5 years of age, it is important that we continue to follow this group in order to determine when and if booster doses will be necessary.

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**Acknowledgments:** The authors acknowledge the support they received from the many Community Health Aides in the study villages and the nurses at the Arctic Investigations Program and the Alaska Native Medical Center who over the years gave their valuable time to the study.

**Grant Support:** Partial support through the Alaska Native Tribal Health Consortium from the Division of Viral Hepatitis, National Centers for Infectious Diseases, Centers for Disease Control and Prevention Cooperative Agreement CA #U50/CCU022279. Merck & Co., Inc., West Point, Pennsylvania, supplied the vaccine and partially funded transportation during the first 10 years of follow-up through an unrestricted grant.

**Potential Financial Conflicts of Interest:** *Grants received:* B.J. McMahon (Merck Pharmaceuticals); *Stock ownership or options (other than mutual funds):* A.J. Parkinson (Merck & Co., Inc.).

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**Appendix Table 1. Number of Missing Observations Due to Persons Who Dropped Out and Were Missing Intermittently in a Study of the Immunogenicity of 3 Doses of Hepatitis B Vaccine Administered during 1981–1982\***

Study Year	Total Persons with Anti-HBs Tests Performed, <i>n</i>	Anti-HBs Determinations Removed for Analysis, <i>nt</i>		Anti-HBs Tests Included in Longitudinal Analysis, <i>n‡</i>	Dropouts, <i>n§</i>				Persons Intermittently Missing, <i>n‡</i>
		Fourth Dose of Vaccine	Seroconverters		Persons Dropped out after Fourth Dose	Seroconverters	Deaths	Persons Lost to Follow-up	
Initial	1436	0	1	1435	0	2	0	0	141
2	1368	0	1	1367	3	2	5	13	188
3	1249	23	1	1225	30	3	12	23	285
4	1222	25	4	1193	37	6	29	37	276
5	1122	36	8	1078	47	10	40	48	355
6	1127	41	8	1078	51	12	44	59	334
7	1077	36	9	1032	55	13	61	80	337
8	1052	49	12	991	61	15	73	119	319
9	1000	50	13	937	67	16	84	153	321
10	960	47	10	903	68	16	88	204	299
11	967	52	11	904	72	16	98	305	183
15	841	48	10	783	76	16	121	582	0

\* Anti-HBs = antibody to hepatitis B surface antigen.

† Persons participated in the follow-up study; however, because they had received a fourth dose of vaccine or had evidence of definite hepatitis B virus infection (seroconverted), the anti-HBs level was removed for analysis.

‡ Numbers in these columns sum to total number of participants (*n* = 1578) for each row in the table.

§ Persons with missing (or removed) data from given follow-up year to the end of the study (counts are cumulative).

|| Six months after third dose and 1 year after first dose of vaccine. A total of 1578 participants received hepatitis B virus vaccine, were enrolled in the study, and participated in at least 1 follow-up blood draw.

**Appendix Table 2. Comparison of Persons Who Participated at 15-Year Serum Draw (*n* = 783) with Members of the Cohort Who Did Not Participate (*n* = 703), Excluding All Who Had Evidence of Definite Hepatitis B Virus Infection or Who Had Received an Additional Dose of Vaccine during the 15-Year Follow-up\***

Demographic Characteristics	Persons with Anti-HBs Level at 15 Years ( <i>n</i> = 783), <i>nt</i>	Persons Not Participating in 15-Year Follow-up ( <i>n</i> = 703), <i>nt‡</i>	<i>P</i> Value
Age class at initial vaccination, % ( <i>n</i> )			
0–4 y	18 (143)	13 (91)	<0.001
5–19 y	49 (383)	59 (417)	
20–49 y	23 (182)	17 (120)	
≥50 y	10 (75)	11 (75)	
Initial antibody GMC (median), <i>mIU/mL</i> §	863 (1191)	850 (1187)	>0.2
Initial responders, % ( <i>n/n</i> )§	95 (677/714)	94 (602/640)	>0.2
11-year antibody GMC (median), <i>mIU/mL</i>	41 (44)	39 (43)	>0.2
Persons with antibody level ≥ 10 <i>mIU/mL</i> at 11-year follow-up, % ( <i>n/n</i> )	74 (445/600)	72 (215/300)	>0.2

\* Anti-HBs = antibody to hepatitis B surface antigen; GMC = geometric mean concentration.

† Does not include persons with evidence of definite breakthrough infection (*n* = 16) or persons with ≥4 doses of hepatitis B virus vaccine (*n* = 76).

‡ Includes 121 persons who were deceased at the time of the 15-year follow-up.

§ Of the 783 participants and 703 nonparticipants, 714 and 640, respectively, had an initial anti-HBs blood draw.

|| Of the 783 participants and 703 nonparticipants, 600 and 300, respectively, had an 11-year anti-HBs blood draw.

**Appendix Table 3. Results from Linear Mixed Model of Levels of Antibody to Hepatitis B Surface Antigen over 15 Years of Follow-up\***

Explanatory Variable	Wald Chi-Square Test Statistic	<i>P</i> Value	Parameter Estimates (95% CI)
Log (initial anti-HBs level)	5335.5	<0.001	0.88 (0.86 to 0.90)
Sex	1.4	>0.2	Female vs. male –0.02 (–0.06 to 0.02)
Age class at initial vaccination†	13.9	0.0009	0–4 y: 0.13 (0.06 to 0.20) 5–19 y: 0.06 (0.01 to 0.11) ≥20 y: reference
Time	375.9	<0.001	–0.07 (–0.08 to –0.06)
Time <sup>2</sup>	247.1	<0.001	0.0032 (0.0028 to 0.0036)
Time × initial anti-HBs level	17.0	<0.001	–0.006 (–0.009 to –0.003)
Time × sex	14.8	<0.001	Female vs. male –0.009 (–0.014 to –0.005)
Time × age class‡	203.0	<0.001	0–4 y: –0.057 (–0.064 to –0.049) 5–19 y: –0.025 (–0.031 to –0.019) ≥20 y: reference

\* Anti-HBs = antibody to hepatitis B surface antigen.

† Wald chi-square test for 0–4 y vs. 5–19 y = 7.5 (*P* = 0.02); for 5–19 y vs. ≥20 y = 4.9 (*P* = 0.03).

‡ Wald chi-square test for 0–4 y vs. 5–19 y = 85.7 (*P* < 0.001); for 5–19 y vs. ≥20 y = 65.3 (*P* < 0.001).