Long-Term Immunogenicity and Efficacy of Universal Hepatitis B Virus Vaccination in Taiwan

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The long-term immunogenicity of universal hepatitis B virus (HBV) vaccine is seldom studied in large-scale prospective community-based populations, especially in adolescents. This study enrolled 1200 children aged 7 years with complete HBV immunization in infancy and determined HBV surface antigen (HBsAg), its antibody (anti-HBs), and HBV core antibody (anti-HBc) annually until the children were aged 14 years. Eleven children had new HBV infections with anti-HBc positivity as the only marker. None became positive for HBsAg or had detectable HBV DNA by polymerase chain reaction. The percentage of protective anti-HBs in 951 children without booster vaccination gradually decreased from 71.1% at age 7 years to 37.4% at age 12 years. Only 1 of the 200 children in the booster group and 2 of the 258 children in the nonbooster group developed new anti-HBc positivity. The results suggest that routine booster vaccination may not be required to provide protection against chronic HBV infection before age 15 years.

Taiwan is hyperendemic for hepatitis B virus (HBV) infection. To control for HBV infection, a nationwide vaccination program was launched in 1984 [1] that resulted in a significant carrier reduction rate in children from 10% to <1% over 10 years [2]. Several studies have documented the long-lasting protective efficacy of HBV vaccination until ages 5–12 years [3–6]; however, little is known of vaccination effectiveness in a community where HBV is hyperendemic. Beginning in 1994, we conducted a prospective follow-up study of primary school-aged children in Taipei, a population representative of the general population in a hyperendemic area. We examined the long-term immunogenicity of primary HBV vaccination and additional booster effects and published preliminary results after 2 years of follow-up [7]. Here, we extended our observations to 7 years of follow-up—14 years after the primary HBV vaccination.

Recent studies show that HBV DNA is frequently detected by polymerase chain reaction (PCR) in patients with chronic liver disease who are positive for hepatitis B core antigen (anti-HBc) but negative for hepatitis B surface antigen (HBsAg) [8, 9]. However, little is known about the HBV DNA status in children with new anti-HBc seroconversion after primary HBV vaccination, we also evaluated the significance of serum HBV DNA in these subjects.

Subjects. A group of 1337 apparently healthy children (696 boys and 641 girls) were recruited at age 7 years from 3 primary schools in Taipei between March and May 1994. All parents provided their child’s vaccination history based on a booklet provided by the health administration authority in Taiwan [2]. Of the 1337 children, 1200 (89.8%) received HBV vaccination, we also evaluated the significance of serum HBV DNA in these subjects.

Primary vaccination schedules. The children were vaccinated with a 5-μg dose of plasma-derived HBV vaccine (Hevac B; Pasteur Institut) at birth and at ages 1, 2, and 12 months. In addition, 0.5 mL (145 IU) of HBV immunoglobulin was given within 24 h of birth to infants whose mothers had HBe antigen or reciprocal serum HBsAg titers ≥2560 by reverse-passive hemagglutinin assay.

Booster schedule. In all, 504 children had HBV surface antibody (anti-HBs) levels <10 sample:negative control ratio (S:N) without anti-HBc and HBsAg positivity at age 7 years. By parent choice, 200 children (109 boys and 91 girls) received a booster dose at age 7 years and were enrolled in the booster group. Of these children, 26 had no protective titer at age 8 years and received, in total, 37 additional booster doses (12 at age 8 years, 3 at age 9 years, 6 at age 10 years, 8 at age 11 years, 4 at age 12 years, and 4 at age 13 years) during the follow-up
period. Of 304 children without a booster dose at age 7 years, 46 received a total of 64 booster doses after age 7 years (33 at 8 years, 10 at 9 years, 4 at 10 years, 8 at 11 years, 7 at 12 years, and 2 at 13 years) and were excluded from analysis. The remaining 258 children (149 boys and 109 girls) were enrolled in the nonbooster group. There were no differences in sex ($P = .49$) or maternal HBV carrier rate ($P = .82$) between the groups. In addition, 3 children with anti-HBs levels $>10$ S:N at age 7 years received booster doses (2 at age 12 years and 1 at age 13 years). Therefore, 249 children received booster doses of vaccine, and 951 had no booster vaccination during the follow-up period.

We used the same dosage of neonatal vaccination for booster vaccination (i.e., recombinant hepatitis B vaccines, 5 $\mu$g of Ricombivax [Merck] or 20 $\mu$g of Engerix [GSK]). As mentioned, we used 5-$\mu$g doses of plasma-derived HBV vaccine before July 1992 for routine vaccination in infants. After July 1992, recombinant vaccine (5 $\mu$g of Ricombivax or 20 $\mu$g of Engerix) was used for infants and children [10, 11].

Serologic analyses. We analyzed serum samples for HBsAg, anti-HBs, and anti-HBc by RIA (Ausab, Ausria II, and Corab; Abbott Laboratories) for subjects at ages 7–12 years and by EIA for subjects at ages 13–14 years. The protective anti-HBs titer was defined as $\geq 10$ S:N for RIA and $\geq 10$ mIU/mL for EIA.

PCR for serum HBV DNA. HBV DNA was extracted by proteinase K treatment, phenol/chloroform extraction, and ethanol precipitation from 20 $\mu$L of serum samples collected at the time of the first anti-HBc–positive result and was amplified by PCR [12]. PCR was carried out with 2 primers specific for the S gene sequence: primer P7 (5′-GTGTTGGCTCTCTGTAA-TTTTC-3′, sense strand) and primer P8 (5′-CGGTAWAAGG-GACTCAGAT-3′, antisense strand, where “W” indicates a mixture of A and T and where “M” indicates a mixture of A and C), which started at map positions 256 (sense strand) and 796 (antisense strand) of the HBV genome, respectively.

Definitions. We defined an “anamnestic response” as one in which children without the protective titer of anti-HBs ($<10$ S:N) at age 7 years developed a protective titer at age 8 years after a booster dose or “natural booster.” Natural booster refers to a participant who had a spontaneous increase in serum anti-HBs titers in the absence of anti-HBc or HBsAg. Participants were considered to have “new HBV infections” if they became positive for HBsAg, anti-HBc, or both after age 7 years.

Significance tests were done by $\chi^2$ test. $P < .05$ was considered to be statistically significant.

Results in study population. Of 1200 subjects, 8 (0.7%) were positive for HBsAg, and 9 had isolated anti-HBc positivity at age 7 and remained positive during the follow-up period. Children who did not complete the long-term follow-up ($n = 463$) did not differ from those enrolled at age 7 in terms of the baseline anti-HBs status ($P = .12$), sex ($P = .6$), and maternal carrier rate ($P = .98$).

Waning anti-HBs levels in children aged $\geq 7$ years. Overall, in 951 children without any booster dose, the percentage of protective anti-HBs decreased gradually from 71.1% at age 7 to 37.4% at age 12 (figure 1A). The average annual decline of protective anti-HBs (from $\geq 10$ S:N to $<10$ S:N) from ages 7–12 years was 10.2%. In the booster study, the anamnestic response developed at age 8 in 120 (60%) of 200 children in the booster group and in 20 (7.8%) of 258 children in the nonbooster group ($P < .01$). The protective anti-HBs rate decreased to 21% in the booster group and to 7.1% in the nonbooster group until age 12 (figure 1B and 1C). The anti-HBs titers at age 13 were not compared with those at age 12, because different laboratory methods were used.

New HBV infections. No new HBsAg-positive infections developed in either the booster or the nonbooster groups. Of 458 children in the booster study, new anti-HBc seroconversion occurred in 1 (0.5%) of 200 children in the booster group and in 2 (0.8%) of 258 children in the nonbooster group. The newly infected child in the booster group did not develop protective anti-HBs titer after the booster dose. In the other case, the child had no protective anti-HBs titer at age 7 years and received a booster vaccination at age 8 years (not included in the booster group). Although she developed an anamnestic response, she had an anti-HBc seroconversion at age 13 years when the anti-HBs titer decreased to 58 mIU/mL.

Of 951 children without any booster vaccination, 17 at age 7 years were positive for HBsAg, anti-HBc, or both. Of the remaining 934 children, 9 gained anti-HBc during the follow-up period, including the aforementioned 2 children in the nonbooster group (table 1).

Eight children who had 2 sequential serum samples positive for anti-HBc were classified as documented seroconverters. Three with only 1 anti-HBc–positive result were classified as possible seroconverters. Of these 3, 1 was lost to follow-up after seroconversion, and 2 seroconverted at age 14 years. No further serologic data are yet available. Ten of 11 newly infected children had anti-HBs titers $<100$ mIU/mL 1 year before the first anti-HBc–positive result, including 8 with titers of 10–100 mIU/mL and 2 with titers $<10$ mIU/mL.

The timing for acquiring anti-HBc positivity was evenly distributed during the 7-year follow-up period, except that there were no new cases at age 11 years. If we were to include the 3
Figure 1. Levels of antibody against hepatitis B surface antigen (anti-HBs) in children aged 7–14 years. The serum levels of anti-HBs declined with age. Anti-HBs titers were tested by RIA for children aged 7–12 years (expressed as sample:negative control ratio [S:N]) and by EIA for children aged 13–14 years (expressed as mIU/mL). Nos. above bars are no. of children followed by age group. A, Children with no booster vaccination (regardless of anti-HBs status at age 7 years). B, Children without protective anti-HBs at age 7 years who received a booster dose of vaccine at age 7 years (anti-HBs level at age 7 years, <10 S:N). C, Children without protective anti-HBs at age 7 years who did not receive booster vaccinations during the follow-up period (anti-HBs level at age 7 years, <10 S:N).

possible seroconverters, on the basis of 11 anti-HBc–positive infections and 7631 person-years of follow-up, the annual incidence of new HBV infection during the 7 years of this study would be 1.44 cases/1000 person-years. Of the 11 newly infected children, sufficient serum samples were available from 10 children for HBV DNA assay. None was positive for HBV DNA by PCR.

Implications of the study. Our data show that many vaccine recipients failed to maintain protective anti-HBs titers, but none became a chronic HBsAg carrier. This phenomenon is
Table 1. Serologic status and serum hepatitis B virus (HBV) DNA in 11 vaccinated children with antibodies against HBV core antibody (anti-HBc) positivity as the only marker of new HBV infection.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age at new HBV infection, years</th>
<th>1 Year before infection</th>
<th>At time of first anti-HBc positive result</th>
<th>HBV DNA</th>
<th>HBsAg carrier mother</th>
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<tr>
<td>1</td>
<td>F</td>
<td>8</td>
<td>27.4</td>
<td>20.4</td>
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<td>P</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>8</td>
<td>159.3</td>
<td>81.0</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>8</td>
<td>11.8</td>
<td>11.9</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>4d</td>
<td>M</td>
<td>9</td>
<td>15.7</td>
<td>NA</td>
<td>NA</td>
<td>Q</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>12</td>
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</tr>
<tr>
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<td>M</td>
<td>13</td>
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</tr>
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<td>24.6</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

**NOTE.** Anti-HBs, HBV surface antibody; HBsAg, HBV surface antigen; N, negative; NA, not available; P, positive; Q, questionable.

a Anti-HBc positivity persisted until the end of follow-up in all 11 children.

b Serum samples collected 1 year before and at the time of new anti-HBc seroconversion were retested for anti-HBs titer by EIA.

c Serum samples collected at the time of the first positive anti-HBc result were tested by polymerase chain reaction for HBV DNA.

d Subjects 4, 10, and 11 were classified as possible anti-HBc seroconverters. Subject 4 had only 1 anti-HBc-positive result at age 9 years and was then lost to follow-up. Subjects 10 and 11 had anti-HBc seroconversion at age 14 years; further serologic data are not yet available.

e Subjects 7 and 9 had anti-HBs titers <10 sample: negative control ratio at age 7 years. Subject 7 received a booster dose at age 7 years and had no anamnestic response. Subject 9 received a booster dose at age 8 years (and was not included in the booster group) and developed protective level 1 year later, but the anti-HBs titer fell to 58.2 mIU/mL before anti-HBc seroconversion.

due to persisting immune memory that affords ongoing protection independent of serum anti-HBs. In the booster study, we found immune memory at age 7 years. Of children in the booster group, 60% had protective titer 1 year after a booster dose. However, we do not know whether the remaining 40% without protective titer at age 8 years were nonresponders or had lost their previously acquired memory.

The participants in the booster study were selected by the criteria of anti-HBs titer <10 S:N and negative HBsAg and anti-HBc positivity at age 7 years. Because of the controversy regarding the need for booster vaccination for this age group, the children in both groups were not randomly assigned. It was determined by the child’s parent as to whether the child received a booster dose. However, since there was no difference in the percentage of maternal HBsAg carriage, the risk of new HBV infection probably would be similar in both groups.

In our study, booster vaccination did not confer additional protection against HBsAg carriage or anti-HBc seroconversion. However, there were some intrinsic biases or limitations to minimize the booster effect in our study. These included small case numbers in both booster and nonbooster groups and possibly poorer booster response for children in the booster group than for children selected by their parents for booster vaccination.

Eight children with chronic HBsAg carriage after immunization were previously studied for the surface-antigen variants in our laboratory, and 25% of HBsAg-positive children had surface gene mutants 10 years after the universal HBV immunization [13]. Eleven vaccinated children in our study cohort had new HBV infection, as determined by isolated anti-HBc seroconversion. Of these children, 5 had HBsAg carrier mothers, which suggests that household contacts with carrier mothers were the predominant transmission route of HBV infection in these children. Nevertheless, infections by carrier siblings could not be eliminated.

Recent studies show that HBV DNA is frequently detectable in persons positive for anti-HBc and negative for HBsAg [8, 9]. Thus, isolated anti-HBc seropositivity may be a marker of a latent virus carrier, not just of past infection. However, HBV DNA could not be detected in the serum of our anti-HBc–reactive children by the sensitive method used in our study. This is in keeping with a recent Italian study [14], but the possibility of HBV replication in the liver cannot be excluded.

Our data indicate that a booster vaccination to children of primary school age does not offer additional protection against new HBV infection and that none of the anti-HBc seroconverters had HBV DNA in their serum. Routine booster vaccination may not be necessary before age 15 years. Since
there is a higher risk of HBV exposure during and after puberty by sexual transmission [15], further follow-up studies beyond adolescence are needed to learn whether the protection is maintained at that time.

References