Determination of Immune Memory to Hepatitis B Vaccination Through Early Booster Response in College Students

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The long-term protection of hepatitis B (HB) vaccination has been debated for years. The purpose here was to evaluate the kinetic changes of antibody to HB surface antigen (anti-HBs) and define immune memory of the HB vaccine among college students who had previously received full neonatal immunization against HB. In all, 127 college students aged 18–23 years born after July 1984 who had completed HB vaccination and were seronegative for all three HB viral markers, including HB surface antigen (HBsAg), antibody to HB core protein (anti-HBc), and anti-HBs, were recruited. They received three doses of HB vaccine at enrollment, 1 month and 6 months after enrollment. Their anti-HBs titers were assayed at enrollment, 7–10 days, 1 month, 6 months, and 7 months following the first dose of HB vaccine. The anti-HBs seroprotective rates for subjects 7–10 days, 1 month, 6 months, and 7 months postvaccination were 20.5%, 75.6%, 94.5%, and 99.2%, respectively. Those who were seroprotective at 7 to 10 days after one dose of HB vaccine booster developed significantly higher levels of anti-HBs at 1 and 6 months than those not developing seroprotective anti-HBs response at an earlier timepoint. **Conclusion:** At least one-quarter of HB vaccinees have lost their immune memory to the HB vaccine when entering college. Immune memory to HB vaccine was identified by early seroconversion, which was present in only 20% of vaccinees in the present study. To ensure higher than 90% anti-HBs seroconversion rates, at least 2 doses of HB booster vaccines are recommended for at-risk youths who received complete HB vaccinations in neonatal or infant periods but are seronegative for HBsAg, anti-HBs, and anti-HBc in adolescence. (HEPATOLOGY 2010;51:1547–1554)

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Universal hepatitis B (HB) immunization has been implemented for more than 20 years in Taiwan and led to remarkable reductions in acute and chronic liver diseases.^{1,2}

The national immunization program of Taiwan was launched in 1984: all neonates or infants born before

Nov 1992 received plasma-derived HB vaccines at birth. They all received standard doses of HB vaccines at birth according to the same standard protocol. The coverage rate of HB vaccines during the past 2 decades in Taiwan has been >90% and data show that the national vaccine coverage rates were more than 95% in 2001 and 2002.^{3,4} It has shown an efficacy of 78%–87% in decreasing the seroprevalence of hepatitis B surface antigen (HBsAg)

Abbreviations: anti-HBc, antibody to hepatitis B core protein; anti-HBs, antibody to hepatitis B surface antigen; BMI, body mass index; GMT, geometric mean titers; HB, hepatitis B; HBsAg, hepatitis B surface antigen.

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carriage in all children,^{5,6} a 75% decrease in the incidence of hepatocellular carcinoma among children 6-9 years of age,¹ and a 68% decline in mortality from fulminant hepatitis and HB-related liver diseases in infants.²

Although this national vaccination program has been very successful, a gradual yearly decline in antibody titers against the HBsAg among vaccinees was noted in several follow-up studies.⁷⁻¹¹ The antibody to HBsAg (anti-HBs) seropositivity rate of the vaccinees decreased from 99% at 1 year to 83% at 5 years, 71.1% at 7 years, 37.4% at 12 years, and 37% at 15-17 years. The seronegative rate for three HB viral markers including HBsAg, antibodies to HB core protein (anti-HBc), and anti-HBs increased from 12.7% at 1 year to 62.6% at 15-17 years. Despite the effectiveness of HB immunization, natural HB infections were seen by detecting anti-HBc in 4.0%-5.7% of vaccine recipients in many studies.^{6,10,12} Case reports of vaccine failure have also been noted.¹³ The causes of failure may be lower vaccination coverage and incomplete HB immunization in the early era of the nationwide HB immunization program or poor response to HB immunization, including vaccine failure.^{14,15}

Regarding immune memory to hepatitis B vaccination, Lu et al.¹⁶ found that breakthrough infections might occur 10 to 15 years later for children who initially had a low response to the HB vaccine. One or more booster immunizations are needed in seronegative subjects 15 years after neonatal immunization with the plasma-derived HB vaccine. A recent study estimated that as high as 26.5% of fully vaccinated adolescents aged 15-18 years may have become immunologically naïve to the HB vaccine, raising concerns about the need for a booster vaccine for high-risk groups in the long run.⁷ An Alaskan study found that among children and adolescents vaccinated with HB vaccines during infancy there was an increased proportion of nonresponders among older adolescents, which may indicate waning immune memory.¹⁷

At the time of the present study the necessity for booster vaccinations for the prevention of HB 15 years postvaccination in the group of young adults who have become seronegative for HB viral markers after complete neonatal HB vaccination was still under debate. Because an increased risk of HB infection is anticipated when adolescents enter into young adulthood through becoming sexually active, breakthrough infections such as fulminant HB might be the main concern instead of the risk of chronic HB carriage. To address this issue, we conducted this study to measure the booster responses after HB vaccination in seronegative young adults who had completed neonatal HB

vaccines in Taiwan before. Moreover, we also tried to define immune memory to hepatitis B vaccination through early booster response in college students from this study.

Subjects and Methods

Study Population. This cohort study was conducted between October 2007 and January 2009. The target population was subjects aged 18-23 years who were born after 1984 when the Taiwanese national HB vaccination program was launched. All subjects in this study were born before 1992. Therefore, all the study subjects received the same plasma-derived HB vaccines and completed HB vaccination during infancy. Their vaccination records must have shown a completed neonatal HB vaccination, and they were seronegative for all three HB viral markers, including HBsAg, anti-HBc, and anti-HBs within 2 years of entry into the study and at study entry. They were recruited through a Student's Health Center Clinic referral, Bulletin Board System posts, and Web-broadcast invitation. The neonatal HB vaccination records were verified through linkage to the Taiwan Center for Disease Control databank. Signed informed consent was obtained from all the participants and their parents or guardians. Pregnant females, persons with a previous history of allergy to HB vaccines, or allergy to yeast were excluded.

Protocol. All participants were tested for HB viral markers at enrollment, even if they had been tested in the previous months, to confirm their serostatus. A questionnaire was completed at enrollment to record sociodemographic factors including age, gender, self-reported family history of HB carriers, self-reported blood type, and so on. The participants then received three intramuscular doses of HB vaccine (Engerix-B, recombinant hepatitis B surface antigen, 20 µg/mL/vial, GlaxoSmithKline, Belgium) at baseline and at the first and sixth months follow-up visits. Their anti-HBs status was checked at baseline, 7-10 days, 1 month, 6 months, and 7 months following the first dose of HB vaccine. Adverse effects associated with the vaccine were also reported within 1 week after each Engerix-B injection.

Body mass index (BMI) cutoffs were adopted as suggested by the Department of Health in Taiwan including slim (BMI <18.5), normal (18.5 ≤ BMI <24), overweight and obese (BMI ≥24) categories. This study was approved by the Ethic Review Committee of the National Taiwan University Hospital (Fig. 1). The primary endpoint of this study was to understand the kinetic changes of anti-HBs. Study subjects were categorized into three groups. Group A

Fig. 1. Study flow chart.

consisted of those who had anti-HBs <10 mIU/mL 7-10 days after 1 dose of HB vaccination, whereas those with anti-HBs between 10-100 mIU/mL were in group B and those with anti-HBs between ≥ 100 mIU/mL were in group C.

Serum HB Viral Markers and Biochemistry. Seromarkers including HBsAg, anti-HBc, and anti-HBs concentrations were determined using enzyme immunoassay kits (HBsAg and anti-HBs by Abbott; anti-HBc by Roche Diagnostics). An anti-HBs titer ≥ 10 mIU/mL was considered seroprotective and titers between 10-100 mIU/mL were considered low. The presence of immune memory to HB was defined as a negative prebooster anti-HBs (<10 mIU/mL) followed by a seroprotective titer (≥ 10 mIU/mL) at 7-10 days after one dose of HB vaccine booster. When calculat-

ing the geometric mean titers (GMT), a titer of 0.1 mIU/mL was used for those lower than 0.1 mIU/mL and of 1,000 mIU/mL for those higher than 1,000 mIU/mL.

Sample Size. The sample size calculation conservatively assumed a 10% placebo response of participants had they not received the actual vaccine. The calculated group sample size necessary to have 90% power to detect a 20% early booster response with actual vaccine ($P < 0.05$, two-tailed) yielded a sample size of 120. We chose to enroll 150 to allow a 20% dropout rate.

Statistical Analysis. Excel and SAS v. 9.1.3 were used for statistical analysis. Chi-square analysis and Fischer's exact test were used for comparing group proportions between seropositive and seronegative participants. GMT and their 95% confidence intervals were

Table 1. Characteristics of 126 Participants by Hepatitis B Surface Antibody (anti-HBs) Status

Anti-HBs Status		Day 7-10(+)	Day 7-10(-) and Month 1(+)	Month 1(-) and Month 6(+)	Month 6(-) and Month 7(+)	P Value
No. cases		26	70	24	6	
Age (years)	<20	9	33	9	2	0.669
	≥20	17	37	15	4	
Sex	Female	11	36	11	3	0.888
	Male	15	34	13	3	
Family history of hepatitis B carrier	Yes	3	10	4	1	0.795
	No	16	40	10	4	
	Unknown	7	20	10	1	
Blood type	A	7	15	5	0	0.319
	B	5	16	7	3	
	AB	0	8	0	0	
	O	13	26	12	2	
	Unknown	1	5	0	1	
Body mass index (BMI, kg/m ²)	<18.5	5	13	2	1	0.489
	18.5 ~ 24	17	53	20	4	
	≥24	4	4	2	1	

also calculated using software developed by T.W. Kirkman.¹⁸ An analysis of variance (ANOVA) test was performed to compare the difference of the three groups at 1, 6, and 7 months categorized by the anti-HBs titers at 7-10 days after the booster. Statistical significance was set at 5%.

Results

Initially, 150 seronegative subjects for the three hepatitis B viral markers (HBsAg, anti-HBs, and anti-HBc) were invited to participate in the study. Among them, five subjects were excluded because of seropositive results upon recheck or dropout. A history of complete neonatal HB vaccination could not be confirmed in 18 cases. Therefore, the remaining 127 cases entered the study to receive HB immunization. There were slightly more male than female participants. The mean age was 19.85 ± 1.06 years. No serious adverse effects were reported following the vaccinations.

After three doses of HB booster vaccines, only one person remained seronegative for anti-HBs. Table 1 shows the characteristics of the 126 participants who were seroprotective for anti-HBs after 7 months by their different response times. There were no significant differences in anti-HBs titers during the four follow-up periods with respect to the demographic or biophysical factors studied. Of note was that none of the participants (0/8) with blood type AB had an early response following one dose of HB vaccine.

The seroprotective rates of anti-HBs for subjects 7-10 days, 1 month, 6 months, and 7 months after receiving their first dose of HB vaccine were 20.5%, 75.6%, 94.5%, and 99.2%, respectively. No gender difference was noted in the anti-HBs level (Fig. 2).

There was no statistical difference found between the response and age either. The anti-HBs titer responses among participants with regard to different time periods are shown in Table 2. The anti-HBs titer response was highest at 7 months, followed by 6 months, 1 month, and then 7 to 10 days.

One month after the first dose of HB vaccine, 24.4% of participants had titers <10 mIU/mL and 75.6% were seropositive, but 29.1% had low titers (<100 mIU/mL). Traditionally, the 24.4% subjects with anti-HBs <10 mIU/mL would be regarded as having lost HB immune memory. However, the clinical significance of those with low seroprotective titers was less clear. They might have mounted a booster response based on existing immune memory. On the other hand, they might have lost the HB immune memory but still produce some anti-HBs after one dose of HB vaccine. To further understand this issue, early immune response were assayed in all the subjects

Fig. 2. Seroprotective rates for anti-HBs after booster doses of Hepatitis B vaccine by gender (N = 127).

Table 2. Hepatitis B Surface Antigen Antibody (anti-HBs) Titer Responses Among 127 Participants at Different Timepoints

	Time																			
	7-10 Days		1 Month						6 Months						7 Months					
			<10 mIU/mL		10-100 mIU/mL		≥100 mIU/mL		<10 mIU/mL		10-100 mIU/mL		≥100 mIU/mL		<10 mIU/mL		10-100 mIU/mL		≥100 mIU/mL	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
<10 mIU/mL	101	(79.5)	31	(24.4)	36	(28.3)	34	(26.8)	7	(5.5)	28	(22.0)	66	(52.0)	1	(0.8)	4	(3.1)	96	(75.6)
10-100 mIU/mL	18	(14.2)	0	(0.0)	1	(0.8)	17	(13.4)	0	(0.0)	1	(0.8)	17	(13.4)	0	(0.0)	0	(0.0)	18	(14.2)
≥100 mIU/mL	8	(6.3)	0	(0.0)	0	(0.0)	8	(6.3)	0	(0.0)	1	(0.8)	7	(5.5)	0	(0.0)	0	(0.0)	8	(6.3)
Total	127	(100)	31	(24.4)	37	(29.1)	59	(46.5)	7	(5.5)	30	(23.6)	90	(70.9)	1	(0.8)	4	(3.1)	122	(96.1)

7-10 days after vaccination. Roughly one-quarter (24.4%) of the subjects had nonprotective anti-HBs (<10 mIU/mL) at 7-10 days and 1 month after a single dose of HB vaccine.

All the study subjects were grouped according to their anti-HBs titer 7-10 days after 1 dose of HB vaccine, namely, those <10 mIU/mL (group A), those between 10-100 mIU/mL (group B), and those ≥ 100 mIU/mL (group C) as shown in Table 3. One month after HB vaccination, essentially all subjects (25/26) in group B and C had anti-HBs titers more than 100 mIU/mL, which was only seen in 34 out of 101 subjects in group A (Table 2). Moreover, subjects in groups B and C had anti-HBs GMT 20- to 30-fold higher than that of group A after 1 month; this striking difference persisted to 6 months but was not seen at 7 months after three doses of HB vaccine. Of note was that groups B and C were comparable throughout the study in terms of their anti-HBs titers.

Discussion

To the best of our knowledge, this is the first prospective study administering three doses of HB vaccines with a 7-month follow-up for youths who had previously received at least three doses of neonatal HB

vaccines. The participants in this study were the oldest cohort studied following the launch of the Taiwanese neonatal HB immunization program with a mean age of around 20 years. The study will shed light on the kinetics of the early booster response, and this will help us understand the length of protection after primary immunization of HB vaccine in infancy.

We showed a high success rate (99.2%) following the three doses of HB vaccines among adolescents and young adults aged around 20 years. Our findings were similar to those from central Taiwan in a younger aged cohort of 12-15 years (97.3%).¹⁰

Loss of HB vaccine immune memory could be easily detected by low anti-HBs (<10 mIU/mL) production following one dose of booster HB vaccination. Defining the presence of HB vaccine immune memory could be problematic because production of higher anti-HBs (>10 mIU/mL) 1 month after booster vaccination may result from primary immune response or anamnestic response. Most studies gave a booster dose of the vaccine to seronegative (anti-HBs <10 mIU/mL) subjects who had completed the HB vaccination in infancy. Blood samples were taken before and 3-4 weeks after vaccination. If the postvaccination serum remained seronegative, this subject was considered to have lost immune memory to HB vaccine antigens.

Table 3. Geometric Mean Titers (GMT) at 1, 6, and 7 Months Categorized by Hepatitis B Surface Antigen Antibody (anti-HBs) Titers at 7-10 Days After Booster

Group	A N = 101	B N = 18	C N = 8	P Value*
	<10 mIU/mL	10-100 mIU/mL	≥ 100 mIU/mL	
Anti-HBs at 7-10 days after 1 dose of HB vaccination				
GMT at 1 month (95% CI) [Range]	22.4 (12.6 ~ 40.2) [0.1 to 907.5]	467.9 (306.5 ~ 714.4) [33.1 to 1000]	637.2 (328.2 ~ 1237.0) [104.4 to 1000]	<0.0001
GMT at 6 months	159.1 (112.3 ~ 225.5) [0.1 to 1000]	548.8 (373.8 ~ 805.7) [95.3 to 1000]	648.4 (249.7 ~ 1684.0) [38.7 to 1000]	0.0021
GMT at 7 months	706.0 (598.4 ~ 832.9) [7 to 1000]	971.0 (912.6 ~ 1033.0) [588.8 to 1000]	807.3 (526.6 ~ 1238.0) [232.3 to 1000]	0.26

*ANOVA test. Figures in parentheses are 95% confidence interval. Figures in brackets are ranges.

However, there was a group of subjects who mounted low-level anti-HBs (10-100 mIU/mL) responses after one dose of the HB vaccine. The interpretation for these subjects was less clear. They might manifest an anamnestic response or have lost immune memory and mounted a primary response. This study aimed to clarify this issue by studying early responses to HB vaccines. Our results demonstrated that early responders (anti-HBs ≥ 10 mIU/mL at 7-10 days after vaccination; groups B and C) eventually developed a significantly higher anti-HBs GMT at 1 month and 6 months compared with the nonearly responders (group A). Almost all early responders had high anti-HBs titer (≥ 100 mIU/mL) after 1 month. This supported the notion that early responders maintained immune memory and thus would have more robust immune responses to HB vaccine compared with the nonearly responders. We also found that the levels of the early response were not critical. Those with early anti-HBs between 10 and 100 mIU/mL (group B) and anti-HBs ≥ 100 mIU/mL (group C) behaved similarly in the subsequent anti-HBs responses. Hence, we believe that a conversion of anti-HBs from < 10 mIU/mL to ≥ 10 mIU/mL 7-10 days after one dose of the HB vaccine booster could be defined as the presence of immune memory.

Participants with an early booster response had titers up to 20 times higher than those who could not mount an early response after 1 month. These findings suggest that when immune memory was present, anti-HBs responses could be induced as early as 1 week following a booster and such responders are likely to have protective titers after a single dose and may not need further doses. However, subjects who do not mount an anamnestic response might still be able to mount a protective response to infection.

The nonresponding rates to plasma-derived HB vaccines have been estimated to be less than 10% according to previous studies.^{7,19} Some of those who had a slow or no response to the second course of HB vaccines might be nonresponders but they are few. In our study, 94.5% subjects had seroconversion after the second dose at second course of HB vaccinations and almost all except one did after three doses of revaccination. With regard to the kinetics of anti-HBs titers, there was a total of 17 cases (13.4%) with unsustained anti-HBs response between doses of HB vaccines in our study. Among them, 15 cases had decreased anti-HBs titer at 6 months, just before the third dose of HB vaccine. Another two cases had a decrease in anti-HBs titer at 7 months, 1 month after the third dose of HB vaccine.

In previous studies, females had a stronger immunogenic response to HB vaccine with higher anti-HBs seropositivity and a reduced chance for HB infection.²⁰⁻²² However, no significant gender difference for HB vaccination response was found in our study or in a recent study in central Taiwan.¹⁰ We also did not detect significant differences in anti-HBs titers during four follow-up periods with respect to age, family history of HB virus carriage, blood type, or BMI (see Table 1). However, it is interesting to note that out of eight participants with blood type AB none had an early booster response. Although the sample size was small, further studies to explore the relationship between blood type and booster response may be warranted.

There remain persistent arguments about the role of T-cell immune memory associated with HB vaccines. We have estimated that 10% to 26.5% of fully vaccinated adolescents may have lost their HB vaccine-conferred booster response using an enzyme-linked immunospot assay to estimate memory T-cell immune response, together with HBsAg-specific IFN- γ - or IL-5-secreting peripheral blood mononuclear cells assays.⁷ In Thailand, 87 high-risk individuals who had received a complete course of recombinant HB vaccine 18-20 years earlier were investigated for their HB virus immune memory. Overall, 58.6% of participants were seropositive for humoral immunity and 50.6% were positive using the enzyme-linked immunospot assay for cellular immunity. It was concluded that a second booster dose should be considered, especially in high-risk groups.²³ In the present study, only 20.5% of the previously vaccinated subjects had an early booster response; they may be potentially vulnerable to HB virus infection.

A difference between immune responses to plasma-derived vaccines and recombinant vaccines has been suggested before. Floreani et al.²⁴ found a faster decay rate of anti-HBs with recombinant vaccines. Kao et al.¹⁰ studied students at a junior middle school of a rural township in central-southern Taiwan. After a booster dose the percentage of anamnestic responses increased with a trend toward the younger cohort born after 1992 ($P < 0.001$). The recombinant vaccine showed fast disappearance rates (62.7%) of the surface antibody against HB 12-15 years after vaccination, but provided better anamnestic responses after a booster dose. However, the cohort effects of these differences could not be excluded. In our study all the study subjects received the same plasma-derived HB vaccines and completed HB vaccination during their infancy. Moreover, recombinant HB vaccines were used in the

second course of vaccinations in this study. Because of this homogeneity, this study could not answer if there is any association between response to the second course of HB vaccine and different dosage and types of HB vaccines at birth. Only two subjects received hepatitis B immunoglobulin (HBIG) at birth. Hence, it was not feasible to examine the relationship between HBIG and subsequent long-term immunity as suggested before.²⁵

Our study implies two possible strategies for youth who received complete HB vaccination in neonatal or infant period but are seronegative for HB seromarkers. The first strategy is to check the anti-HBs 1 week after the first booster dose. If there is immune memory based on early anti-HBs seroconversion, no further vaccine doses would be needed. If negative, however, two subsequent doses are needed to ensure seroprotection in more than 90% of vaccinees. A second strategy is to give at least two doses (1 month apart) to ensure the seropositive rate is higher than 90% without further testing of anti-HBs. A response rate higher than 90% is probably sufficient to minimize the risk of acquisition in a highly immunized population with good herd immunity. Both strategies need substantial resources and efforts. The cost-effectiveness of these two strategies warrants further evaluation. In the meantime, surveillance of acute HB should continue to see if further vaccinations are needed.²⁶

Some limitations of this study should be noted. First, there was no study arm to examine the decay in GMT over time with a single dose of HB vaccine. In addition, our study was not designed to detect natural seroconversion from seronegative to seropositive among adolescents and young adults who had completed their neonatal HB immunization. Finally, we did not address the possible presence of T-cell memory among the seronegative patients.

In conclusion, at least one-quarter of HB vaccinees have lost their immune memory to the HB vaccine when entering college. Immune memory to HB vaccine could be identified by early seroconversion, which was present in only 20% of vaccinees in this study. To ensure higher than 90% anti-HBs seroconversion rates, at least two doses of HB booster are recommended for at-risk youths who received complete HB vaccinations in neonatal or infant periods but are seronegative for HBsAg, anti-HBc, and anti-HBs in adolescence.

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References

1. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997;336:1855-1859.
2. Kao JH, Hsu HM, Shau WY, Chang MH, Chen DS. Universal hepatitis B vaccination and the decreased mortality from fulminant hepatitis in infants in Taiwan. *J Pediatr* 2001;139:349-352.
3. Hsu HM, Chen DS, Chuang CH, Lu JC, Jwo DM, Lee CC, et al. Efficacy of a mass hepatitis B vaccination program in Taiwan. Studies on 3464 infants of hepatitis B surface antigen-carrier mothers. *JAMA* 1988;260:2231-2235.
4. Chien YC, Jan CF, Kuo HS, Chen CJ. Nationwide hepatitis B vaccination program in Taiwan: effectiveness in the 20 years after it was launched. *Epidemiol Rev* 2006;28:126-135.
5. Tsen YJ, Chang MH, Hsu HY, Lee CY, Sung JL, Chen DS. Seroprevalence of hepatitis B virus infection in children in Taipei, 1989: five years after a mass hepatitis B vaccination program. *J Med Virol* 1991;34:96-99.
6. Chen HL, Chang MH, Ni YH, Hsu HY, Lee PI, Lee CY, et al. Seroepidemiology of hepatitis B virus infection in children: ten years of mass vaccination in Taiwan. *JAMA* 1996;276:906-908.
7. Lu CY, Ni YH, Chiang BL, Chen PJ, Chang MH, Chang LY, et al. Humoral and cellular immune responses to a hepatitis B vaccine booster 15-18 years after neonatal immunization. *J Infect Dis* 2008;197:1419-1426.
8. Lee PI, Lee CY, Huang LM, Chang MH. Long-term efficacy of recombinant hepatitis B vaccine and risk of natural infection in infants born to mothers with hepatitis B e antigen. *J Pediatr* 1995;126:716-721.
9. Lin YC, Chang MH, Ni YH, Hsu HY, Chen DS. Long-term immunogenicity and efficacy of universal hepatitis B virus vaccination in Taiwan. *J Infect Dis* 2003;187:134-138.
10. Kao JT, Wang JH, Hung CH, Yen YH, Hung SF, Hu TH, et al. Long-term efficacy of plasma-derived and recombinant hepatitis B vaccines in a rural township of Central Taiwan. *Vaccine* 2009;27:1858-1862.
11. Ni YH, Huang LM, Chang MH, Yen CJ, Lu CY, You SL, et al. Two decades of universal hepatitis B vaccination in Taiwan: impact and implication for future strategies. *Gastroenterology* 2007;132:1287-1293.
12. Chang HC, Yen CJ, Lee YC, Chiu TY, Jan CF. Seroprevalence of hepatitis B viral markers among freshmen—20 years after mass hepatitis B vaccination program in Taiwan. *J Formos Med Assoc* 2007;106:513-519.
13. Boot HJ, van der Waaij LA, Schirm J, Kallenberg CG, van Steenberghe J, Wolters B. Acute hepatitis B in a healthcare worker: a case report of genuine vaccination failure. *J Hepatol* 2009;50:426-431.
14. Lee GC, Hwang LY, Beasley RP, Chen SH, Lee TY. Immunogenicity of hepatitis B virus vaccine in healthy Chinese neonates. *J Infect Dis* 1983;148:526-529.
15. Zuckerman JN. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol* 2006;78:169-177.
16. Lu CY, Chiang BL, Chi WK, Chang MH, Ni YH, Hsu HM, et al. Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. *HEPATOLOGY* 2004;40:1415-1420.
17. Samandari T, Fiore AE, Negus S, Williams JL, Kuhnert W, McMahon BJ, et al. Differences in response to a hepatitis B vaccine booster dose among Alaskan children and adolescents vaccinated during infancy. *Pediatrics* 2007;120:e373-382.
18. Kirkman TW. 1996. Statistics to use. <http://www.physics.csbsju.edu/stats> Accessed 20 April 2009.
19. Zuckerman JN. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol* 2006;78:169-177.

20. Dentico P, Buongiorno R, Volpe A, Zavoiani A, Pastore G, Schiraldi O. Long term immunogenicity safety and efficacy of a recombinant hepatitis B vaccine in healthy adults. *Eur J Epidemiol* 1992;8:650-655.
21. Fang JW, Lai CL, Chung HT, Wu PC, Lau JY. Female children respond to recombinant hepatitis B vaccine with a higher titer. *J Trop Pediatr* 1994;40:104-107.
22. Chen CC, Yen CH, Wu WY, Hu SW, Chen SC, Bell WR, et al. Epidemiology of hepatitis B virus infection among young adults in Taiwan, China after public vaccination program. *Chinese Med J* 2007;120:1155-1158.
23. Chinchai T, Chirathaworn C, Praianantathavorn, Theamboonlers A, Hutagalung Y, Bock PH, et al. Long-term humoral and cellular immune response to hepatitis B vaccine in high-risk children 18-20 years after neonatal immunization. *Viral Immunol* 2009;22:125-130.
24. Floreani A, Baldo V, Cristofolletti M, Renzulli G, Valeri A, Zanetti C, et al. Long-term persistence of surface antibody against hepatitis B after vaccination against HBV: an 18-year experience in health care workers. *Vaccine* 2004;22:607-610.
25. Boxall EH, A Sira J, El-Shuhkri N, Kelly DA. Long-term persistence of immunity to hepatitis B after vaccination during infancy in a country where endemicity is low. *J Infect Dis* 2004;190:1264-1269.
26. Kao JH, Chen DS. Hepatitis B vaccination: to boost or not to boost? *Lancet* 2005;366:1337-1338.