

A two-dose hepatitis B vaccine regimen: proof of priming and memory responses in young adults¹

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This study shows that two doses of a recombinant hepatitis B vaccine (10 µg or 20 µg of HBsAg per dose), administered 6 months apart to young, healthy adults, can induce an antibody (anti-HBs) response similar to that expected with the standard three-dose regimen of this vaccine given at intervals of 0, 1, and 6 months. While only 46-67% of the vaccinees displayed a protective anti-HBs titer of ≥ 10 mIU ml⁻¹ prior to the receipt of the second dose at 6 months, virtually all were primed as 97-99% of the subjects developed such a titer when tested a month after the second dose. Among vaccinees given 10 or 20 µg doses, respectively, the secondary rise in antibody following the second dose yielded geometric mean titers (GMTs) of 1103 and 2538 mIU ml⁻¹, respectively. The study further demonstrated that a two-dose regimen of vaccination induced strong immunologic memory for HBsAg, as a booster dose of vaccine given 2 years later resulted in a rapid and vigorous anamnestic antibody response. © 1998 Elsevier Science Ltd. All rights reserved

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Infection caused by hepatitis B virus (HBV) can be prevented through immunization with vaccine composed of viral surface antigen (HBsAg) and aluminum salt adjuvant^{1,2}. A series of three or four doses given by intramuscular injection is indicated for the hepatitis B vaccines currently licensed in the United States, with a minimally acceptable immune response defined as an anti-HBs titer of > 10 mIU ml⁻¹³. Although the standard three-dose or four-dose regimen of hepatitis B vaccine is highly immunogenic for most healthy individuals, it seemed plausible that a series utilizing only two doses might be effective for at least some populations. With the two-dose schedule, the initial dose must fulfil the 'priming' role now allocated to the initial two doses of the standard schedules. An effective two-dose vaccination regimen would have the benefit of reducing the number of clinic or physician visits and injections needed to immunize against HBV infection, with

corresponding reductions in administrative costs and the possibility of improved compliance.

The present study was undertaken primarily to test the concept that a two-dose regimen of recombinant hepatitis B vaccine can induce an anti-HBs response comparable to that expected with the standard three-dose regimen. A secondary objective was to ascertain whether subjects immunized with a two-dose regimen retain immunologic memory for HBsAg 2 years after the initial dose of vaccine. The initial anti-HBs responses of healthy, non obese young adults given two doses of a recombinant hepatitis B vaccine (10 µg or 20 µg dosage of HBsAg) 6 months apart, were evaluated 1 month after the second dose, while retention of immunologic memory was assessed from the antibody response to a booster dose given approximately 2 years after the initial dose.

MATERIALS AND METHODS

The study was conducted at the University of Kentucky Medical Center. Written informed consent was obtained from all subjects. The study was reviewed and approved by the Institutional Review Board of the University of Kentucky.

Study population

Healthy, non-pregnant, non obese (weight-height index [WHI] <95th percentile adjusted for gender⁴)

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adults 18–39 years of age, who were seronegative for hepatitis B serologic markers (HBsAg, anti-HBc, anti-HBs) and not previously vaccinated with any hepatitis B vaccine were eligible for the study. Females were permitted to participate only if they had a negative serum or urine pregnancy test within 7 days prior to each scheduled injection of vaccine and agreed to use birth control for at least one month after each vaccine injection. Persons with known or suspected hypersensitivity to vaccine components, known or suspected immunologic impairment (including use of immunomodulatory drugs), or who within the previous 3 months received serum immunoglobulins or other blood-derived products, were excluded.

Study design/vaccination regimen

This was an open, randomized study with subjects assigned (1:1) to receive a primary course of either two 10 μg or two 20 μg doses of the yeast recombinant hepatitis B vaccine, RECOMBIVAX HB[®] (Merck), administered 6 months apart. Two investigational lots of vaccine were used, one containing 10 μg HBsAg (lot#1329/C-Y231) and the other 20 μg HBsAg (lot#1330/C-Y232) in a 1.0 ml volume. Each 1.0 ml dose also contained 0.5 mg aluminum (as aluminum hydroxide) as an adjuvant and 1:20000 thimerosal as a preservative. Vaccine was given by intramuscular injection in the deltoid using a 23 gauge needle, 1 or 1.5 inches in length depending on the size of the volunteer's arm.

Within each dosage group, subjects were further randomized to have blood samples taken 1 month or 2 months after the first vaccine dose, while all subjects had blood samples taken at 3 months, 6 months (just prior to the second dose), and 7 months (1 month after the second dose). A booster dose of vaccine was offered approximately 2 years after the initial dose (approximately 18 months post the second vaccine dose) with the intent of evaluating the anamnestic response 1 and 3–4 weeks later in those vaccinees who had had developed $\geq 10 \text{ mIU ml}^{-1}$ of anti-HBs at 7 months.

Anti-HBs

Serum samples were tested for anti-HBs at the Merck Research Laboratories, West Point, PA, using the AUSAB[®] radioimmunoassay test kit (Abbott Laboratories, North Chicago, IL), with seroconversion defined as any detectable antibody ($\geq 2.1 \text{ S/N}$) and seroprotection defined as a titer $\geq 10 \text{ mIU ml}^{-1}$. An anamnestic response to booster vaccination was defined as a greater than or equal to fourfold rise in antibody titer measured in the serum sample obtained approximately a week after the booster dose in subjects with detectable antibody prior to the booster or as the development of $\geq 10 \text{ mIU ml}^{-1}$ of anti-HBs in subjects with no detectable antibody prior to boosting.

Vaccine safety

Safety was assessed by having vaccinees record body temperature 4–6 h after each injection and daily thereafter for 5 days. They were also instructed to record

any injection site reactions or systemic complaints daily for 5 days after each injection of vaccine.

Study objectives

The primary objective of this study was to determine the seroprotection rate (% with anti-HBs titer $> 10 \text{ mIU ml}^{-1}$) at 7 months in subjects given a two dose course of vaccination 6 months apart. The associated hypothesis was that $\geq 95\%$ of the vaccinees would develop this level of anti-HBs. A secondary objective was to estimate the proportion of vaccinees who would seroconvert for anti-HBs 3–6 months following a single priming dose of vaccine, with the associated hypothesis in this case being that $\geq 80\%$ of the vaccinees would develop detectable antibody. Finally, the study was intended to assess the persistence of immunologic memory for HBsAg in vaccinees with $\geq 10 \text{ mIU ml}^{-1}$ of anti-HBs at month 7 by measuring the response to a booster dose of vaccine given approximately 2 years after the first dose.

Statistical analysis

The 95% exact confidence intervals were calculated for the percent of subjects who developed detectable anti-HBs titers ($\text{S/N} \geq 2.1$) or who developed $\geq 10 \text{ mIU ml}^{-1}$ of antibody in the two study groups. Rates between the two groups were compared using Fisher's exact test. GMTs were computed by taking the natural exponent of mean log titer values. Since the minimum detectable titer of the assay is 0.6 mIU ml^{-1} , any undetectable titers ($< 0.6 \text{ mIU ml}^{-1}$) were assigned an arbitrary value of 0.3 mIU ml^{-1} for the calculation of GMTs. Comparisons of GMTs between groups were performed using analysis of variance models⁵. Titers were log-transformed in the analysis. Statistical significance was determined at the two-sided 0.05 level throughout the analysis.

RESULTS

Subject accounting

Two hundred subjects were randomized in the study, 100 to each dosage group (10 μg or 20 μg) (Table 1). Ninety-eight subjects received both 10 μg doses of vaccine while 97 received both 20 μg doses. Among these, there were 18 subjects (7 in the 10 μg group and 11 in the 20 μg group) who did not meet one or more of the prescribed criteria for inclusion in the analysis of immune response to the primary course of vaccination (reasons included age at entry > 39 years, prevaccination serum not available, WHI > 95 th percentile, or possible prior HBV infection). However, safety data have been summarized for all vaccinees who completed report cards (Table 2).

A 10 μg booster dose of vaccine was given approximately 2 years after the initial dose to a total of 171 subjects (Table 1). One hundred and forty-four of these (73 in the 10 μg group and 71 in the 20 μg group) were eligible for assessing the immune response to booster vaccination (i.e. were known to have developed $\geq 10 \text{ mIU ml}^{-1}$ of anti-HBs at 7 months and met all other criteria inclusion in analyses of immune response to vaccination) (Table 3).

The study population was predominantly Caucasian (95%), 59% of the subjects were female, and the mean age at entry was 25 years (Table 1). Subjects assigned to the two dosage groups were similar in terms of age, gender, race, body mass index, proportion completing the two-dose regimen, and proportion receiving booster vaccine dose.

Table 1 Characteristics of study participants by dosage group

Attribute	Dosage group ^b	
	10 µg	20 µg
No. enrolled		
Dose 1	100	100
Dose 2	98	97
Booster ^c	83	88
Age		
Range	18-39	18-40
Mean	25.1	25.5
Median	23.0	23.0
Gender		
Female	54%	64%
Male	46%	36%
WHI		
Range	17.5-50.3	17.8-50.9
Mean	27.4	28.8
Median	26.8	26.0

^a10 µg booster dose given at approximately 2 years to all available subjects.

^bThere were no significant differences between dosage groups with respect to age, gender or WHI ($P > 0.05$).

Safety

Vaccination was well tolerated, with most adverse experiences being local and mild. There were no serious adverse experiences attributable to vaccination and only one subject withdrew from the study because of a reaction. That reaction appeared to be a possible acute allergic response to a 20 µg initial vaccination. The patient developed fever and a rash several hours after the injection. Three patients withdrew because of pregnancy (two in the 10 µg dose) and another three were withdrawn because of poor compliance.

Table 2 summarizes the frequencies of injection site reactions (soreness, tenderness, and itching), systemic complaints (fatigue, osteomuscular pain, headache, and nausea), and elevated temperature ($\geq 100^\circ\text{F}$, oral) reported to occur any time within a 5 day period following a dose of vaccine. The frequencies of these events were analyzed for each vaccination and dosage. There were no significant differences with successive vaccinations or between the two dosages of vaccine.

Anti-HBs responses

Table 3 shows the anti-HBs responses to primary vaccination and to subsequent booster vaccination of subjects in each dosage group who met the inclusion criteria for analysis of immune response. With respect to the study objective of primary interest, 90 subjects in the 10 µg dosage group and 84 in the 20 µg group met

Table 2 Injection site reactions, systemic complaints and elevated temperatures occurring within 5 days of vaccination

Event	Dosage	% (Proportion) of vaccinees ^{a,b}		
		Dose 1	Dose 2	Dose 3 (Booster)
Injection site reactions	10 µg	15.0% (15/100)	18.9% (18/95)	17.2% (11/64)
	20 µg	10.3% (10/92)	13.5% (13/96)	29.7% (22/74)
Systemic complaints	10 µg	16.0% (16/100)	13.7% (13/95)	12.5% (8/64)
	20 µg	16.5% (16/97)	12.5% (12/96)	18.9% (14/74)
Elevated temperature ($\geq 100^\circ\text{F}$)	10 µg	1.0% (1/92)	3.2% (3/95)	5.0% (3/60)
	20 µg	3.2% (3/92)	5.3% (5/95)	4.2% (3/72)

^aDenominators are the numbers of vaccinees who completed report forms.

^bThere were no significant differences in the frequencies of injection site reaction, systematic complaints or elevated temperature with successive vaccinations or between the dosages of vaccine ($P > 0.05$).

Table 3 Anti-HBs responses of young adults to a two-dose regimen of RECOMBIVAX HB[®] (10 µg or 20 µg HBsAg) at zero and six months followed by a booster dose at 2 years

Time after Dose 1 (interval)	10 µg			20 µg		
	% (Proportion) with antibody			% (Proportion) with antibody		
	% Antibody +ve	$\geq 10 \text{ mIU ml}^{-1}$	GMT (mIU ml^{-1})	% Antibody +ve	$\geq 10 \text{ mIU ml}^{-1}$	GMT (mIU ml^{-1})
1 mo. (PD1) ^a	50% (22/44)	11% (5/44)	1.6	55% (22/40)	20% (8/40)	2.0
2 mos. (PD1) ^a	66% (31/47)	23% (11/47)	2.8	86% (38/44)	54% (24/38)	9.0
3 mos. (PD1)	74% (58/78)	51% (40/78)	7.6	80% (64/80)	68% (54/80)	16
6 mos. (PD1)	78% (71/91)	46% (42/91) ^b	7.4	87% (75/86)	67% (58/86) ^b	16
7 mos. (PD2)	97% (87/90)	97% (87/91) ^b	1103 ^b	99% (83/84)	99% (83/84) ^b	2538 ^b
2 yrs. (PRB)	90% (66/73)	75% (55/73)	39	94% (67/71)	89% (63/71)	117
+1 wk. (POB)	98% (52/53)	96% (51/53)	1526	100% (63/63)	98% (62/63)	3448
+3-4 wks. (POB)	100% (63/63)	100% (63/63)	6213	100% (69/69)	100% (69/69)	14776
Proportion with an anamnestic response 1 wk. POB		79.3% (42/53)			87.3% (55/63)	

PD = postdose, PRB = prebooster, POB = postbooster.

^aDifferent subsets of the vaccinee population sampled at 1 and 2 months, respectively (see Study design/vaccination regimen).

^bThe proportion of vaccinees with $\geq 10 \text{ mIU ml}^{-1}$ of anti-HBs at 7 months (97% versus 99%) was not significantly different between treatment groups ($P > 0.05$), while the GMTs (1103 mIU ml^{-1} versus 2538 mIU ml^{-1}) were significantly different ($P = 0.023$). The proportion of vaccinees with $\geq 10 \text{ mIU ml}^{-1}$ of anti-HBs at 6 months (46% versus 67%) was significantly different between treatment groups ($P = 0.006$).

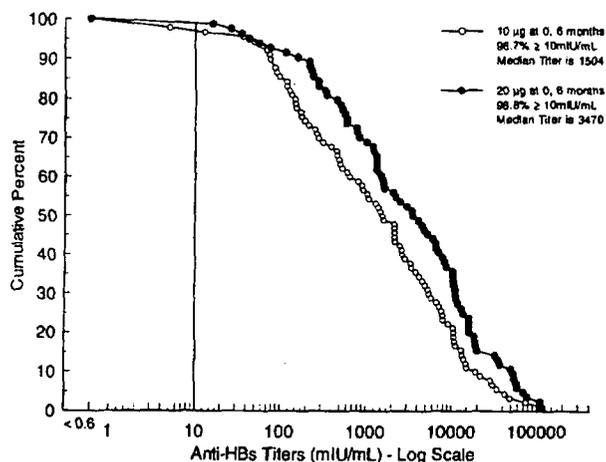


Figure 1 Reverse cumulative distribution of anti-HBs level at 7 months in adults given 10 µg or 20 µg doses of RECOMBIVAX HB[®] at 0 and 6 months

all eligibility criteria, received both doses of vaccine, and had a 7 month blood sample tested for anti-HBs. Seroprotection rates in the two groups were similar, with 97% (95% CI; 90%, 99%) of subjects in the 10 µg group developing ≥ 10 mIU ml⁻¹ of antibody compared to 99% (95% CI; 94%, 100%) of subjects in the 20 µg group. However, the GMT in the 20 µg group was significantly higher than in the 10 µg group (2538 mIU ml⁻¹ versus 1103 mIU ml⁻¹; $P = 0.023$) (Table 3). Figure 1 shows the overall distribution of the 7 month anti-HBs titers in subjects given either dosage of vaccine. The 20 µg dosage generally stimulated higher titers than the 10 µg dosage across a broad titer range, but it is interesting to note that there is little separation between the two groups at titers < 100 mIU ml⁻¹.

Prior to the second dose of vaccine, only 46% of subjects in the 10 µg group and 67% of subjects in the 20 µg group achieved ≥ 10 mIU ml⁻¹ of anti-HBs ($P = 0.006$). But it is apparent that a single dose of vaccine served to prime the immune response in much larger proportions. Three to 6 months after the first dose of vaccine, 74–78% of subjects given a 10 µg dose and 80–87% of subjects given a 20 µg dose had detectable antibody. The cumulative seroconversion rates (i.e. the proportion with antibody at 3 and/or 6 months after the first dose) were even higher and similar across

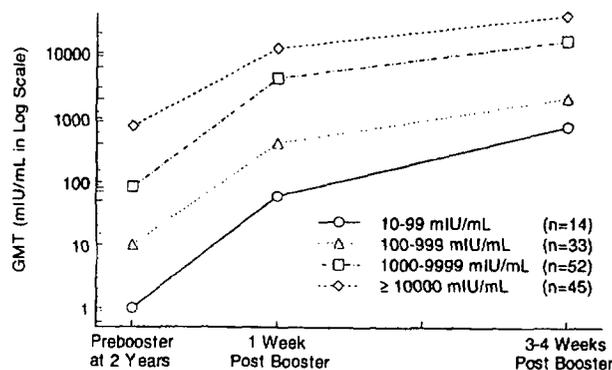


Figure 2 Anamnestic Antibody responses to booster vaccination at two years by initial level of anti-HBs at 7 months

the two dosage groups, being 84% (95% CI; 74%, 90%) and 90% (95% CI; 81%, 95%) for vaccinees in the 10 µg and 20 µg groups, respectively.

Table 3 also shows how anti-HBs persisted in serum samples obtained approximately 2 years (range 20.6–25.1 months; median 24.8 months) after the initial dose of vaccine in 144 vaccinees (73 in the 10 µg group and 71 in the 20 µg group) who met all eligibility requirements for the study, received both doses of the primary vaccination series, had ≥ 10 mIU ml⁻¹ of anti-HBs when tested at 7 months, and were then given a 10 µg booster dose of vaccine following the blood sample at 2 years. Prior to the booster dose, the proportion of vaccinees retaining ≥ 10 mIU ml⁻¹ of anti-HBs had declined to 75% in the 10 µg group and 89% of the 20 µg group with GMTs of 39 mIU ml⁻¹ and 117 mIU ml⁻¹, respectively. Approximately a week after the booster (median 8 days; range 7–11 days), 96% of subjects in the 10 µg group and 99% of those in the 20 µg group had ≥ 10 mIU ml⁻¹ of anti-HBs, and this was associated with substantial increases in GMT to 1526 mIU ml⁻¹ and 3448 mIU ml⁻¹, respectively (Table 3). These results met the predefined criteria of an anamnestic response for 79% of the vaccinees in 10 µg group and 87% of those in the 20 µg group. The rise in antibody continued beyond the first week, so that based on a serum sample taken three to four weeks after the booster (median 23 days; range 19–64 days) 100% of the vaccinees tested had ≥ 10 mIU ml⁻¹ of anti-HBs. The GMTs by this time reached 6213 mIU ml⁻¹ in the 10 µg group and 14776 mIU ml⁻¹ in the 20 µg group, representing increases over the prebooster baseline of 158-fold and 127-fold, respectively.

As in previous studies, an effect of gender on immune response was observed in this study⁶. One month after the second dose, female subjects had a higher seroprotection rate (100% versus 94%) and a higher GMT (3816 mIU ml⁻¹ versus 536 mIU ml⁻¹) than male subjects. After adjusting for treatment effect, these differences were still statistically significant with a P -value of 0.024 for seroprotection rate using a Cochran-Mantel-Haenszel test^{7,8}, and a P -value of 0.001 for GMT using an analysis of variance model. Older age subjects tended to have lower titer levels, but the effect was not significant in this study with its relatively narrow age range of 18–39 years.

Figure 2, which combines results from both treatment groups, further illustrates the anamnestic character of the antibody response to booster vaccination in each of four subgroups comprised of vaccinees whose antibody levels after the primary two-dose series were 10–99, 100–999, 1000–9999, or ≥ 10000 mIU/mL, respectively. Vaccinees in the lowest responder group retained very little antibody at two years. However, this group displayed a 60-fold rise in GMT within a week after receiving a booster vaccination, increasing to 768-fold after three to four weeks, a level 16-fold greater than that attained following the primary series. A large fold-rise in the level of antibody after booster was characteristic of all four groups, although subjects in the ≥ 10000 mIU/mL category, who retained more antibody at the time of boosting, had lower fold-rises than the others. Vaccinees in this group had a 16-fold rise in antibody one week after boosting, increasing to

51-fold after three to four weeks, a level 1.6-fold greater than that attained following the primary series.

DISCUSSION

Young healthy adults respond very well to a standard three-dose schedule of hepatitis B vaccine. For example, a prior series of clinical studies involving several hundred young healthy adults showed that 98–99% of vaccinees given 10 µg doses of RECOMBIVAX HB[®] at intervals of 0, 1, and 6 months developed ≥ 10 mIU ml⁻¹ of anti-HBs with GMTs of 1365–1834 mIU ml⁻¹. The present study sought to test the idea that the first dose of a two-dose series of vaccine can elicit a primary immune response as effectively as the first two doses of a three-dose series, so that a second dose given 6 months later will induce a final antibody response comparable to that expected with a standard three-dose regimen. The results of the study support this concept. The seroprotection rates of 97% and 99% together with GMTs of 1103 mIU ml⁻¹ and 2538 mIU ml⁻¹ among subjects given two 10 µg or two 20 µg doses of RECOMBIVAX HB[®] 6 months apart in this study are similar to the seroprotection rates of 98–99% accompanied by GMTs of 1365–1834 mIU ml⁻¹ observed in previous studies of the standard three-dose regimen of RECOMBIVAX HB[®].

While the end results are similar, there are differences between the two- and three-dose regimens with respect to the early antibody response. In prior studies of the standard three-dose regimen, 82–88% of younger adults had a protective anti-HBs titer of ≥ 10 mIU ml⁻¹ 3 months after the initial dose (2 months after the second dose in the series), while only 51% and 68% of subjects in the present study developed this level of antibody 3 months after a single 10 µg or 20 µg dose of vaccine, respectively (Table 3)⁹. Thus, the standard 0, 1, and 6 months schedule would seem to be the preferred choice for persons at elevated risk of exposure to HBV within a 6 month period or for those who might not return for the last dose of vaccine in the series.

Still, it is important to emphasize that 84–90% of the vaccinees in this study did produce a detectable level of antibody (S/N ≥ 2.1) in the interval 3–6 months after a single dose of vaccine, showing that one dose served to induce a primary immune response in most cases. Indeed, most of the vaccinees who lacked any detectable antibody at the time of the second dose then responded in a way suggesting that they had also been 'primed' by the initial dose of vaccine. Among 29 subjects negative for anti-HBs 6 months after the initial dose, 25 (86%) had a titer ≥ 10 mIU ml⁻¹ when tested a month after the second dose of vaccine. The secondary nature of the response is suggested by the rise in GMT for this subgroup, which changed from 0.6 mIU ml⁻¹ (range <0.6–2.2 mIU ml⁻¹) at 6 months to 137 mIU ml⁻¹ (range <0.6–48408 mIU ml⁻¹) after the second dose. By contrast, the primary anti-HBs response of seronegative vaccinees 1 month after the initial dose of vaccine was quite muted, with GMTs reaching only 1.6 mIU ml⁻¹ and 2.0 mIU ml⁻¹ in the 10 µg dose and 20 µg dosage groups, respectively (range <0.6–110 mIU ml⁻¹).

The level of anti-HBs titer does wane over time after vaccination^{10,11}. In a group of 288 healthy adults, who had ≥ 10 mIU ml⁻¹ of anti-HBs after a three-dose course of RECOMBIVAX HB[®] administered at intervals of 0, 1 and 6 months, there was a 21-fold decline in GMT at year two with 84% retaining a titer of ≥ 10 mIU ml⁻¹¹⁰. Subjects given a two-dose course of vaccine in the present study displayed a similar pattern with declines of 28-fold and 22-fold in GMT and retention of ≥ 10 mIU ml⁻¹ of anti-HBs by 75% and 89% of vaccine responders in the 10 µg and 20 µg groups, respectively, 2 years after the initial dose of vaccine.

Loss of antibody following vaccination does not imply loss of immunity so long as vaccinees retain immunologic memory for HBsAg¹¹. This study shows that vaccinees retained immunologic memory over a 2 year period for at least one correlate of immunity (i.e., humoral antibody). Among those who initially had ≥ 10 mIU ml⁻¹ of anti-HBs a month after the second dose of vaccine, 79–87% displayed a clear anamnestic antibody response when given a booster dose 2 years after initial vaccination, and 100% developed ≥ 10 mIU ml⁻¹ of anti-HBs within 3–4 weeks of boosting accompanied by a large increase in GMT. Similar secondary responses to booster vaccination have been demonstrated in healthy responders initially given a three-dose course of RECOMBIVAX HB[®] at intervals of 0, 1 and 6 months^{12,13}. It is especially significant that the anamnestic response to booster vaccination in the present study was robust in vaccinees with a relatively low titer after the initial two-dose series of vaccination. Ninety percent (9/10) of subjects with titers of only 10–99 mIU ml⁻¹ at 7 months had an anamnestic response, while 83% (88/106) of those with an initial titer > 100 mIU ml⁻¹ displayed such a response a week after booster vaccination.

We conclude from this study that a single dose of RECOMBIVAX HB[®] primes the immune response in a very large proportion of healthy younger adults so that a second dose 6 months later induces a protective anti-HBs response comparable to that expected with a standard three-dose course of vaccination. Furthermore, the study has shown that subjects who responded to the two-dose regimen of vaccine, including those with relatively low titers (< 100 mIU ml⁻¹), retain immunologic memory for HBsAg after 2 years that should provide effective protection against HBV infection even when the antibody level falls below 10 mIU ml⁻¹.

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