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Absence of anti-hepatitis B surface antibody after vaccination does not necessarily mean absence of immune response

Received: 16 June 2000

Abstract A small number of subjects vaccinated against hepatitis B do not produce anti-hepatitis B surface (HBs) antibody levels detectable by commercial assays. Others lose detectable anti-HBs at some time after vaccination. The absence of clinical hepatitis despite potential exposure to hepatitis B virus (HBV) in both kinds of subjects suggests that they might be protected by low antibody levels. However, besides anti-HBs, T helper response and memory cells which may be induced by the vaccine are certainly also important for immunity against HBV. In the present study, samples from vaccinated subjects, found to be anti-HBs negative in an initial assay, subsequently showed positive results in, respectively, 25%, 36% and 38% of the cases, when a second, third and fourth assay was used. In addition, 360 samples from “nonresponders” and from vaccinees who had lost anti-HBs, the reactivity of which was under the enzyme-linked immunoassay-cut-off value were compared to that of nonvaccinated controls. The absorbances were found to be significantly higher in the nonresponders (0.038) and in the vaccinees having lost anti-HBs (0.041), than in the controls (0.025). Such findings contribute to explaining why so-called nonresponders as well as vaccinees who have lost anti-HBs nevertheless appear to be protected.

Key words Hepatitis B vaccine · Anti-hepatitis B surface antibody · Hepatitis B infection · Serology

Introduction

Hepatitis B vaccines were introduced in 1981 as plasma-derived vaccines and, in 1986, as the recombinant vaccines that are still in use. The quality of the protection conferred by such vaccines can now be estimated on the basis of the 19 years that have elapsed since their initial introduction.

The efficacy of the vaccination has been assessed with the help of the anti-hepatitis B surface antibody (anti-HBs) levels obtained. Levels reached 1 month after the last injection of the primovaccination schedule have often been considered. Subjects with no anti-HBs at this point, as measured by commercial enzyme-linked immunoassays (EIA), are said to be “nonresponders”. Their proportion of the population depends on various factors, including gender, age, body mass index, smoking habits and immunogenetic factors [12, 14]. The risk for such subjects of becoming infected with HBV and/or of developing clinical hepatitis is not well defined.

Since the earliest studies [6, 16], an anti-HBs titer higher than 10 mIU/ml has been accepted as protective. Despite its arbitrary character, this limit has benefited from a consensus that has proven useful for comparing the immunogenicity of various vaccine preparations or of vaccination schedules. The risk of developing hepatitis for subjects having anti-HBs levels between 0 and 10 mIU/ml is, however, not better known than in subjects having 0 mIU/ml. It seems that there are practically no clinical cases of hepatitis which occur today in subjects who have undergone the entire vaccination schedule, including the nonresponders and the subjects having anti-HBs between 0 and 10 mIU/ml. Two exceptional cases, one clinical and one with enzyme elevations only, have been reported [1, 10]. This suggests that protection can be achieved thanks to undetected levels of anti-HBs or to another type of immune response.

Such consideration also applies to vaccinees, who have lost a previously demonstrable anti-HBs level. In
such subjects, there has never been a case of clinical hepatitis described.

Here, we propose the hypothesis that the nonresponders, as well as vaccinees who have lost detectable anti-HBs levels, might possess antibody levels that are too low to be detected by a commercial EIA but, however, are still sufficient to protect. In the present study, we tried to confirm this hypothesis by (1) testing negative samples with additional commercial EIA, and (2) using the absolute values of absorbances rather than the calculations based on the EIA cut-off values.

**Material and methods**

**Serum samples**

A first group of 468 samples were obtained from 299 vaccinated subjects, including:

- 146 samples from 146 subjects vaccinated 5 years previously
- 58 samples from 19 subjects, also primovaccinated 5 years previously, but having received a booster injection 3, 7, 15 or 30 days before
- 134 samples taken from 134 subjects 30 days after one initial injection of vaccine and 134 additional samples from the same 134 subjects, 30 days after a second injection.

The 299 subjects were hospital employees included in a vaccination study [2].

A second group of 360 samples obtained from 308 vaccinated subjects (hospital employees, police officers and travelers), all of whom were also without anti-HBs detectable by a commercial EIA, consisted of:

- 118 samples from 118 nonresponders (74 men and 44 women, aged 25–63 years, median 43 years), of whom 36 were after primovaccination and 82 were after primovaccination and revaccination. Of these 118 nonresponders, 58 were subsequently to be given an additional injection.
- 90 samples from 90 vaccinees (52 men and 38 women, aged 23–63 years, median 36 years), who had lost anti-HBs. Of these subjects, 80 were to receive a subsequent reimmunization.
- 152 samples from 100 subjects (40 men and 60 women, aged 19–39 years, median 24 years), 15 or 30 days after a first injection of vaccine.

The results obtained in the second group were compared to those of 100 control samples, obtained from the same 100 subjects as before, but taken before vaccination. These were day 0 samples of a vaccination study [8]. All 100 subjects were known to be without previous HBV infection (also anti-HBc negative).

**Methods**

All 468 samples were tested with EIA 1 and 2. If results were negative with both EIA, further testing occurred with a third and, if necessary, a fourth EIA. The EIA used were the COBAS CORE Quant.EIA (Hoffmann-La Roche, Basel, Switzerland), the Abbott AUSAB EIA (Abbott, Illinois, USA), the Enzymun-Test (Boehringer-Mannheim, Indianapolis, USA), and ETI-AB-AUK (Sorin Biomedica, Saluggia, Italy). However, as this study was not designed to compare anti-HBs EIA specificity and sensitivity, and since new versions have been introduced since then, the kits are designated in the results section as EIA 1–4, according to the order used. The EIA were used according to the manufacturers instructions and their cut-off calculated as prescribed for the kit.

For the second group, presence of anti-HBs was assessed using the absolute values of the absorbance obtained with one commercial EIA (Cobas Core Roche Anti-HBs Quant EIA, Basle, Switzerland), without considering the cut-off value of the test. The median absorbance of each subgroup was compared to that of the control group of nonvaccinated subjects.

The exact Fischer’s test (chi-square method) was used to calculate the coefficient of probability. Median absorbances which are asymmetrically distributed were compared, using a nonparametric test (Mann-Whitney).

**Results**

In the first group of 468 samples, 262 samples from vaccinated subjects were found anti-HBs negative with EIA 1 and 213 with EIA 2. Sensitivity of EIA 1 (74.9%) was lower than that of EIA 2 (94.2%, P < 0.01). In the 262 samples negative with EIA 1, the successive use of a second, a third and a fourth EIA enabled one to respectively reduce the rate of “nonresponse” from 100% to 75%, 64% and 62% (Table 1). If the sequence of testing is changed, beginning with a more sensitive EIA (EIA 2), the rate of nonresponse is reduced from 100% to 92%, 79% and 76%, using three additional EIA.

The 262 samples found to be negative with EIA 1 consisted of four subgroups of samples taken from vaccinated subjects. Table 1 shows that the subgroup of the 49 samples taken from true nonresponders was reduced by only 12% when using the three additional EIA, whereas the three other subgroups were reduced by 44–50%.

The samples, that became positive when an additional EIA was used, had only low levels of anti-HBs in the additional assay(s). These levels were less than 10 mIU/ml in all of the 49 samples taken from nonresponders. They were also less than 10 mIU/ml in the other three subgroups, except in nine samples, that had between 10 and 100 mIU/ml.

Table 2 shows the absorbances (A), obtained in the subjects from the second group, using the EIA routinely used for measuring anti-HBs. The A values were all under the cut-off of the assay, which was 0.080. The median A of the nonvaccinated controls was 0.025 and that of the 118 nonresponders to vaccination, 0.038. These 118 subjects consisted of 36 negative after primovaccination and 82 after revaccination: their A values were, respectively, 0.040 and 0.037, in both cases significantly higher than those of controls. The subjects having lost anti-HBs after vaccination, as well as those not yet having produced detectable anti-HBs levels, also had A elevations of the same order of magnitude.

Table 2 (fourth line) shows that only four subjects (4%) of the control group were above the median A + 2SD of this group (i.e., above 0.025 + 2 × 0.0106 = 0.0462). About one third of the nonresponders were above this limit.

Table 3 shows that the A values of nonresponders, subsequently given an additional vaccine injection, were higher in those who finally responded to the booster than in those who did not. The observation was the same for nonresponders to primovaccination and to revaccination. Table 3 also shows that, of 80 vaccinees who had lost anti-HBs, 67 who later responded to a