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Hepatitis B vaccines

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1. Introduction

Hepatitis B vaccines have now been available for over 20 years. The World Health Organization has recommended that hepatitis B vaccination should be included in routine immunization for all children, worldwide. As a result, various immunization strategies have been developed for routine infant vaccination, prevention of perinatal transmission and catch-up vaccination for older age groups.

Knowledge of the structure and genomic organization of the hepatitis B virus (HBV), reviewed in Refs. [1,2], has led to the development of immunogenic HBV vaccines with an excellent record of safety and immunogenicity. The purpose of this short overview is to summarize the structure and properties of available licensed HBV vaccines relevant to the hepatitis B consensus conference and to discuss potential applications for some of the newly developed vaccines.

All available HBV vaccines contain the hepatitis B envelope protein. Hepatitis B surface antigen(s) (HBsAg) is composed of three related envelope proteins which are synthesized by the alternate use of three translational start codons and a common stop codon. The HBsAg proteins include a major polypeptide of 226 amino acids (aa) designated small HBs (SHBs) previously also called major surface protein, in a non-glycosylated (p24) and glycosylated (gp27) form. The middle-sized protein (MHBs), which shares the 226 aa of the p24 region at the C terminus and has an additional 55 aa residue at the N terminus, is termed pre-S_2 corresponding to gp33 and gp36. The large HBs protein (LHBs) contains, in addition to the S and pre-S_2 domains, the pre-S_1 domain of 119 aa (p39, gp42) [1,2,4–6]. In the native envelope, all the proteins SHBs, MHBs, and LHBs are covalently linked to one another by intermolecular disulfide bonds between the S domains and partially embedded in membrane lipids. A relatively high proportion of LHBs will favor formation of filamentous HBsAg particles, while presence or absence of the MHBs domain will not affect the morphology of the HBsAg particle. The envelope of the wild-type complete HBV particle has a composition similar to that of secreted HBsAg filaments, with a high proportion of LHBs. Thus, a vaccine containing HBsAg filaments should be able to induce an immune response to all three-envelope antigen components.

Originally four major subtypes of the HBV envelope protein, namely *adw*, *adr*, *ayw*, and *ayr* were serologically identified. These were further divided into several subgroups. The frequency of these subtypes is distinct in different geographic areas. To date, at least seven genotypes (A–G), coding for various subtypes are known. The antigenic *specificity a* is common to all HBV subtypes, while *d/y* and *w/r* are mutually exclusive. Antibodies to the a determinant provide protection against infection by any of the HBV subtypes. However, several mutants of the envelope protein have been described following active or passive immunization with HBV vaccines or hepatitis B immunoglobulin (HBIG) [3].

The biologic function of the various envelope proteins and the relative significance of the immune response to each of its components (S, pre-S_2, and pre-S_1) are only partially understood [4,5]. Induction of immune memory against HBsAg and generation of anti-HBs antibodies are essential for long-term protection against HBV. In acute HBV infection, followed by resolution of viremia, detection of anti-HBs in serum, which represents the humoral immune response to the SHBs, is delayed for several weeks. In contrast, Pre-S_1 and pre-S_2 antigens and antibodies appear and disappear early after acute infection, sometimes in a biphasic pattern [15]. The pre-S antigens seem to be important in inducing T-cell help for production of anti-HBs. Thus, T-cell recognition requires presentation to T cells of HBV antigenic determinants, which must be processed by antigen presenting cells prior to expression on the surface of T cells in association with HLA antigens. Milich and co-workers have characterized the T-cell mediated immune response to pre-S antigens and have shown that enhanced immunity to HBsAg can be induced using pre-S antigens in non-responder mice resistant to SHBs antigen [5,11,13]. This observation led to development of

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new vaccines containing pre-S antigens also called 'third-generation HBV vaccines'.

Pre-S antigens(s) seem to induce neutralizing antibodies, which block attachment, endocytosis and possibly membrane penetration of HBV into the hepatocyte. The pre-S_1 domain of the large envelope protein contains a 21–47 aa sequence, important for attachment to the hepatocyte. The pre-S_2 domain has four putative functions. It is a proteolysis sensitive site and it has a 5–16 aa sequence which can block a human serum albumin receptor-binding site. It also has an activated protein kinase binding site as well as a permeabilization site, which may be important in transfer of HBV particles into the cytosol. Pre-S antigens, and particularly pre-S_1, express highly immunogenic T- and B-cell epitopes, a property which has potential applications in third generation vaccines [4,7–9]. Furthermore, synthetic peptides derived from aa sequence 5-32 may protect chimpanzees against HBV challenge [10]. It was suggested that the pre-S_2 antigen plays a role in penetration of HBV into its target cell, which may be prevented through an adequate anti-pre-S_2 response [9]. However, the precise mechanism of how HBV enters the hepatocyte is far from understood. There is also some evidence that HBV may attach to physiologically important receptors through its pre-S_1 site, reacting with interleukin-6, asialoglycoprotein, or transferrin [1–15].

These combined properties seem to contribute to the enhanced immunogenicity of pre-S_2 [5,11–13] in a glycosylated and possibly in a non-glycosylated form.

Antibody responses after exposure to HBsAg following infection with wild-type HBV or after immunization are well characterized. Anti-HBs antibodies can be either subtype specific or common to all genotypes of HBsAg, for example, against the a determinant, which is a neutralizing epitope present in all HBV vaccines in use. After natural infection, pre-S_1 and pre-S_2 antibodies appear first, and before anti-HBs, but also disappear rapidly [15]. Their detection has been inconsistent because of varying specificities and sensitivity of the experimental assays used [5,6,12,15,67,68]. Several murine monoclonal antibodies developed against HBV envelope proteins, and directed against the a epitope, were shown to neutralize chimpanzee infectious doses of both *ad* and *ay* HBV subtypes. By convention, seroconversion to anti-HBs is defined as detection of anti-HBs in an immunological assay at ≥ 2.1 standard deviations from the reading of the negative control, which is usually ~ 1 mIU/ml (2.1–9 mIU/ml). Seroprotection against infection is present when anti-HBs levels are ≥ 10 mIU/ml. Numerous studies have shown that most children and young adults will develop hundreds to several thousand mIU/ml of anti-HBs following three doses of a conventional HBV vaccine. Vaccinees who develop an anti-HBs response above 2.1 and below 10 mIU/ml after three doses, given at 0, 1 and 6 months, are sometimes referred to as hyporesponders and probably are not adequately protected against HBV. Vaccinees who develop an anti-HBs level between 10 and 100 mIU/ml after three doses are referred to as low responders. In the United Kingdom, seroprotection against HBV infection was recently re-defined at anti-HBs levels ≥ 100 mIU/ml. This approach has public health implications and may require redefinition of non-responsiveness to routine immunization. Anti-HBs levels tend to fall with time, but primed immune memory will respond to challenge with wild-type virus as well as to booster inoculation with HBsAg through an anamnestic anti-HBs response. Currently, there is no reason to offer booster doses to vaccinees who developed anti-HBs titers > 100 mIU/ml following immunization.

Detection of antibodies to pre-S antigens remains a research tool. Several assays were evaluated to monitor anti-pre-S2 and anti-pre-S1 antibodies but none of them was considered satisfactory for clinical monitoring in humans.

2. Hepatitis B vaccines

In the late 1970s, two vaccines against HBV were developed in the United States and France, both containing purified HBsAg obtained from serum of HBsAg carriers [16–18]. These plasma-derived vaccines contained HBsAg, that had been subjected to a combination of aggressive biophysical and biochemical treatments, which led to partial disruption of the surface antigen. The final purified HBsAg was subjected to formaldehyde treatment and adsorbed to alum. A United States product contained 22 nm HBsAg particles devoid of the pre-S proteins while a French HBV vaccine contained additional small and inconsistent amounts of pre-S_2 and pre-S_1 antigens. Later, similar vaccines were produced in Korea and China. Plasma-derived vaccines have been shown to be highly immunogenic, efficacious, and safe [19–21] (see Table 1).

Concerns about safety of blood products, as well as the inconsistency as a source of raw material and the advances of recombinant DNA technology led to the development of *second-generation* recombinant vaccines produced in yeast [18,21–24]. Most experience available to date comes from using two recombinant vaccines, Engerix-B (SmithKline Biologicals, Belgium) and RECOMBIVAX HB-Vax II (Merck & Co., USA) [22,23]. These two vaccines contain non-glycosylated SHBs p24, which must be released from the yeast during the manufacturing process [24]. All licensed HBV vaccines in conventional use contain alum ($AlOH_3$) as an adjuvant. Although a potent B cell stimulator, alum is ineffective in inducing a Th_1 response. New more potent adjuvants are at different stages of development and should induce a more rapid and enhanced anti-HBs response. Furthermore, Th_1 inducing adjuvants may be evaluated in patients already infected with HBV. Although the yeast-derived particles of different vaccine manufacturers seem to have similar physical properties, their immunogenicity differs as shown in animal potency studies [25]. The recommended pediatric and adult dose per

Table 1
Development of hepatitis B vaccines, worldwide

Type	Name (manufacturer)	Envelope antigen	Remarks
Plasma-derived, SHBs	Hepatavax-B (Merck & Co., USA)	SHBs	HBsAg, 5–40 μg/dose
	Hevac B (Pasteur)	SHBsAg, (± MHBs*)	HBsAg, 5–20 μg/dose
	KGC (Korea Green Cross)	SHBs[a]	
Recombinant, yeast-derived*	RECOMBIVAX HB (Merck & Co.)	SHBsa HBsAg	HBsAg, 2.5–10 μg/dose
	Engerix-B (SmithKline, Belgium)	SHBs[a] HBsAg	HBsAg, 10–20 μg/dose
	TGP 943 (Takeda Chem, Japan)[d]	SHBs[a], MHBs HBsAg	10 μg/dose
Recombinant, mammalian	Gen Hevac B (Pasteur, France)[b]	SHBs, MHBs	HBsAg + pre-S_2, 20 μg/dose
cell-derived	Bio-Hep-B/Sci-B-Vac (Bio-Technology General, Israel)[c,e]	SHBs, MHBs, LHBs	HBsAg, pre-S_2, pre-S_1 2.5–10 μg/dose
	AG-3 (Hepagene, Hepacare) (Medeva, UK, Evans UK)[c,f]	SHBs, MHBs, LHBs	HBsAg, pre-S_2, pre-S_1 10–20 μg/dose

* Additional yeast-derived vaccines are produced in Japan, Korea, Cuba and Germany (*Hansenula polymorpha*).
[a] SHBs-p24.
[b] Contain non-glycosylated and glycosylated p24, gp27, gp33, gp36.
[c] Contain non-glycosylated and glycosylated p24, gp27, gp33, gp36, p39, gp42.
[d] Licenced in Japan.
[e] Licenced in Israel.
[f] Licenced in western Europe.

injection for Engerix-B is double the dose for RECOMBIVAX HB Vax II. So far, over a billion doses of recombinant vaccines have been administered worldwide, with an excellent record of safety and immunogenicity.

Cost-benefit analyses have strongly supported the introduction of universal immunization against HBV to newborns [26,27]. Indeed, over 140 countries have already introduced universal vaccination against HBV for newborns or individuals at risk. Furthermore, combination vaccines, using tetravalent diphtheria-tetanus-pertussis and hexavalent hepatitis B vaccines [28], or combined A and B vaccine [29], have been developed.

Despite the extraordinary efficacy of second-generation HBV vaccines, immunization failure may occur and can sometimes be explained by variables such as improper storage or administration, advanced age, obesity, renal failure, chronic liver disease and especially, immunosuppression. Another important factor that may affect non-responsiveness to SHBs immunization seems to be genetically determined resistance [30,31]. The ability to produce antibodies in response to immunization with HBsAg is controlled by autosomal dominantly expressed HLA class II molecules [30–33]. Hohler and co-workers have reported enhanced expression of DRB 1*3, DRB 1*7 and DRB 1*14 in non-responders to an HBV vaccine [33]. In contrast, response to vaccination is related to DRB 1*13, which seems to have a promoting effect on anti-HBs seroconversion. It was suggested that the differential association of DRB 1*13 and DRB 1*14 might allow identification of non-responsiveness to immunization with HBsAg. Milich and others have shown that non-responsiveness to SHBs immunization in mice can be circumvented through immunization with pre-S proteins [32]. Furthermore, immunization with synthetic MHBs (pre-S_2 or pre-S_2/S) peptides, as well as with yeast and mammalian CHO cell-derived pre-S_2/S vaccines, may induce neutralizing antibodies and protect chimpanzees against HBV [10].

Yeast (and plasma)-derived HBV vaccines are highly efficacious in preventing HBV infection and in reducing the incidence of persistent infection as well as of hepatocellular carcinoma. In a recent report, a total of 181 clinical studies were reviewed in which 24 277 individuals were immunized with Engerix-B and 8627 with RECOMBIVAX HB-Vax II [34]. The recommended pediatric dose per injection was 10 and 5 μg for both vaccines and the adult dose was 20 and 10 μg, respectively. Seroprotection (> 10 mIU/ml) was achieved in 95.8 and 94.3%, respectively using the licenced three-dose schedule at 0, 1 and 6 months. Children and adolescents (1–19 years) achieved the highest seroprotection rates, namely 98.6 and 98.8% respectively. Thus degree of non-response in children and young adults is low. Accelerated immunization schedules using two to four doses are effective although anti-HBs titers are lower when intervals between injections are reduced.

In summary, serum-derived and recombinant HBV vaccines have gained world-wide acceptance based on demonstration of efficacy, safety and cost effectiveness.

3. Do we need more immunogenic hepatitis B vaccines?

Currently available vaccines are immunogenic, efficacious and safe. Yet there is justification for producing more immunogenic vaccines that could eventually be more efficacious in defined groups such as non-responders to yeast-derived vaccines or travelers and health care workers who need rapid protection even after a priming dose of vaccine. The main target groups for such a vaccine should consist of genetically, overweight and age associated non-responders to conventional immunization, immunosuppressed and dialysis patients, as well as patients with

chronic liver disease. A more immunogenic vaccine may also enable a reduction in the number of injections required for long-term protection against HBV, which could be administered alone or in combination with an HAV or other childhood vaccines, thus reducing the cumulative load of alum used as adjuvant in vaccines.

There is also the issue of vaccine-induced mutants. HBV-envelope variants (escape mutants) have been described in Italy, Singapore, Gambia and the United States, in recipients of serum-derived, as well as recombinant, HBV vaccines [3,35–37]. Similar mutants have also been described in liver transplant patients receiving polyclonal or monoclonal HBIG for protection against reinfection with HBV [35,38]. The common reason for generation of such mutants is a single point mutation in one of the amino acids coding for the determinant of HBsAg (often aa 145). It is likely that inclusion of pre-S_2 and pre-S_1 epitopes in future HBV vaccines may eliminate or reduce the generation of vaccine associated escape mutants.

Several novel HBV vaccines were reported in the last decade, including:

(a) Yeast-derived Pre-S/S vaccines
(b) Mammalian cell-derived pre-S/S vaccines
(c) DNA vaccines
(d) Polypeptide micelle vaccine derived from HBsAg
(e) Expression of immunogenic HBV peptides in vaccinia virus
(f) Synthetic polypeptides containing immunogenic surface or core epitopes
(g) Anti-idiotype vaccines
(h) Oral immunization with a recombinant salmonella gene product containing HBcAg epitopes

The following discussion will concentrate on pre-S/S HBV vaccines with a focus on mammalian cell-derived vaccines. Mammalian cell-derived vaccines expressing pre-S/S antigens have undergone clinical trials and three of them are already licensed in a number of countries [39–66]. These vaccines were developed following the pioneering studies of Neurath, Milich, Gerlich and Tiollais on the role of the pre-S antigens in neutralization and prevention of HBV [8,9,14,32].

4. Pre-S/S hepatitis B vaccines

4.1. Yeast-derived pre-S_2/S HBV vaccines

Vaccines containing pre-S_2/S particles expressed in yeast have been developed in Japan, the United States and Belgium [42–44]. A preliminary study with one experimental pre-S_2/S vaccine developed in the United States, suggested a somewhat higher immunogenicity and good tolerability for the pre-S_2/S vaccine as compared to the S vaccine, in male volunteers. A larger scale comparative trial did not confirm the preliminary observation. More encouraging results were reported from Japan, where a yeast-derived pre-S_2/S recombinant vaccine was reported to produce a faster seroconversion rate, in non-responders to conventional vaccination and in overweight Sumo wrestlers [42–45]. However, efforts to produce a yeast-derived pre-S/S vaccine have not resulted in a licensed product in the Western Hemisphere.

4.2. Rationale for developing mammalian cell-derived HBV vaccines

Choosing a host cell for expression of a recombinant vaccine depends upon the safety, efficacy, potential for scaling-up production and cost effectiveness. Early expression systems used initially for production of recombinant HBsAg, included *Escherichia coli*, yeast (*Saccharomyces cerevisie a or Hansenula polymorpha*), insect-derived baculovirus, and vaccinia virus. The yeast has been developed to an excellent expression system for SHBsAg. It is cost-effective, easy to scale up, and its HBsAg product was shown to be well tolerated, safe and immunogenic. Nevertheless, it has a number of disadvantages, including the fact that the antigen is internal and the yeast must be opened to release the recombinant product. Furthermore, the yeast is unable to provide the same post translational modifications, protein folding, macromolecular assembly, and glycosylation, as observed in infected human hepatocytes, properties which are important for inducing enhanced immunogenicity, and which are present in mammalian cells. Indeed, studies in mice and in humans suggest an immunogenic advantage of mammalian cell-derived envelope particles (pre-S_2/S or pre-S_1/pre-S_2/S), as compared to yeast-derived non-glycosylated small surface antigen [25,40,46–47]. Available information suggests that Chinese hamster ovary (CHO) cell line and mouse-cell line-derived pre-S/S hepatitis B vaccines seem to be more immunogenic on the T-cell level (even with the use of alum as an adjuvant). Such vaccines were shown to generate T-cell help leading to higher seroconversion rates and anti-HBs titers at lower doses, as compared to yeast-derived SHBsAg [46–54]. Concern has been raised as to the safety of mammalian cell line proto-oncogenes, which theoretically may be able to induce neoplastic transformation in the vaccinee. Temin has already stated that the risk must be very low [55]. Current CHO-derived HBV vaccines have a residual DNA content estimated at < 10 pg/dose, which is one tenth of the WHO recommended limit and one billions of the dose shown to induce tumors in mice [56]. The current safety record of mammalian cell-derived biological products, and especially CHO-derived antigens, is excellent, and a number of such products are already licensed. Specifically, significant adverse effects were not reported in four clinical trials, in which 920 newborns and adults received the pre-S_2/S CHO-derived vaccine [57] and in

a number of clinical trials using either CHO or mouse-derived mammalian cell.

4.3. Mammalian cell-derived pre-S_2/S HBV vaccines

Early observations using serum-derived vaccines which contained low levels of pre-S_2 antigen suggested an enhanced immunogenicity as compared to recombinant vaccines [58]. A 'third-generation' mammalian cell-derived vaccine was first developed at the Pasteur Institute in transfected CHO cells, expressing S and pre-S_2 antigens [39]. A large-scale clinical trial with the CHO-derived vaccine was conducted in French Polynesia starting in 1988 [57]. Newborns received three or four doses of 20 μg Gen Hevac B, given at different time intervals. The vaccine was well tolerated without significant adverse events. Moreover, seroconversion rates were 88–98% after the first and second dose, respectively, implying an extraordinary immunogenicity. Out of 582 vaccinated children, four children became HBsAg carriers (most probably as a result of intrauterine infection), as compared with the theoretically expected 38 HBsAg carriers in non-vaccinated individuals. Sixteen percent of vaccinees seroconverted to anti-HBc despite vaccination, but did not develop clinical disease or persistent viral infection. This result was obtained without the coverage of hepatitis B immune globulin, and presumably these anti-HBc^+ children were born to HBV infected or recovered mothers, since some of them lost anti-HBc over time. The high immunogenicity of this new vaccine was also demonstrated in a study where 120 chronic uremic pre-dialysis patients received four doses of either 20 (g/dose of the pre-S_2/S Gen Hevac B vaccine or 5 μg/dose of the Pasteur plasma-derived vaccine [59]. The new recombinant vaccine elicited higher seroconversion rates (94%), as compared to the plasma-derived vaccine (76%) in this population of vaccine-resistant patients.

4.4. Mammalian cell-derived pre-S_1/pre-S_2/S HBV vaccines

Two such vaccines, Bio-Hepb B/Sci B Vac [42,48,50,53,54,56,57,62–68] and Hepacare [43,49,51,52,55] were developed in CHO cells and in a mouse cell line, respectively, both expressing pre-S_1/pre-S_2/S epitopes. Both vaccines were shown to induce a rapid and augmented anti-HBs response with seroconversion appearing earlier as compared to yeast-derived vaccines [65]. Furthermore, non-response to conventional SHBs containing vaccines was bypassed as shown in a limited number of clinical trials. The role of such vaccines in protecting defined risk groups against HBV infection is under discussion. Available evidence already suggests that the high seroconversion rates observed after the priming dose with a pre-S_1/pre-S_2/S vaccine may enable reduction in the number of recommended doses from three to two (with a 1–5-month interval between injections).

5. Summary

Yeast-derived hepatitis B vaccines, containing the small HBV envelope protein SHBAg, are immunogenic, safe and cost-effective in prevention of hepatitis B virus infection in neonates, children and adults. Newly developed pre-S/S hepatitis B vaccines may play a role in inducing fast and augmented seroconversion rates in special risk groups.

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