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Effect of Revaccination Using Different Schemes among Adults with Low or Undetectable Anti-HBs Titers after Hepatitis B Virus Vaccination▽

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Our objective was to investigate the effect of various reimmunization schemes for hepatitis B in adults with low or undetectable anti-HBs titers. Over 2 years, 10 μg of *Saccharomyces cerevisiae*-recombinant hepatitis B virus (HBV) vaccine (synthesized in China) was used in at least one standardized scheme to immunize 2,310 healthy male and nonpregnant female adults. Of these, 240 subjects tested negative for hepatitis B markers. These 240 subjects were equally divided into 4 groups. The first group, designated Engerix-40, was revaccinated with 40 μg Engerix-B; the second, Engerix-20, was revaccinated with 20 μg Engerix-B; the third, Chinese-20, was revaccinated with 20 μg Chinese-made yeast-recombinant vaccine; and the last group, Chinese-10, was revaccinated with 10 μg Chinese-made yeast-recombinant vaccine. Blood samples were collected before and 1, 2, 8, and 12 months after the first injection. The anti-HBs-positive conversion rates of the Engerix-40, Engerix-20, and Chinese-20 groups were higher than that of the Chinese-10 group ($P < 0.01$). Over time, the anti-HBs conversion rate increased in all groups, but values were significantly different from those for the other groups only in the Chinese-10 group ($P < 0.001$). The anti-HBs geometric mean titers (GMTs) of the Engerix-40, Engerix-20, and Chinese-20 groups were higher than in the Chinese-10 group ($P < 0.05$). Increased doses raise and maintain anti-HBs titers in subjects with low or undetectable titers after HBV vaccination.

Viral hepatitis B is a worldwide public health problem, and there are no specific drugs to treat hepatitis B virus (HBV) infection. For susceptible populations, the most effective preventive measure is to improve immune competence by immunizing with a hepatitis B vaccine (7). Yet, 5 to 15% of subjects have low or undetectable anti-HBs titers after an entire course of Heptavax-B vaccination following standardized immunization programs (0, 1, and 6 months), as recommended by the WHO (17, 26). Subjects with low or undetectable anti-HBs titers remain susceptible to HBV (23).

Many studies have probed for the reasons why subjects fail to develop adequate anti-HBs titers after hepatitis B vaccination (5, 6, 9), but no formal recommendations regarding standardized, normalized reimmunization programs have been made. To develop an effective enhanced hepatitis B vaccination program, we reimmunized 240 subjects with low or undetectable anti-HBs titers using 4 schemes and report and compare the results here.

MATERIALS AND METHODS

(i) Subjects. Between September 2006 and August 2009, 2,310 healthy male and nonpregnant female adults were selected from among outpatients at the Infectious Department of the Third Affiliated Hospital, Sun Yat-sen University. The subjects were immunized by hypodermic injection of 10 μg Chinese-made *Saccharomyces cerevisiae*-recombinant HBV vaccine using at least 1 standardized scheme over 2 years. Of the original 2,310 immunized outpatients, 240 healthy subjects tested negative for hepatitis B markers (HBV surface antigen [HBsAg], anti-HBs, HBeAg, anti-HBe, and anti-HBc), as detected by enzyme-linked immunosorbent assay (ELISA), and were enrolled in this study. The enrollees had no history of hepatitis and had normal liver function. Informed consent was obtained from all subjects.

(ii) Research methods and revaccination schemes. The 240 healthy adult subjects were randomly divided into 4 groups of 60, and each group was assigned to a different revaccination scheme. Revaccination was administered in each group by intramuscular injection into the deltoid muscle at 0, 1, and 6 months. Members of the Engerix-40 group, 32 males and 28 females with a mean age of 30.56 ± 10.47 years (range, 18 to 62 years), were revaccinated each time with 40 μg imported yeast-recombinant hepatitis B vaccine (Engerix-B). Members of the Engerix-20 group, 31 males and 29 females with a mean age of 31.83 ± 11.43 years (range, 20 to 61 years), were revaccinated each time with 20 μg imported yeast-recombinant hepatitis B vaccine (Engerix-B). Members of the Chinese-20 group, 28 males and 32 females with a mean age of 31.33 ± 9.76 years (range, 19 to 60 years), were revaccinated each time with 20 μg Chinese-made yeast-recombinant hepatitis B vaccine. Members of the Chinese-10 group, 29 males and 31 females with a mean age of 28.95 ± 10.28 years (range, 19 to 62 years), were revaccinated each time with 10 μg Chinese-made yeast-recombinant hepatitis B vaccine.

The baseline statistical differences between subjects in the 4 groups were insignificant ($P > 0.05$).

(iii) Sources of vaccines. The Chinese-made yeast-recombinant hepatitis B vaccine (10 μg and 20 μg) was produced by Shenzhen Kangtai Biological Products Co., Ltd. (batch no. 20051131, 5 μg/0.5 ml). The imported yeast-recombinant hepatitis B vaccine (20 μg Engerix-B) was produced by Shanghai Glaxo-SmithKline Biological Products Co., Ltd. (batch no. XHBVB270AA, 20 μg/ml). The vaccines were used within the dates of validity.

(iv) Blood collection and detection. Five milliliters of venous blood was collected from all subjects before the first injection, ~28 to ~30 days after the first injection (time 1 [T1]), ~28 to ~30 days after the second injection (T2), 2 months after the third injection (T8), and 13 to 15 months after the first injection (T12). Anti-HBs was detected according to the manufacturer's instructions for the anti-HBs assay kit (Abbot Axsym AUSAB) by a single investigator in a single laboratory, using the same equipment and methods; the reagents were purchased from the same supplier.

(v) Definition of anti-HBs levels. Anti-HBs titers of <10 mIU/ml were considered negative; anti-HBs titers of ≥10 mIU/ml were considered positive.

(vi) Statistical analysis. The statistical software SPSS 13.0 was used for statistical analysis. Mean comparisons between groups were made by single-factor

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TABLE 1. Comparison of anti-HBs-positive conversion rates among the four groups

Group	No. (%) of subjects who converted to HBs positivity at:				χ^2	*P*
	T1	T2	T8	T12		
Enrerix-40	53 (88.33)[a,b]	57 (95.0)[a,b,c]	59 (98.33)[a,b,c]	58 (96.67)[a,b,c]	5.663	0.148
Engerix-20	46 (76.67)[a]	48 (80.0)[a]	51 (85.0)[a,b]	52 (86.67)[a,b]	2.578	0.461
Chinese-20	42 (70.0)[a]	43 (71.67)[a]	39 (65.0)	38 (63.33)	1.292	0.731
Chinese-10	25 (41.67)	22 (36.67)	39 (65.0)	33 (55.0)	11.917	0.008
χ^2	33.214	53.324	28.282	36.925		
P	<0.001	<0.001	<0.001	<0.001		

[a] Versus Chinese-10, $P < 0.01$.
[b] Versus Chinese-20, $P < 0.05$.
[c] Versus Engerix-20, $P < 0.05$.

variance analysis, and the chi-square (χ^2) test was used to compare ratios. Differences were considered statistically significant with *P* values of <0.05 (bilateral).

RESULTS

Comparison of anti-HBs-positive conversion rates among four reimmunization schemes. The anti-HBs-positive conversion rates for the 4 groups are shown in Table 1. With each revaccination in the series, the number of anti-HBs-positive subjects increased in the Engerix-40, Engerix-20, and Chinese-20 groups, albeit insignificantly. Thirteen months after revaccination, these rates fell. However, with each revaccination, the number of anti-HBs-positive subjects increased significantly in the Chinese-10 group (between T1 and T8, T2 and T8, and T2 and T12; *P* was <0.05 for all). After 3 revaccinations, this rate in the Chinese-10 group peaked (65.0%) but remained lower than that of the other groups.

As shown in Table 1, the anti-HBs-positive conversion rates for the Engerix-40, Engerix-20, and Chinese-20 groups were higher at each successive testing for anti-HBs titers than in Chinese-10 recipients (*P* was <0.01 for all, except that *P* was >0.05 at T8 and T12 between Chinese-20 and Chinese-10). This rate in the Engerix-40 group was higher than in the Engerix-20 and Chinese-20 groups (*P* was <0.05, but *P* was >0.05 at T1 between Engerix-40 and Engerix-20). Anti-HBs-positive conversion rates after revaccination did not differ significantly between the Engerix-20 and Chinese-20 groups at T1 and T2, unlike at T8 and T12 ($P < 0.05$).

Comparison of antibody GMTs. The anti-HBs geometric mean titers (GMTs) in the 4 groups over time are shown in Table 2. With each successive assay, the GMTs in the Engerix-40, Engerix-20, and Chinese-20 groups were higher than in the Chinese-10 group (*P* was <0.05, but *P* was >0.05 at T8 and T12 between Chinese-20 and Chinese-10). The GMT in the Engerix-40 group was higher than in the Chinese-20 group at T8 and T12 (*P* was <0.05, but *P* was >0.05 at T1 and T2). GMTs did not differ at any time point between the Engerix-40 and Engerix-20 groups or between Engerix-20 and Chinese-20.

Adverse reactions. No adverse reactions developed in the Engerix-20 and Chinese-10 groups. Four patients developed swelling at the injection site in the Chinese-20 group, 2 of whom experienced arm pain at the site of injection. This resulted in an adverse reaction rate of 5.8% (4/69). Only 1 Engerix-40 recipient developed swelling at the injection site. There were no other adverse reactions.

DISCUSSION

Protective levels of anti-HBs are not generated in a certain percentage of persons who undergo an immunization program with hepatitis B vaccine, i.e., the GMT of anti-HBs fails to reach 10 IU/liter (11). In healthy populations, the proportion of subjects with low or undetectable anti-HBs titers after HBV vaccination is approximately 2% to 15% (3), rendering them susceptible to hepatitis B virus. We should improve low or undetectable anti-HBs titers after HBV vaccination to control HBV infections in the general population.

For subjects with low or undetectable anti-HBs titers after HBV vaccination, several methods have been developed to overcome this nonresponse (15). The current chief countermeasures include using more-immunogenic epitopes (24) or replacement vaccines (18, 22), increasing the number of vaccinations and doses, changing the method of vaccination, and combining the vaccine with an adjuvant or immunoregulant

TABLE 2. Comparison of GMTs between the four groups

Group	GMT (mIU/ml) at:			
	T1	T2	T8	T12
Engerix-40	433.92 ± 396.88[a]	392.62 ± 317.99[a]	468.45 ± 329.11[a,b]	434.58 ± 335.18[a,b]
Engerix-20	373.82 ± 394.04[a]	431.95 ± 377.45[a]	427.87 ± 332.10[a,b]	350.75 ± 265.07[a]
Chinese-20	389.85 ± 406.17[a]	378.50 ± 339.93[a]	287.97 ± 283.29	232.97 ± 236.65
Chinese-10	105.73 ± 186.65	192.18 ± 323.61	229.30 ± 304.94	194.10 ± 251.62
F value[c]	10.383	5.906	7.85	9.634
P	<0.001	0.001	<0.001	<0.001

[a] Versus Chinese-10, $P < 0.01$.
[b] Versus Chinese-20, $P < 0.01$.
[c] *F* value, Fisher value.

(14, 16, 25, 27). The addition of preS2 antigen or core antigens (HBcAg) to the HBV surface antigen (HBsAg) provides additional protective immunity.

Nevertheless, many of these methods have failed to significantly reverse nonresponse to HBV epitopes. Many issues related to low or undetectable anti-HBs titers after HBV vaccination require in-depth exploration, including the number of revaccinations that is necessary to create an effective response, how long the antibody persists after revaccination, and the rates of protection and efficacy.

In our study, 3 revaccination doses (10 μg, 20 μg, and 40 μg) from different manufacturers (imported and made in China) were used to inoculate 240 nonresponders in 4 groups, and the reimmunization effects were observed. Baseline data on the subjects in the 4 groups were comparable. Positive anti-HBs conversion rates in the Engerix-40, Engerix-20, and Chinese-20 groups were higher than in Chinese-10 recipients ($P < 0.01$). Such rates in the Engerix-40 group exceeded those in the Engerix-20 and Chinese-20 groups ($P < 0.05$), indicating that the anti-HBs response rates in nonresponders after hepatitis B vaccination can be improved by any of the 4 schemes that we implemented: 10 μg of Chinese-made recombinant hepatitis B vaccine (the previous standard dose), 20 μg of Chinese-made recombinant hepatitis B vaccine, and either 20 μg or 40 μg of the imported Engerix-B recombinant hepatitis B vaccine. The immunization effects of increased vaccination doses were better than merely revaccinating.

Although anti-HBs-positive conversion rates after revaccination did not differ significantly between the Engerix-20 and Chinese-20 groups at T1 and T2, there were significant differences in such rates at T8 and T12 between Engerix-20 and Chinese-20 recipients ($P < 0.05$). This result indicates that Chinese-made recombinant hepatitis B vaccines are inferior to Engerix in generating anti-HBs seroconversion, as shown in Table 1. As revaccination doses and time increased, anti-HBs-positive conversion rates rose in the Engerix-40, Engerix-20, and Chinese-20 groups, albeit insignificantly. Therefore, an increase of 1 or 2 revaccinations might suffice for subjects with low or undetectable anti-HBs titers. Positive anti-HBs conversion rates failed to rise after 3 revaccinations, compared with 2 revaccinations.

As time between revaccinations increased, however, positive anti-HBs conversion rates increased in Chinese-10 recipients (P was <0.05 between T1 and T8, T2 and T8, and T2 and T12). After 3 revaccinations, positive anti-HBs conversion rates in the Chinese-10 group peaked (65.0%) but remained lower than in the Engerix-40, Engerix-20, and Chinese-20 groups. One year after revaccination, positive anti-HBs conversion rates declined.

At each time point after revaccination, GMTs in the Engerix-40, Engerix-20, and Chinese-20 groups were higher than in Chinese-10 subjects (P was <0.05, but P was >0.05 at T8 and T12 between Chinese-20 and Chinese-10), indicating that the effects of increments in the revaccination scheme were better than revaccination with the original dose.

Engerix-B (the comparator) is a noninfectious recombinant DNA hepatitis B virus vaccine. The active ingredient is purified hepatitis B virus surface antigen (HBsAg), produced in yeast cells (*Saccharomyces cerevisiae*) by recombinant DNA technology involving several physicochemical steps. It adsorbs to aluminum hydroxide and is hydrated. HBsAg assembles spontaneously, in the absence of chemical treatment, into spherical particles that are 20 nm in diameter on average, containing nonglycosylated HBsAg polypeptides and a lipid matrix that consists primarily of phospholipids. These particles have the characteristic properties of natural HBsAg. The HBV component is formulated in phosphate-buffered saline. Each pediatric dose of 1 ml has 20 μg of antigen that is adsorbed to 0.5 mg of aluminum hydroxide, to which thimerosal is added (at a 1:20,000 dilution) as a preservative.

The Chinese-made yeast-recombinant hepatitis B vaccine also consists of purified surface antigen (Ag) from HBV, obtained by culturing genetically engineered *Saccharomyces cerevisiae* yeast cells that express the surface Ag gene of the virus. Each 0.5-ml pediatric dose contains 5 μg of Ag adsorbed to ~0.35 to ~0.62 mg/ml of aluminum hydroxide, to which 50 μg/ml thimerosal is added (12, 21).

Both the yeast-recombinant hepatitis B vaccine that is made in China and Engerix-B can effect satisfactory immunization. Adverse reactions in the Chinese-20 group were more severe than in groups subjected to other schemes, which might have been due to the additional liquid that was required to reach the same dose as that of Engerix-B. Also, adjuvant contents were increased in the Chinese-made hepatitis B revaccinations, explaining why their adverse reactions were relatively more severe.

Protective antibody levels can be reached in most subjects with low or undetectable anti-HBs titers after HBV vaccination (22). However, there are differences in positive anti-HBs conversion rates in such subjects, according to several reports (1, 2, 19), which might be related to factors such as age, body mass index, characteristics of vaccination before revaccination, and the method and dose of revaccination.

Low or undetectable anti-HBs titer status after HBV vaccination could not be eliminated by effective, strengthening immunization. In our study, we found that 3.33% of subjects in the 40-μg group had no antibody responses and that nearly 50% had no antibody responses in the 10-μg group after reimmunization. Therefore, to maximize the protective effects of the hepatitis B vaccine, anti-HBs-positive rates should be detected in a timely manner after primary vaccination, and strengthening immunization should be given to nonresponders (10). Vaccinating with a larger dose of hepatitis B vaccine may improve protection rates, a pattern that has been observed in studies on immunization effects in HIV-infected adults (4). Nevertheless, further clinical studies should be performed to determine whether increases in immunization dose should be continued.

Studies have shown that although GMTs increase in subjects with low or undetectable anti-HBs titers after revaccination, they are not sufficiently high and cannot be sustained. The antibody response rate can be increased by increments in hepatitis B vaccine, but the appropriate increment and the length of time for which the immunity persists after revaccination have not been determined (8). Whether we need to provide an additional booster dose after 3 doses remains a separate debate (20). Therefore, further studies should be performed on these matters.

Anti-HBs is the only easily measurable correlate of vaccine-induced protection. However, we have known that vaccinated

persons are still protected against HBV infection despite declines in anti-HBs to <10 mIU/ml. This phenomenon of continued vaccine-induced protection is thought to be the existence of immune memory. Studies have shown that of children who respond to a primary vaccine with antibody levels of >10 mIU/ml, 15% to 50% have low or undetectable concentrations of anti-HBs 5 to 15 years after vaccination. The mechanism of immune memory may work through selective expansion and differentiation of clones of antigen-specific B and T lymphocytes (13). Although data indicate that a high proportion of vaccine recipients retain immune memory and would develop an anti-HBs response upon exposure to HBV, direct measurement of immune memory is not yet possible. The phenomenon of immune memory in adults is also not yet understood. All these observations need further study.

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