Antibody to Hepatitis B Surface Antigen (Anti-HBs) Induced by a Recombinant Hepatitis B Vaccine Consisting of Subtype adr Antigen is Underestimated on the World Health Organization (WHO)-standardized Assay

Key words: hepatitis B vaccine, hepatitis B surface antigen subtype

The protective efficacy of hepatitis B vaccination against hepatitis B virus (HBV) infection is well established. In the USA and Europe, a serum or plasma level of antibody to hepatitis B surface antigen (anti-HBs) above 10 milli-international units per milliliter (mIU/ml) standardized by the World Health Organization (WHO) reference preparations is generally considered protective (1, 2). In Japan, however, since passive hemagglutination (PHA) methods have been successfully adopted for evaluating anti-HBs, a level of above 10 mIU/ml seems to be not collectively regarded as protective. For instance, Yoshida and Saito indicated that health-care personnel (HCP) with an anti-HBs level below 100 mIU/ml should be revaccinated (3). One reason for such inconsistency may be differences in the sensitivity and specificity of assay methods (2), including mismatches of hepatitis B surface antigen (HBsAg) between vaccines or infected HBV and assay systems (4).

To examine whether HBsAg subtypes of vaccines affect anti-HBs measurements, we compared anti-HBs titers determined by PHA and by WHO-standardized chemiluminescentimmunoassay (CLIA) in serum samples from individuals who received yeast-derived recombinant HB vaccines consisting of major surface protein of different subtypes. The study was conducted by the Health Care Center of Toyama Medical and Pharmaceutical University and was approved by the University Human Investigation Committee, and included 656 students. Every participant gave written informed consent. In 2000, 437 students, 228 men and 209 women (18 to 31 years of age; mean 19.3±2.0) received the vaccine including subtype <jyw-HBsAg (Yoshitomi Pharmaceutical, Osaka). In 2001, 219 students, 119 men and 100 women (18 to 36 years of age; mean 19.4±2.3) received the vaccine containing subtype adr-HBsAg (Fujisawa Pharmaceutical, Osaka). There were no significant differences in gender and age between the two vaccinee groups. All recipients were tested for serum anti-HBs one month after completion of the standard three-dose vaccination series at 0, 1, and 6 months. Anti-HBs levels in given samples were tested simultaneously by PHA (Institute of Immunology, Tokyo) and by CLIA (Dainabot, Tokyo).

Results in a range of PHA titer of less than 64 are shown in Fig. 1. Recipients of each vaccine responded well, with only 2 <jyw-vaccinees and 3 adr-vaccinees showing anti-HBs levels below PHA titer 8. Overall, samples from adr-vaccinees tended to demonstrate lower CLIA measurements than those from <jyw-vaccinees. In a range of PHA titer of 8 to 16, which are taken to be protective in Japan, all 9 <jyw-vaccinees, but only 1 of 6 adr-vaccinees, showed CLIA measurements above 10 mIU/ml. When the PHA titer was over 64, lower CLIA measurements in adr-vaccinees than in <jyw-vaccinees were significant. The median CLIA measure-
mets (mIU/mL) in samples from adr-vaccinees versus (vs.) ayw-vaccinees with PHA titers of 64, 128, 256, 512, 1,024, 2,048, and 4,096 were as follows; 136 vs. 69 (p<0.01), 442 vs. 177 (p<0.0001), 744 vs. 356 (p<0.0001), 2,270 vs. 737 (p<0.0001), and 9,040 vs. 4,940 (p<0.01) (Wilcoxon rank sum test). Such differences were confirmed by repeated testing at two different laboratories. Thus, at the PHA titers set as internal standards, CLIA measurements underestimated the adr-vaccine-induced anti-HBs when compared with the ayw-vaccine-generated antibody.

There are differences in assay principals between PHA and CLIA, such as solid-phase materials to which HBsAg is bound and procedures for measuring anti-HBs which reacts with the solid-phase antigen. However, according to the manufacturers' information, the sensitivity and specificity for determining anti-HBs concentration in each system have been strictly inspected. Other obvious differences between the two methods are subtypes of the solid-phase HBsAg and possibly of the control anti-HBs derived from human plasma or serum. The solid-phase HBsAg in PHA is of subtypes adr+adw, while that in CLIA is of ad+ay. Although detailed information is not available, the control anti-HBs in the two systems would be derived from individuals of different races, indicating that different HBV subtypes generate the control anti-HBs in each system. Regarding amino acid sequence of the major surface protein of the vaccines, 19 of 226 amino acids are different between the two vaccines used in this study. Taken together, we consider that the major reason for our results is that anti-HBs induced by the adr-vaccine reacts less well with subtypes ad+ay antigens in the CLIA system than the antibody generated by the ayw-vaccine. Findings similar to ours have shown that anti-HBs generated by the pre-S2 protein-containing adr-vaccine was underestimated by a WHO-standardized radioimmunoassay. Because antibody to the a epitope of the major surface protein is believed to afford protection against HBV irrespective of subtypes, the anti-a amount would represent a precise value and affect the anti-HBs measurements. However, as standardized assay methods for the anti-a levels have not been available, this issue is currently beyond our present scope.

The clinical significance of our findings would be that HCP who had received the adr-vaccines and showed an anti-HBs concentration below 10 mIU/ml (of course below 100 mIU/ml) on WHO-standardized assays may be "unnecessarily" enrolled in HB vaccine dosing at booster schedules or HB immunoglobulin (HBIG) administration at occupational exposures, even though they possess a protective anti-HBs amount based on the consensus in Japan. Indeed, the protective anti-HBs amount should be determined according to the magnitudes of HBV exposure, but a positive anti-HBs test by PHA, which is represented by titer of 8 to 16, has been adopted as the minimum protective level in Japan, as has been indicated by The Japanese Society of Gastroenterology. Frequent administrations of HB vaccine or HBIG are considered undesirable not only because of costs but also because of possible adverse events (1, 2).

In summary, we consider it important to specify HB vaccine products and anti-HBs assay methods in discussing issues on HB vaccination to avoid confusion among facilities.

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