Rapid seroprotection against hepatitis B following the first dose of a Pre-S₁/Pre-S₂/S vaccine

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Background/Aims: Will immunization with an experimental Pre-S₁/Pre-S₂/S hepatitis B vaccine (Bio-Hep-B™) induce faster seroprotection using fewer doses as compared with a yeast derived S vaccine (Engerix B®).

Methods: Healthy volunteers, n = 36, mean age 23 y, randomized to receive 2 or 3 doses of both vaccines given months 0 and 6, or 0, 1 and 6.

Results: Following primary immunization, seroprotection occurred in 6, 39, 53 and 60% in the Bio-Hep-B™ group at weeks 1, 2, 3 and 4, compared with 0, 12, 18 and 12.5% in the Engerix-B® vaccinees, respectively. Six months following injection of the first dose, seroprotection was 70 and 25% in Pre-S/S and S vaccinees respectively. Area under the curve in vaccinees of Bio-Hep-B™; versus Engerix-B® showed mean anti-HBs level of 365 ± 166 and 85 ± 48 mIU/ml £ day respectively (P < 0.012). At month 7, 100% seroprotection was achieved in both groups while anti-HBs rose from 81 to 28 800 mIU/ml and from 12 to 923 mIU/ml in recipients of Bio-Hep-B™ and Engerix-B® respectively (P < 0.025).

Conclusions: Bio-Hep-B™ induces rapid seroprotection against hepatitis B in 60–70% of vaccinees, within 4–24 weeks after the first dose. Two instead of the conventional three doses of the Pre-S/S vaccine may be sufficient to induce adequate seroprotection.

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1. Introduction

Chronic hepatitis B (HBV) infection is a serious global problem affecting approximately 300 million individuals worldwide [1]. Active immunization against HBV has made enormous progress in the past two decades. Following development of the first generation serum derived HBV vaccines, second generation yeast derived recombinant HBV vaccines were introduced starting in 1986. Over 110 countries have already introduced universal vaccination against HBV to their immunization programs. The yeast derived HBV vaccines, which contain the non-glycosylated small S HBV surface antigen (rHBsAg) have an excellent record of safety and immunogenicity in young and immunocompetent individuals immunized by three doses given at 0, 1 and 6 months i.m. [2,3]. However, in immunosuppressed individuals such as in patients receiving radiation, chemotherapy or dialysis, the immunogenicity of these vaccines is significantly reduced. Furthermore, the current practice of immunization with three doses of an HBV vaccine is not always implemented. In developing and even developed countries the second or third dose of the vaccine is frequently missed due to low compliance or logistic problems. Thus, there is a rationale for developing more immunogenic HBV vaccines that should provide faster protection immediately after injection of the first dose and require fewer doses for achievement of protection. Indeed, such candidate vaccines are now in various phases of development [4–6]. These vaccines are produced in mammalian cells and contain, in addition to the small non-glycosylated S envelope protein, glycosylated S and Pre-S₂ antigens [4]. Two of these experimental vaccines also contain the Pre-S₁ antigen [5,6]. Available data suggest that inclusion of Pre-S proteins in addition to the small surface antigen and the physical properties of the viral surface particles produced
in mammalian cells, contribute to the enhanced immunogenicity of these new, third generation vaccines [4,5].

**Bio-Hep-B/Sci-B-Vac™** is such a third generation vaccine produced in HBV transfected Chinese Hamster Ovary (CHO) cells, which secrete the entire spectrum of the envelope proteins of the virus (namely, S, Pre-S1 and Pre-S2) [5,7,8]. The vaccine contains glycosylated and nonglycosylated forms of the 3 surface antigens absorbed to alum. The addition of the Pre-S antigens and their glycosylated form was shown by Milich et al. [9] to enhance the immune response against the small envelope protein. The experimental Pre-S1/Pre-S2 HBV vaccine has previously shown to induce an enhanced humoral immune response against the hepatitis B surface antigen (HBsAg) in mice as compared with two second generation, yeast derived vaccines [5]. Furthermore, Bio-Hep-B™ has been shown to bypass genetically determined non-responsiveness to vaccination in mice resistant to immunization with the small S protein [5]. The enhanced immunogenicity of Bio-Hep-B™ has now been demonstrated repeatedly in human subjects [8–14]. However, no controlled trials were conducted thus far to assess the pace at which seroprotection against HBV is achieved following the first dose of the vaccine.

### 1.1. Aim

To test the hypothesis that the mammalian cell derived vaccine Bio-Hep-B™, 10 µg/dose, will induce faster seroprotection against HBV using one and two doses as compared with the conventional licensed yeast derived vaccine Engerix-B®, 20 µg/dose, given three times over a period of 6 months.

### 2. Materials and methods

Thirty-six healthy volunteers (20 males, 16 females), mean age 23 years (range 19-28 years) without evidence of systemic disease and tested negative for anti-HBc and anti-HBs antibodies, were randomized by draw to receive either 2 i.m. doses at 0 and 6 months of Bio-Hep-B™ (10 µg/dose) (Biotechnology General, Rehovot, Israel) or Engerix-B® (Smith Kline–Beecham, Belgium) (20 µg/dose). In addition, two similar groups were randomized to receive 3 doses of either Bio-Hep-B™; or Engerix-B® at 0, 1 and 6 months. Bio-Hep-B™, also known as Sci-B-Vac, is an experimental mammalian cell derived Pre-S1/Pre-S2/S HBV vaccine [7,8], while Engerix-B® is a yeast derived vaccine containing only the S protein licensed for administration in 3 doses given at 0, 1 and 6 months [3]. Anti-HBs antibody levels as measured for protection against HBV infection, were tested at baseline day 0, weeks 1, 2, 3, and 4 after the first dose, at 6 months after the first dose (just prior to the second injection) and at 2 and 4 weeks following the second or third doses using an AUSAB® assay (Abbott, Chicago, IL). Seroprotection was defined at an anti-HBs level of >10 mIU/ml. The clinical trial was approved by the Hadassah Institutional Review Board and all participants in the study gave written informed consent.

#### 2.1. Statistical methods

Area under the curve (AUC) of anti-HBs levels was calculated by PC info software (retriever data systems) compared in vaccinees of both vaccines using the Wilcoxon non-paired rank sum test. Serum anti-HBs levels were compared using the Student t-test.

### 3. Results

#### 3.1. Immunogenicity of hepatitis B vaccines during the first month post priming

During the first 4 weeks following the priming vaccine dose, seroprotection was observed in 6, 39, 53 and 60% of vaccines in the Bio-Hep-B™ group (n = 19) at weeks 1, 2, 3 and 4 respectively. In contrast, seroprotection was observed in only 0, 12, 18 and 12.5% of recipients of Engerix-B® (n = 17) at 1, 2, 3 and 4 weeks post-primary immunization, respectively. Mean levels of anti-HBs were 18.1 ± 6.8 and 4.1 ± 2.3 in the Bio-Hep-B™ and Engerix-B® vaccinees, respectively, at 4 weeks after the first injection. AUC of anti-HBs levels in the first month was calculated for each volunteer (Table 1) expressed in mIU/ml × day. This parameter provides more accurate information on the individual anti-HBs protection level during the selected period of observation as compared with the more conventional quantitative anti-HBs assay. Comparison of AUCs in the vaccinees of Bio-Hep-B™ vs. vaccinees of Engerix-B® showed a mean level of 365 ± 166 and 85 ± 48 mIU/ml × day respectively (P = 0.012).

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<td>38</td>
<td>Mean (±SE) 365.11 ± 166</td>
<td>Mean (±SE) 85.44 ± 48</td>
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*P = 0.012 (Wilcoxon non-paired test). |

Expressed in mIU/ml × day.
3.2. Immunogenicity of hepatitis B vaccine six months post priming

At 6 months post-priming following an injection of a single dose, seroprotection was already 70% in Pre-S/S vaccinees (mean anti-HBs titer of 81 mIU/ml) as compared with 25% in the yeast derived S vaccinees (mean anti-HBs titer of 12 mIU/ml) (Fig. 1). After one booster injection at 6 months, 100% seroprotection was achieved in all groups with anti-HBs levels rising 356-fold to 28 800 mIU/ml and 77-fold to 923 mIU/ml in recipients of Bio-Hep-B™ and Engerix-B®, respectively (Fig. 2) (P < 0.025 when comparing two doses of 10 μg/dose Bio-Hep-B™ vs. two doses of 20 μg/dose Engerix-B®). A second injection of Engerix-B® at 1 month (but not of Bio-Hep-B™) in addition to the 6 month booster, was required to reach similar anti-HBs levels for both groups of vaccinees at 7 months. Both vaccines were well tolerated, and no significant adverse events were observed in recipients of Bio-Hep-B™ or Engerix-B®.

4. Discussion

HBV is one of the most common causes of liver disease. According to an estimate by the World Health Organization, over 2 billion people worldwide have been infected with the virus during their lifetimes. The HBV's envelope is composed of three proteins: the small (S), middle (Pre-S2) and large (Pre-S1) antigens, derived from a single open reading frame in the HBV genome [15]. In the native viral envelope, all three proteins are covalently linked to one another by intermolecular disulphide bonds and are partially embedded in membrane lipids. The biological significance of the Pre-S envelope proteins is only partially understood. It has been suggested that the Pre-S2 domain plays a role in the binding of HBV of the hepatocyte [16], and that Pre-S2 might be involved in the penetration into the hepatocyte. These theories suggest that effective anti Pre-S1/Pre-S2 activity may be useful in prevention of HBV infection.

Indeed, Neurath et al. [17] has shown that antibodies against Pre-S1 are HBV neutralizing. They suggested that the Pre-S1 domain should be considered for inclusion into HBV vaccines. Others showed that a synthetic Pre-S2 peptide vaccine given to chimpanzees raised antibodies that bound to viral particles and protected the animals from challenge with hepatitis B virus [18].

The currently used licensed yeast derived recombinant vaccines against HBV have an excellent record of safety and immunogenicity. However, three doses, given at months 0, 1 and 6, are required to induce maximal seroprotection in healthy children and young adults. Even with three doses of these vaccines, some healthy populations remain non-responders. Vaccination failure rates increase with age, obesity, and systemic diseases such as kidney disease or other causes of immune suppression [19,20]. Furthermore, non-responsiveness to conventional vaccination may also be genetically determined [21]. As a result, the number of non-responders and hyporesponders to HBV vaccination is relatively large, ranging between 6–8% at 18 years old and rising by 5–10% for every decade post-30 years of age. Thus there is a need for development of a more potent vaccine to be used in the non-responders and hyporesponder populations.

The present controlled study was conducted in young adults to confirm the results observed in children that the humoral anti-HBs immune response to a primary dose of the Pre-S/S vaccine is faster as compared with the yeast derived S vaccine. Vaccinees’ sera were tested weekly during 4 weeks after the first priming dose of either Bio-Hep-B™ or Engerix-B® and then at weeks 24 and 28 for detection of seroconversion. Bio-Hep-B™ demonstrated an enhanced immunogenicity during the first month after immunization as shown by a significantly higher calculated AUC for anti-HBs, and a higher seroprotection rate achieved using a 50% lower HBsAg dose as compared with vaccinees receiving Engerix-B®. This increased immunogenicity may be useful especially when rapid immunization against HBV is required, as when vaccinees do not show up for the conventional week 4 booster immunization. Andre et al. [22] reported that seroconversion rates (anti-HBs > 1mIU/ml) in healthy subjects after vaccination with Engerix-B® at 0,
1 and 6 months were 43, 96 and 99.5% for 1, 6 and 7 months. In the present study, when seroprotection was defined more stringently at serum anti-HBs levels of >10 mIU/ml, seroprotection for Engerix-B® vaccinees was 12.5, 25 and 100% at 1, 6 and 7 months using the 20 μg/dose injection given at 0 and 6 months. In contrast, in Bio-Hep-B™ vaccinees (injected at 0 and 6m with 10 μg/dose) seroprotection was 60, 70 and 100 at 1, 6 and 7 months using a 50% lower dose of HBsAg. These results suggest an enhanced human immune response for Bio-Hep-B™ following the first vaccine dose.

Thus, a single dose of 10 μg Bio-Hep-B™ was sufficient to induce seroprotecting anti-HBs levels in 70% of vaccinees at 6 months post priming. Our results also suggest that two doses of Bio-Hep-B™ were sufficient to induce similar anti-HBs levels as observed after three doses of Engerix-B®. In the present study, there were no non-responders in either group, which may be due to the age of the volunteers and the small sample size. It is important to note that presently all licensed hepatitis B vaccines are approved for prevention of HBV provided that three doses of the vaccines are given at 0, 1 and 6 months. The present communication wishes to challenge this concept.

To date, the presence of antibodies to Pre-S1 and Pre-S2 following immunization with Bio-Hep-B™ has been documented only in mice and rabbits [15]. Available assays for measurements of anti-Pre-S1 antibodies in humans have a high background activity and are not yet reliable enough. Therefore, we must await the development of more reproducible anti-Pre-S1 and Pre-S2 assays, which is in progress.

The enhanced immunogenicity of the new vaccine suggests that single dose immunization against hepatitis B may be an achievable objective. Indeed, 70% of Bio-Hep-B™ vaccinees had protecting anti-HBs levels at 6 months after a single dose of 10 μg HBsAg. We postulate that the remaining 30% of vaccinees who did not have detectable anti-HBs antibodies using a conventional assay, may have in fact already undergone priming of their immune memory and will respond to a new HBV challenge with development of adequate anti-HBs levels. This assumption is based on the 100% seroconversion rate observed 1 month following a booster dose at 6 months post priming. Further studies to prove the presence of immune memory to HBsAg in these 30% of vaccinees who have not yet seroconverted after the first vaccine dose are in progress. Preliminary data suggest that lymphocytes taken from these initially anti-HBs negative subjects respond in-vitro to stimulation with recombinant HBsAg. (D. Shouval et al., personal observation).

Finally, to date, eighteen clinical studies using Bio-Hep-B™ at a conventional three dose schedule have been conducted in adults, children and neonates. In these studies, 10,220 vaccine doses have been administered to 3432 subjects [10–14]. In these studies 591 healthy adults and 439 children completed a 12 month serologic follow-up of anti-HBs after immunization with three doses at 0, 1 and 6 months. A rapid seroconversion rate was observed at 2 and 6 months following the first and second injections (at 0 and 1 month) of 5 or 10 μg/dose ranging between 45–96% and 54–98% in adults and children, respectively [8]. The high immunogenicity of the experimental vaccine was also manifested in the generation of unusually high anti-HBs titers in children [8].

We conclude that the Pre-S1/Pre-S2/S HBV vaccine induces earlier seroprotection against HBV, during the initial 4 weeks post priming as compared to the yeast derived vaccine tested. Thus two, instead of the conventional three doses of the Pre/S/S vaccine, are sufficient to induce protection against HBV. This enhanced immunogenicity may be useful especially when rapid seroprotection against HBV is required.

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References


