Excellent Response Rate to a Double Dose of the Combined Hepatitis A and B Vaccine in Previous Nonresponders to Hepatitis B Vaccine

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(See the editorial commentary by Diepolder, on pages 297–8.)

Background. Hepatitis B vaccine has been shown to be highly efficient in preventing hepatitis B. However, 5%–10% of individuals fail to develop protective levels (>10 mIU/mL) of antibodies to hepatitis B surface antigen (anti-HBs) and are considered to be nonresponders.

Methods. A total of 48 nonresponders and 20 subjects naive to the HBV vaccine received a double dose of combined hepatitis A and B vaccine (Twinrix) at 0, 1, and 6 months. The levels of anti-HBs and antibodies to hepatitis A virus (anti-HAV) were determined before vaccination and 1 month after each dose.

Results. Among 44 nonresponders, protective anti-HBs levels were found in 26 (59%) after the first dose and in 42 (95%) after the third dose. Among the control subjects, the corresponding figures were 10% and 100%, respectively. All subjects seroconverted to anti-HAV. The titers of both anti-HBs and anti-HAV were lower in the previously nonresponsive subjects (P < .01).

Conclusion. Revaccination of nonresponders to the standard hepatitis B vaccine regimen with a double dose of the combined hepatitis A and B vaccine was highly effective. This is most likely explained by the increased dose, a positive bystander effect conferred by the hepatitis A vaccine, or both.

Hepatitis B virus (HBV) is widely spread, and >350 million people are estimated to be chronic carriers [1]. HBV can cause acute hepatitis, which in some cases leads to acute liver failure. Approximately 5% become chronic carriers and are at risk of developing liver cirrhosis and hepatocellular cancer. Transmission of HBV can be prevented by vaccination [2]. After immunization, a serum titer of antibodies to hepatitis B surface antigen (anti-HBs) of ≥10 mIU/mL has been shown to be effective in preventing disease and is the generally accepted level for determining that a vaccine response has occurred [3].

Health care personnel are at risk of acquiring HBV infection, and many authorities recommend vaccina-
In some smaller studies, a high response rate has been noted. In one study, nonresponders to intramuscular vaccine were vaccinated with repetitive intradermal doses every second week (with a maximum of 4 doses), and 17 (94%) of 18 responded to this regime [14]. In another study, 62 (89%) of 70 public safety workers who had been previously vaccinated intradermally responded after 3 additional doses of standard-dose intramuscular vaccine [12]. A higher vaccine dose is recommended in immunocompromised subjects, such as patients undergoing hemodialysis [19]. To protect individuals at high risk for hepatitis B exposure against becoming infected with HBV, there is a need for effective regimes that can make nonresponders respond to hepatitis B vaccine. The combined hepatitis A and B vaccine has been shown to be very effective in inducing seroconversion to both hepatitis A virus (HAV) and HBV in healthy vaccinees, [20]. Some studies have shown even higher anti-HAV levels with the combined vaccine than with the monovalent vaccine in healthy adults [21], whereas other studies have not been able to show this [22, 23]. The aim of the present study was (1) to see whether a high dose of hepatitis B antigen, normally used for immunocompromised patients, in combination with hepatitis A vaccine could induce protective anti-HBs titers (≥10 mIU/mL) in healthy nonresponders to hepatitis B vaccine and (2) to investigate the humoral immune response to hepatitis B vaccine in nonresponders, compared with that in vaccine-naive individuals.

METHODS

Subjects. Health care workers known to be nonresponders to hepatitis B vaccine were asked to participate in a prospective study. Participants were chosen from a group of vaccinees who had participated in a previous study of intradermal hepatitis B vaccine given at 0, 1, and 6 months in the Department of Infectious Diseases, University Hospital, Linköping, Sweden. All vaccinees in the previous study had been vaccinated by 2 nurses who had vast experience with intradermal immunization. Nonresponders were defined as those who had received at least 4 doses of recombinant hepatitis B vaccine (Engerix-B; 0.1 mL intradermally; GlaxoSmithKline) without developing anti-HBs titers of ≥10 mIU/mL; after measurement of anti-HBs, all had received a forth dose followed by new anti-HBs testing, which still showed anti-HBs titers of <10 mIU/mL. All participants received oral and written study information, and all gave oral informed consent for participation in the study. Healthy adults who were >18 years of age and seronegative for HAV and HBV were chosen from people who contacted the department for vaccination to serve as the reference group. The reference group consisted mainly of health care workers and students in health care science. Exclusion criteria for the study were the presence of serious diseases affecting the immune system and pregnancy. All were negative for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen, anti-HBs, and anti-HAV before inclusion. All subjects in the study were vaccinated intramuscularly at 0, 1, and 6 months with 2.0 mL of combined hepatitis A and B vaccine (Twinrix; GlaxoSmithKline) at the Department of Infectious Diseases, University Hospital, Linköping. Blood samples were drawn 5 times during the study period: before the first dose, immediately before the second dose, 1 month after the second dose, immediately before the third dose, and 1 month after the third dose. Adverse events were registered in a questionnaire administered after each dose; additionally, nurses inquired about adverse events each time a sample was drawn and before each new dose. The study was approved by the Ethics Committee of the Health University, Linköping, Sweden.

Methods of virological serology. All testing of HBV markers were performed at the clinical microbiology laboratory of University Hospital, Linköping. Measurement of anti-HBs levels was done using the AxSYM AUSAB assay (Abbott Laboratories). Samples with anti-HBs concentrations reported as >1000 mIU/mL were diluted using the automated dilution protocol. Samples with anti-HBs concentrations reported as >25,000 mIU/mL by the automated dilution protocol were diluted using a manual dilution of 1:25. The amount of anti-HBs in samples was determined using a calibration curve. Measurement of anti-HAV levels was done using the AxSYM HAVAB 2.0 quantitative assay (Abbott Laboratories). The dynamic range of this assay is 0–100 mIU/mL. For anti-HAV levels of >100 mIU/mL, a standard dilution protocol described by the manufacturer was used. For anti-HAV levels of >20,000 mIU/mL, no further dilution protocol existed. Therefore, no further dilution was done. These titers are referred to as 20,000 mIU/mL in the statistical analyses.

Statistical analyses. The study was designed as an open trial. We estimated that, with a study group of ~50 nonresponders and a reference group of 20 previously unvaccinated healthy individuals, we would be able to answer our questions—that is, we would be able to show differences between the groups (but not similarities), and the reference group would show whether the vaccination schedule would work in a normal population. Differences in demographic data between the groups were analyzed by t test. For comparing titers after each dose, the Mann-Whitney rank-sum test was used; for response rates after each dose, Fisher’s exact test was used. We also analyzed the influence of such cofactors as smoking, age, BMI, and sex on the results. For this analysis, we used a general linear model. The model was selected by beginning with a full model that included all interactions allowed by the data; then reducing interactions, starting from highest order stepwise; and then reducing the main effects if they were not part of the interactions. For anti-HBs titers, we choose to do the calculations with logarithmic data, because of the wide variation in levels and because the effect for anti-HBs seemed to be multiplicative rather than additional; we used numeric values for anti-HAV titers. Correlation between log anti-HBs and anti-HAV titers after 3 doses was measured. The
Minitab software package (version 13) was used for the statistical analyses.

RESULTS

Patient demographics. A total of 48 nonresponders were enrolled, of whom 44 completed the study. Of the 4 who did not complete it, 1 moved abroad during the study period, 1 did not show up for further vaccination for unknown reasons, 1 was impossible to evaluate because the subject’s blood samples were accidentally misplaced in the laboratory, and 1 experienced severe tiredness after the first dose and did not receive any further doses (this occurrence was reported to the Swedish Medical Products Agency, which concluded that it could have been a vaccine-related adverse event). Twenty healthy HAV- and HBV-nonimmune adults were enrolled in the reference group, all of whom completed the study. Apart from the tiredness described above, no serious adverse events were seen in either the nonresponder group or the reference group.

Demographic data are shown in table 1. Because all of the subjects in the nonresponder group and the majority of those in the reference group were either health care workers or students in health care science, there was an overrepresentation of women in this study (reflecting that more women than men work in health care). In the nonresponder group, 13 subjects were smokers, 1 had stopped smoking immediately before inclusion, and an additional 4 had stopped 4–5 years before entering the study. The corresponding figures for the reference group were 1 smoker and 2 previous smokers. For 2 persons in the nonresponder group and 1 in the reference group, smoking data were not available. Mean BMI was higher in the nonresponder group (mean, 25.5; range, 20.0–35.6) than in the reference group (mean, 24.4; range, 19.3–30.9), as was the mean age (50 [range, 21–69] years in the nonresponder group vs. 34 [range, 20–59] years in the reference group) (P < .001 for both comparisons).

Antibody responses. Of the 44 nonresponders who completed the study, 26 (59%), 35 (80%), and 42 (95%) developed anti-HBs levels of >10 mIU/mL after the first dose, the second dose, and the complete 3-dose schedule, respectively. In the reference group, 2 (10%) of 20, 19 (95%) of 20, and 20 (100%) of 20, respectively (figure 1). Of the 2 nonresponders, 1 did not have a detectable anti-HBs level, and 1 had an anti-HBs level of 8.5 mIU/mL. Both of these individuals were smokers. After 3 doses, a total of 35 nonresponders (80%) developed anti-HBs titers of >100 IU/mL. All subjects in the reference group had an anti-HBs titer of >100 IU/mL after 3 doses. All subjects in both groups responded to hepatitis A vaccine and developed anti-HAV. Taken together, these data indicate that previous nonresponders show an anamnestic response to revaccination, given that significantly more subjects developed protective anti-HBs levels after the first injection (26/44 [59%] vs. 2/20 [10%]; P < .001, Fisher’s exact test). Thus, despite the absence of protective levels of anti-HBs from the previous vaccination, the immune systems of these subjects were primed to HBsAg, suggesting that they may, in fact, have been protected against infection. The present revaccination schedule was highly effective in inducing seroconversion, given that there was no statistical difference in frequency after 3 doses (42/44 vs. 20/20; difference not significant). However, anti-HBs as well as anti-HAV levels were significantly lower in the nonresponder group than in the reference group (P < .001 and P = .001, respectively). This suggests that nonresponder status with respect to HBsAg may not be an isolated immunological event but rather reflect a more general phenomenon. Anti-HAV and anti-HBs levels after each dose for all individuals are shown in figures 2 and 3, respectively, with the median marked for all doses and groups.

Table 1. Summary of demographic data.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex, no.</th>
<th>Age, mean (range), years</th>
<th>BMI, mean (range)</th>
<th>Smoking, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponders</td>
<td>5/39</td>
<td>50 (21–69)</td>
<td>25.5 (20.1–35.6)</td>
<td>13</td>
</tr>
<tr>
<td>Reference group</td>
<td>2/18</td>
<td>34 (20–59)</td>
<td>24.4 (19.3–30.9)</td>
<td>1</td>
</tr>
<tr>
<td>p</td>
<td>1.0</td>
<td>&lt;.001</td>
<td>.310</td>
<td>.003</td>
</tr>
</tbody>
</table>

NOTE. BMI, body mass index.

Figure 1. No. of vaccinees who achieved a protective level (≥10 mIU/mL) of antibodies to hepatitis B surface antigen (anti-HBs) after each dose in the nonresponder group and in the reference group. P values for differences in the response rate between the 2 groups are given (Fisher’s exact test).
When analyzing the influence of other factors known to correlate with the rate of response to a complete hepatitis B vaccination schedule by use of a general linear model, we found that both a high BMI \((P < .001)\) and smoking \((P = .004)\) were associated with lower anti-HBs levels and that a high BMI was associated with lower anti-HAV levels \((P = .016)\). The effect of these factors seemed to be more pronounced in the nonresponder group than in the reference group for both anti-HBs and anti-HAV. A correlation between anti-HBs and anti-HAV titers after 3 doses was found, with a correlation coefficient of 0.648 for log anti-HBs and anti-HAV titers \((P < .001)\).

**DISCUSSION**

The absence of protective levels of antibodies (i.e., nonresponder status) after hepatitis B vaccination is a clinical problem, and several methods to correct it have been tested [12–18]. In the present study, we used a high dose of the combined hepatitis A and B vaccine and obtained an excellent seroconversion rate (95%) among nonresponders. Other studies have shown similarly high response rates. In one study, intradermally vaccinated nonresponders were inoculated with 3 doses of intramuscular vaccine, and a response rate of 89% was achieved; however, nonresponders in this study had received only 3 doses of intradermal vaccine, and only 49% were primary responders [12]. In another study, repetitive intradermal doses were given as described above to nonresponders to at least 5 doses of hepatitis B vaccine, and 94% responded [14]. One could conclude from these studies and from our present data that the response seems to be dose dependent. We also found that 59% of the nonresponders developed a protective anti-HBs level after the first dose, compared with only 10% in the reference group. This strongly suggests that the immune system of nonresponders was primed by the previous round of vaccination. Subsequently, some or most nonre-

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**Figure 2.** Levels of antibodies to hepatitis A virus (HAV) after each dose in the nonresponder group and in the reference group. Lines indicate medians. The difference in anti-HAV level between the reference group and the nonresponder group after 3 doses was statistically significant \((P < .001)\), Mann-Whitney rank-sum test.

**Figure 3.** Levels of antibodies to hepatitis B surface antigen (anti-HBs) after each dose in the nonresponder group and in the reference group. Lines indicate medians. \(P\) values for differences in anti-HBs level after each dose between the 2 groups are given (Mann-Whitney rank-sum test).
sponders might actually be protected against hepatitis B despite the absence of protective anti-HBs levels, consistent with previous observations [24]. Only 2 of the nonresponders failed to produce protective levels of anti-HBs, and of these only 1 was completely negative for anti-HBs, suggesting a true nonresponder status. Thus, true nonresponder status seems to be quite rare, whereas most subjects who fail to respond to the first round of hepatitis B vaccination are merely poor responders.

In the present study, we also confirmed the effects of previously noted factors predisposing to nonresponder status. For example, both of the nonresponders who failed to respond to revaccination were smokers. However, many others had this risk factor, suggesting that smoking alone cannot explain their poor anti-HBs responses. Other known risk factors for nonresponder status seemed to affect the anti-HBs titers to a greater degree in the nonresponder group than in the reference group, indicating that other factors, such as genotype, could influence the results.

Our results suggest that a high dose of HBsAg in combination with the hepatitis A antigen could offer a clinically relevant approach to correcting nonresponder status in persons who are not already immune to hepatitis A and for whom protection against hepatitis B is essential. It has been shown that, in healthy individuals, anti-HBs levels are higher when the combined vaccine is used than when a vaccine containing HBsAg alone is used [25]. It is therefore likely that, with the combined hepatitis A and B vaccine (Twinrix), the hepatitis A component might act as an adjuvant for the hepatitis B response. Our present data certainly favor this hypothesis. Titers are of less importance according to present consensus recommendations, which state that once the titer reaches ≥10 mIU/mL there is no need for further testing or booster doses in immunologically competent persons [26].

A potentially important observation was that both anti-HBs and anti-HAV titers were significantly lower in the nonresponder group than in the reference group. This would imply that nonresponder status after the first round of hepatitis B vaccination reflects a more general inability to rapidly generate high levels of antibodies in response to vaccination. Our findings suggest that, in these individuals, the reduced ability to respond to hepatitis B vaccine could be associated with a generally reduced capacity to produce vaccine-induced antibodies. To test this hypothesis, it would be of interest to investigate whether nonresponders or low responders to hepatitis B vaccine also have poor response to other vaccine antigens after immunization. Another explanation for the reduced levels of both anti-HBs and anti-HAV in the nonresponder group could be that, whereas the hepatitis A component acts as an adjuvant to the hepatitis B response, the hepatitis B antigen (or immune response) had a negative effect on the hepatitis A response. Additional studies are needed to elucidate this issue.

In conclusion, in light of the present results, we propose that a double dose of the combined hepatitis A and B vaccine can be safely and effectively used to induce protective levels of anti-HBs in nonresponders to the traditional hepatitis B vaccine among those nonimmune to hepatitis A.

Acknowledgments

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References

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