Review Article

Hepatitis B Vaccine and Immunoglobulin: Key Concepts

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Abstract

Hepatitis B virus (HBV) immunization is safe and has been accepted worldwide as a routine practice. The target of such vaccination is to induce the immune response in the host, resulting in the prevention of replication of HBV. There are several immunological and clinical factors which determine the clinical efficacy and safety of the HBV vaccine. In this article we have highlighted the response of the host immune system to HBV vaccination (immunogenicity), efficacy, and safety of the vaccine, issues with booster dosing, paths of development (preclinical and clinical) of the HBV vaccine, novel and upcoming strategies for improvement of HBV vaccination, and the concept of therapeutic HBV vaccination. The different aspects and regulatory recommendations pertaining to HBV vaccine development are also discussed. The new strategies for improvement of HBV vaccination include pre-S1 and pre-S2 portions of the HBV surface antigen, increasing the antigen dose, accelerated vaccination schedules, alternative vaccination route, use of adjuvants like immunostimulatory DNA sequences, etc. Therapeutic vaccination is being explored for initiation of a multifunctional and multispecific T cell response against the major HBV antigens and also effective activation of humoral immunity for viral control.

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Introduction

Human beings are the sole major reservoir of hepatitis B virus (HBV) and hence a complete control strategy by HBV vaccination could lead to virus eradication.[1] Despite major development and advances in antiviral therapy, primary prevention of infection by vaccination is of utmost importance in public health.[2] Global vaccination is, in fact, the most economical method employed to reduce the problem of HBV infection.[3]

The goal of active immunization against HBV is to boost the immunity in the host resulting in loss of HBV surface antigen (HBsAg) and continued control of HBV replication. Vaccination strategies against HBV include administration of traditional HBsAg vaccine, human anti-HBV surface antibody (anti-HBs), T cell vaccine, DNA vaccines, apoptotic cells expressing HBV antigens, and viral vectors expressing HBV proteins.[4] Parenteral HBV immunoglobulin is occasionally used to provide instant protection until an effective response in the host immune system occurs and also among individuals who do not form an effective immune response to conventional HBV vaccination.[5]

In 1991, the World Health Organization (WHO) endorsed that all the countries should integrate HBV vaccination in their national immunization programs,[3] and this vaccine should be given on day 0 and at the end of 1 month and 6 months.[6] Infant immunization is considered an effective strategy to prevent HBV infection and this has been incorporated in the national immunization programs of most of the countries.[7] However, catch-up strategies, adult vaccination and dealing with special populations are also important.[8]

With regard to HBV protection, both monovalent and combined vaccines were found to provide similar seroprotection or vaccine response rates.[9,10] HBV vaccines are available as a single-antigen formulation and in combination with other vaccines. The single antigen vaccines are recommended for use at birth. The combined vaccines are usually not recommended at birth ('Pediarix' for individuals aged 6 weeks–6 years and 'Twinrix' for individuals aged ≥18 years).[11] The recommended doses of hepatitis B vaccine, by group and vaccine type, is enumerated in Table 1.[11] The schematic representation of the mechanism of action of HBV vaccine is depicted in Fig. 1.

A brief history of the HBV vaccine

The first HBV vaccine (a heat-treated form of HBV) was developed by Blumberg and Millman[12] in 1969. The United States Food and Drug Administration approved a plasma-derived HBV vaccine produced by Merck Pharmaceuticals in 1981 that involved inactivation of viral particles in the blood which had been collected from HBsAg-positive donors. In 1986, the subsequent generation of genetically engineered (or DNA recombinant)—a highly purified HBV vaccine—was synthetically prepared without containing any of the blood products.[13] In the present time, all recombinant vaccines which contain HBsAg are expressed in yeast *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Pichia pastoris* or mammalian (Chinese hamster ovary) cells.[14]

Table 1. Recommended doses of hepatitis B vaccine, by group and vaccine type

Population	Age (years)	Single-antigen vaccine				Combination vaccine			
		'Recombivax'		'Engerix'		'Pediarix'[*]		'Twinrix'[†]	
		Dose (µg)	Volume (mL)	Dose (µg)	Volume (mL)	Dose (µg)	Volume (mL)	Dose (µg)	Volume (mL)
Normal individuals	Birth–10	5	0.5	10	0.5	10[*]	0.5	NA	NA
	11–15	10[$]	1	NA	NA	NA	NA	NA	NA
	11–19	5	0.5	10	0.5	NA	NA	NA	NA
	≥20	10	1	20	1	NA	NA	20[†]	1
Hemodialysis patients and other immune-compromised persons	<20	5	0.5	10	0.5	NA	NA	NA	NA
	≥20	40	1	40	2	NA	NA	NA	NA

Abbreviation: NA, not applicable.
[*]'Pediarix' is approved for use in persons aged 6 weeks through 6 years (prior to the 7th birthday).
[†]'Twinrix' is approved for use in persons aged ≥ 18 years.
[§]Adult formulation administered on a two-dose schedule.

Adequate response after HBV vaccination

Several possible questions can be raised concerning the characteristics of anti-HBs antibodies:[15] A) Is there a titer effect (higher antibody titer affords greater protection)? B) Is there a delay in the antibody response? C) Is the antibody persistent or boosters required? D) Does the vaccine-stimulated anti-HBs offer the same protection as naturally arising anti-HBs?

Several clinical trials have been performed to investigate the most optimal and effective vaccine dose and schedule of vaccination in different subject groups like adults, infants and neonates, and immune-suppressed patients.[16] The ideal vaccine will produce sufficient titer with minimal delay, remain persistent and offer the same protection as naturally acquired anti-HBs.[17] The antibody titer after vaccine administration ranges from <10 IU/L (non-responder) to >10000 IU/L.[18] At least three doses are necessary for a minimally acceptable immune response (anti-HBs antibody titer ≥10 IU/L estimated 1–2 months after administering the last dose of the vaccine).[19] The anti-HBs titer declines very fast, within 12 months, and comparatively slowly thereafter.[16] Advanced models with mathematical algorithms can predict such a declining trend.[20]

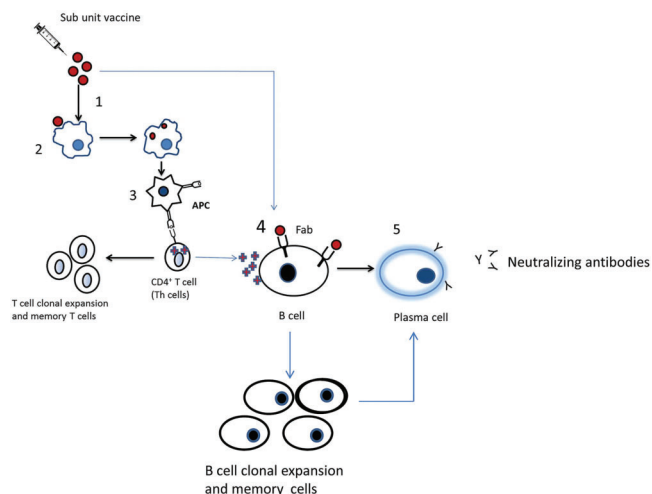## HBV vaccination

Healthy individuals

In healthy individuals, the HBV vaccine is given at baseline, 1 month and 6 months. A booster dose may not be needed in apparently healthy individuals.

Liver transplanted patients

For liver transplanted patients, the classic schedule is 0, 1 month, 6 months and booster dose in the subsequent 1 or 2 years based on serology is recommended.[21] In liver transplant candidates, the rate of seroconversion is significantly low compared to healthy individuals. The low rate of seroconversion can be attributed to disease severity as well as immunosuppressant medication use. To overcome vaccine failure approaches like accelerated schedule, increased dose and repeated vaccinations have been tried with improved success.[22–24] Earlier individuals undergoing liver transplant for chronic hepatitis B infection were solely dependent on antivirals and/or hepatitis B immune globulin (HBIG), but newer vaccines have been developed to reduce the use of HBIG and also with an aim for better protection against reactivation.

Poor response to HBV vaccination

Unresponsiveness to HBV vaccination is considered as a serum anti-HBs titer <10 IU/L after an initial course of

Fig. 1. Schematic representation of the mechanism of action of HBV vaccine. 1. The injected HBV vaccine containing HBsAg proteins are engulfed and processed by the antigen presenting cells. 2. The antigen presenting cells process the antigen and attach the same to the surface of the antigen presenting cells. 3. The antigen presenting cells present the antigen to the T helper cells, leading to clonal expansion of the T cells as well as production of memory T cells. 4. The antigen can be recognized directly by B cells, producing a weak immune response, with binding of the antigen to the Fab region on the B cell receptor and secondary signaling from cytokines released by T-helper cells; B cells begin somatic hypermutation at the Fab region, which further increases the corresponding fit between the Fab region and the antigen. 5. The B cells mature to plasma cells to produce neutralizing antibodies. They also undergo clonal expansion and memory cell formation for future defense.

vaccination.[25] A flaw in the T cell compartment-specific for HBsAg is the principal factor.[16] Hindering effect on anti-HBs response is caused by old age, smoking, body mass index, gender, concomitant chronic disease,[16] gluteal vaccination, hemodialysis, immunodeficiency,[15,26] chronic HBV infection, celiac disease, inappropriate storage conditions, etc.[27] Cirrhotic patients might be at higher risk for flares from immune-mediated hepatitis after HBV vaccination.[4] The absence of a satisfactory response may be due also to genetic predispositions linked to the major histocompatibility complex. Considering the other factors, a meta-analysis has shown that there was no statistical correlation between alcoholism or different vaccination schedules and response to HBV vaccination.[26]

HBsAg mutants

There are numerous reports on vaccine failure due to HBV mutants,[16] although the prevalence of such mutants is uncertain. Most of the antibodies which appear after the natural course of infection as well as vaccination are directed towards the determinant epitope cluster which is located in the major hydrophilic domain of HBsAg. Some mutants may also arise in the regions targeted by vaccine and thus affect HBsAg antigenicity. The effect of such HBsAg mutants on public health vaccination programs are governed by their prevalence, infectivity, pathogenicity, and cross-immunity.[16]

These mutations are mostly due to single point mutations in the α determinant of the surface antigen (S gene mutations), which is the target site of neutralizing antibodies. Ultimately these antibodies cannot bind to these mutants. These mutants are of prime concerns as the vaccinated people are affected by these mutants.[28]

Available evidence suggests that mutants are selected by vaccines. A survey of hepatitis B surface variant infection in children conducted in Taiwan shows that the prevalence of vaccine escape mutant significantly raised from the prevaccination to postvaccination era. The prevalence was 7.8% in 1984, which increased to 19.6% in 1989 and 23.1% in 1999. The prevalence was higher in the vaccinated children group (32%) compared to the unvaccinated group (9%).[29] Another study in an Italian population found the prevalence of G145R mutation to be 3.1% among 256 patients studied from 2007–2011.[30] In China, it has been observed that post mass vaccination in children the prevalence of escape mutants in children increased from 6.5% in 1992 to 14.8% in 2005, with the predominant mutation being G145R.[31]

Long-term efficacy of HBV vaccines

There are many issues regarding the duration of protection by the HBV vaccine. Most studies concerned with long-term protection were conducted with the plasma-derived vaccine and not with the recombinant one. Although antigen content of both these vaccines is the same, some physical and chemical properties may vary.[15,17] Between 8–42% of the people with protective antibody following vaccination lost it within 5 years. However, the time taken for the disappearance of antibody shows wide variation.[32] Although the possibility of developing HBsAg among subjects who respond to an HBV vaccine is almost nil, risks persist for developing anti-core HBV antigen (anti-HBcAg) conversion with the decline of anti-HBsAg titer. However, it was demonstrated that after a usual three-dose HBV vaccination, approximately 90% (range: 74–100%) of the subjects who received the vaccine remained protected for ≥30 years regardless of the anti-HBs antibody titer.[33]

Booster dosing

For many years, booster immunizations were advocated for individuals having increased risk of HBV infection (mostly occupational), when the anti-HBs titer reaches the minimal protective titer ≥10 IU/L (in most countries) or 100 IU/L (in the UK).[16] Generally, anti-HBS titer above 10 mIU/mL is regarded as protective. Some countries have kept anti-HBs levels of 100 mIU/mL as protective, to provide greater confidence that a specific response has been established. Some assays are also not specific at lower levels.[34]

Currently, many national authorities no longer advocate regular boosters but recommend the serological control 1–3 months after termination of the HBV vaccination schedule.[16] In a statement by the European Consensus Group on HBV Immunity, a list of recommendation was laid.[35] Booster is not mandated for immunocompetent subjects responding effectively to the initial vaccination course. Periodic testing of anti-HBs antibodies and booster injection is needed for immune-compromised patients when the titer is <10 IU/L. Longstanding monitoring should check the non-appearance of breakthrough episodes to detect carrier state after >15 years. Additional doses need to be given to those individuals who inadequately responded to the primary vaccination course. Booster doses can be tried in cases of breakthrough infections.

Safety of HBV vaccines

HBV vaccines are mostly safe. However, absolute contra-indication to HBV vaccination is hypersensitivity to yeast or any vaccine constituent. In addition, anaphylaxis, deranged liver enzymes, erythema multiforme, arthritis, multiple sclerosis, Guillain-Barré syndrome, neuritis, thrombocytopenia, optic neuritis, transverse myelitis, and alopecia have been reported. Few reports of arthritic, neurological, gastrointestinal and immunological adverse reactions subsequent to HBV vaccination are available.[36] However, surveillance studies conducted in the USA have demonstrated that there was no association between serious adverse events and HBV vaccination. The Centers for Disease Control and Prevention has ruled out any confirmed evidence that HBV vaccine causes chronic illness, including multiple sclerosis, rheumatoid arthritis, chronic fatigue syndrome, or autoimmune disorders.[36] The Global Advisory Committee on Vaccine Safety has also confirmed that HBV vaccination is very safe.[37]

Adjuvants in recombinant HBV vaccines

Modern recombinant vaccines are very refined and contain less antigenic components (lesser immunogenicity). As a result, adding adjuvants is essential to induce a better immune response. Among the adjuvants, aluminum salts are widely used. These salts can form insoluble particles, cause retention and release of vaccine antigens gradually like a depot, and thereby induce innate immunity.[14] The various adjuvant systems used with recombinant HBV vaccines are AS01B (liposomal), AS01E (liposomal), AS02A (oil in water emulsion), AS02B (oil in water emulsion), AS02V (oil in water emulsion), AS03 (α-tocopherol and squalene in oil in water emulsion), AS04 (0.5 mg/dose of aluminum phosphate/hydroxide in sodium chloride and water), etc.[14]

Preclinical development of HBV vaccine[38]

The different preclinical models for HBV vaccine evaluation includes cell lines (e.g. HepG2, Huh7 HepAD38, HepaRG) which are useful for replication and transcription studies. These can be cultured indefinitely. However, this model can elicit distorted and inadequately functional innate immune responses and results in loss of liver architecture. Primary human hepatocytes are also useful to study HBV replication and transcription, although these cannot be cultured indefinitely. Distortion of liver architecture is also a drawback in this model. The duck model is an ortholog model and is useful to study the life cycle of the virus and drug metabolism. Chronic infection and covalently closed circular DNA (referred to as cccDNA) formation can be studied. However, there is a lack of proper research tools to use this model. The tupaia (treeshrew) model is also useful to study HBV infectivity and viral lifecycle. However, proper research tools for use of this model are lacking. The woodchuck (groundhog) model is also an ortholog model and is useful for studying infection of HBV and viral lifecycle, cccDNA formation, drug metabolism, and carcinogenicity. There is also a lack of good tools to cultivate this model. The mouse is not an effective model to study HBV infectivity, yet this model can still be utilized for immunotherapy and drug metabolism studies. The chimpanzee is an ideal model for studying experimental HBV infection, viral lifecycle, immune responses, and drug metabolism as well as to perform vaccine research. However, the disease is less severe in this species than in humans. Ethical concerns also prevail using this model. The WHO guideline is available for preclinical assessment of an HBV vaccine.[39] As confirmatory studies with recombinant HBsAg vaccines have been performed already in the chimpanzee model, extensive preclinical studies are no longer required for HBsAg protein-based new vaccines.

Clinical development of the HBV vaccine

The HBV vaccine trials are carried out in individuals who are anti-HB or HBsAg negative.[17] An ideal population to study an HBV vaccine is the one with a higher risk of infection that can be attributed mainly to environmental circumstances. Further, the infected individuals should develop and retain the antigen for a sufficient time. HBsAg detection could help to identify any infection and the subjects would not develop anti-HB without producing HBsAg first.[17] An HBV vaccine developmental plan should consider the target population and the sociocultural aspects, the risk for the target disease and vaccine, the incidence of HBV infection and related environmental factors, the proper dose and route of administration, induction of herd immunity, and regulatory requirements.[40]

Sample size calculation

The detailed calculation of sample size in conducting a classical HBV vaccine trial has been described by Lustbader *et al.*[17] The infection rate escalates as the months of exposure increases. Therefore, the required sample size is a decreasing function of time. Assuming constant error probabilities of 0.01, the sample sizes required for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months of exposure are 355, 167, 106, 60, 59, 51, 42, 33, 32, 27 and 24 respectively in each group. It is unlikely that there would ever be all the volunteers available at one time to initiate the trial and hence a staggered start design might be required.[17]

Regulatory recommendations for clinical development of HBV immunoglobulins

WHO mentions the use of HBV immunoglobulin in newborn infants whose mothers are HBsAg-positive, in anyone following exposure to the percutaneous or mucous membrane with HBsAg-positive blood or body fluids, following sexual exposure to an HBsAg-positive person, or to protect from recurrent HBV infection following liver transplantation. The HBV immunoglobulins are generally considered as adjuvants to the vaccine.[41] A recent study showed a better response rate in HBV perinatal transmission in the group who received both immunoglobulin and HBV vaccine.[42]

The European Medicines Agency has published its guidelines on the clinical development of HBV immunoglobulins.[43] The European Medicines Agency recommends batch-to-batch consistency, after a manufacturing process change, such that it has to be demonstrated that the change has not compromised the quality, efficacy, and safety of the new vaccine. The pharmacokinetic profile ought to be tested in $\geq$ 20 adult healthy volunteers who are negative for HBsAg, anti-HBs, and anti-HBc. A pharmacokinetic study in children is not mandatory because data from adults can be mathematically extrapolated to children. The dose should be consistent with the current regulations on human plasma-derived HBV immunoglobulin. If the dose varies, supporting clinical data is needed. For efficacy evaluation, the sample size should be adequate to provide confirmatory data and the recommended baseline data include disease status (acute or chronic HBV), presence of any coinfection with other viral infections (e.g. hepatitis C, D, and human immunodeficiency virus), use of any exposure immunosuppressive agents and antiviral drugs, time elapsed between liver transplant and start of prophylaxis, and circulating HBV DNA titer.

If HBV immunoglobulin is developed as a monotherapy for a particular indication then the anti-HBs titers should be maintained at >500 IU/L and >100–150 IU/L, and for patients with and without active viral replication respectively. For HBV immunoglobulin together with antiviral therapy, targets for trough titers of HBV immunoglobulin should be justified. Intersubject variability of anti-HBs and use of antivirals should be reported. The primary end-point should be the proportion of patients showing recurrence to HBV infection as demonstrated by positive results for HBsAg and/or hepatitis B e antigen (HBeAg). Secondary end-points will include anti-HBs titer, circulating HBV DNA titer, time to recurrence of HBV infection, and overall survival. Supportive evidence of efficacy can include liver histopathology reports. A pretreatment serum sample from each subject should be stored at -70°C for future tests. All the end-points need to be estimated prior to HBV immunoglobulin administration and then periodically for $\geq$12 months. It is recommended to accumulate long-term data on recurrence and overall survival with either a risk management plan or a postapproval efficacy study. All adverse events need to be recorded for analysis and reporting. The effect of passive transmission of hemagglutinins (anti-A/anti-B) should be assessed in patients who have received high doses of HBV immunoglobulin. Data in the elderly population are not needed, as the benefit/risk may be extrapolated from adult data.

HBIG in liver transplantation

HBIG is indicated to prevent reinfection in individuals who are undergoing liver transplantation due to hepatitis. If the HBIG is developed with indication for prevention of hepatitis B recurrence following liver transplantation then the clinical development to evaluate efficacy should be done in patients who have undergone liver transplant for liver failure caused by hepatitis B. In the same setting, additional data like antigen-driven complement fixation, opsonisation, phagocytosis, antibody-dependent cell mediated cytotoxicity may also be submitted. The new immunoglobulin for the above-said indication should be studied with at least 25 participants and also with the intended mode and route for administration. The European Medicines Agency accepts open-label uncontrolled studies for clinical development and also recommends to use end-points measuring the proportion of patients who develop a recurrence of hepatitis B as demonstrated by positive results for HBsAg and/or HBeAg, titer of anti-HBs, titer of circulating hepatitis B virus DNA, time to recurrence of hepatitis B, and overall survival.[43]

Regulatory recommendations for clinical development of recombinant HBV vaccines

The WHO guidelines on clinical investigation of HBV immuno-globulins recommends that new or significantly modified recombinant HBV vaccine formulations should have extensive product characterization, immunogenicity testing, safety testing and proof-of-concept studies in animals. Variations in manufacturing, alteration in vaccine formulation or change in the route of administration require immunogenicity studies together with adequate animal safety/toxicological studies.[39,44] Pre-clinical investigation of HBV vaccines should follow WHO guidelines.[39] As no effects, apart from those on immunity, are expected with sole HBV vaccines, safety pharmacological studies are also not required. Toxicology studies should be performed as per WHO guidelines.[39] Such studies should also reflect the intended clinical use of the vaccine in special populations like neonates and children. The assessment of immune responses should rely on the anti-HBsAg antibody titer in serum, using a validated and standardized assay.

If a vaccine is proposed to contain a novel antigen dose and/or an adjuvant, that should be studied in all target populations. New HBV vaccines are needed to be compared directly with at least one licensed vaccine for which there is sufficient experience. If there are substantial changes in manufacturing, the new vaccine should be compared to an approved existing one. New HBsAg vaccines ought to be initially tested in healthy adult volunteers. Once immunogenicity is demonstrated in adults, further research should be conducted in the younger target populations, as per the intended use. For clinical trials, enrolment should usually be restricted to subjects with a negative history of HBV vaccination or disease and also negative for HBsAg, anti-HBs, and anti-HBc. The dose of recombinant HBsAg requires justifications based on pre-clinical studies and, if necessary, formal dose-ranging studies in adults are needed. Studies in neonates may include those who are born to HBsAg-positive and/or -negative mothers based upon the study's objectives. Studies in infants may be limited to those born to HBsAg-negative mothers (with no birth dose of HBV immunoglobulin), with/without a prior birth dose of vaccine.

Studies should determine the proportion of seronegative individuals who achieve anti-HBs antibody titer ≥10 IU/L at approximately 4 weeks after completion of an initial course of HBV vaccination. For the comparison between a new vaccine and reference vaccines, the protocol and analysis should predefine a well-justified noninferiority margin[45] to compare the proportion of individuals with ≥10 IU/L anti-HBs. The protocols should plan for secondary analysis of the proportion of subjects who achieve ≥100 IU/L anti-HBs and should present reverse cumulative distributions.[46] Secondary analyses should also compare the geometric mean titer between vaccines, and studies may plan for formal comparison of geometric mean titer ratios.

Some of the clinical studies should be performed with different lots manufactured using the same process. A proper trial to demonstrate lot-to-lot consistency is not normally required unless any particular concern is present.[44] Research may be conducted to assess immunogenicity, efficacy, and safety of new recombinant HBsAg-containing vaccines in populations at risk or not responding adequately to vaccination in view of the potential requirement for a higher antigen dose and/or adjuvant. The chance for possible immune interference between HBV vaccines and other routine coadministered vaccines needs to be investigated. The immunogenicity, efficacy, and safety of a new vaccine, when formulated with other components (combination vaccine), should be assessed.

Recently vaccines with a novel adjuvant are under development with the target of achieving better seroprotection rates. The conventional HBV vaccines used aluminium hydroxide as an adjuvant, whereas the new recombinant vaccine ('HEPLISAV-B') uses cytosine phosphoguanine oligonucleotide synthesized using bacterial DNA. This new recombinant vaccine was approved by the United States Food and Drug Administration in November 2017 after the trials demonstrated increased seroprotection rates over the conventional vaccines.[47,48]

Strategies for improvement of the HBV vaccine

Current HBsAg vaccines cannot elicit an adequate immune response in patients with chronic hepatitis B, as there are a high load of viral antigens in the circulation which will induce immunological tolerance. To circumvent this potential barrier, pre-S1 and pre-S2 polypeptides are used in the vaccine. The gene coding for HBV surface antigen consists of pre-S1, pre-S2, and S regions.[49] The main approach for improvement is to supplement HBsAg with the pre-S1 and pre-S2 portions of HBsAg.[50] These polypeptides are present at a very low level in patients with chronic hepatitis B, as they are the important domains in the mature virions only. In addition, antibodies induced by the pre-S1 and pre-S2 region can prevent the entry of virions into the host hepatocytes. Therefore, pre-S1 and pre-S2 region can be used as the alternate strategies to improve the immunological response against HBV.[51]

The additional approaches include increasing the antigen dose, accelerated vaccination schedules, alternative (intradermal) vaccination route, use of new adjuvants like immunostimulatory DNA sequences, etc.[14] Combined hepatitis A and B vaccination is an interesting tactic to enhance the heterologous immunogenicity.[52] Additionally, immunostimulatory sequences are unmethylated CpG motifs with oligodeoxynucleotide sequences, which is one of the pathogen-associated molecular patterns (PAMPs) that will activate the Toll-like receptors present on the antigen presenting cells. This will subsequently activate the innate and adaptive immune responses. These immunostimulatory sequences

can be used as adjuvants in the HBV vaccine to elicit a strong immune response.[53]

Therapeutic HBV vaccines

The accomplishment of prophylactic HBV vaccination depends on neutralization of the invading HBV by antibodies, and eventually effective viral control. Instead, the key target of therapeutic vaccination is to induce a multifunctional and multispecific T cell response against the major viral antigens as well as to activate humoral immunity.[54] An ideal therapeutic vaccine would also induce neutralizing antibodies. However, it is controversial whether a therapeutic vaccine can achieve virus elimination or sustained viral control.[54] For HBV, both innate and adaptive immunity against the virus should be triggered. Therapeutic vaccination against chronic HBV infection aims to overcome the immunosuppression induced by high viral load, tolerogenic liver environment, and T cell dysfunction. Therapeutic HBV vaccine based on protein or peptides against include administration of HBsAg with or without HBcAg (highly immunogenic) and vaccination with immune dominant HLA-A2-restricted HBcAg18–27 peptide epitope. The HBcAg18–27-Tapasin interaction leads to increased production of cytokine IFN-γ and interleukin-2, it also enhances HBV-specific cytotoxic T lymphocytes which play a vital role in hepatitis B virus clearance. In animal models, the same peptide has shown enhancement of specific cytotoxic T lymphocyte activity induced by the fusion protein-reduced HBV DNA and HBsAg levels, and decreased the expression of HBsAg and HBcAg in liver tissue of HBV transgenic mice.[55]

Genetic vaccination is also being tried with replication incompetent recombinant viral vector vaccines or with HBV DNA that are genetically engineered. Cell-based vaccine methods involve the transfer of peptide-containing antigen-presenting cells, autologous dendritic cells and transmission of functional HBV-specific CD8^{+} cells carrying HBV-specific T cell or chimeric antigen receptors.[54]

In the near future, therapeutic vaccines are expected to offer great relief in individuals living with chronic hepatitis B. Therapeutic vaccines in hepatitis offer a great platform for biomedical research. A study conducted by Zhao *et al.*[56] in a mouse model to find the efficiency of pHBV vaccine in chronic hepatitis B showed that HBV vaccine decreased HBsAg and HBV DNA efficiently and safely in HBV carrier mice.

Researchers have also tried a combination vaccine, termed NASVAC, which contains HBsAg and HBcAg against pegylated interferon, in treatment-naive chronic hepatitis B patients. At the end of 24 weeks, the high proportion of individuals with sustained control of HBV DNA was seen in the combination vaccine-treated group compared to the pegylated interferon group (57.7% vs. 35%; $p<0.01$).[57] The GS-4774 T cell vaccine designed to elicit hepatitis B virus (HBV)-specific T cell response was tried in a phase II trial, to study the safety, tolerability, and efficacy in chronic hepatitis B patients. Though the vaccine was well tolerated, it failed to provide adequate clinical benefits.[58] A trial conducted by Horiike *et al.*[59] evaluated the efficacy of combination therapy of lamivudine and vaccine in patients with chronic hepatitis B. It is found that in a subgroup consisting of HBeAg[+] chronic hepatitis B patients who received lamivudine at a dose of 100 mg daily for 12 months and also vaccine containing 20 μg of HBsAG, intradermally, once every 2 weeks for 12 months, showed a higher proportion of individuals having a reduction in HBV DNA compared to those in the lamivudine alone group (100% vs. 48%; $p<0.05$).

Conclusions

Immunization against HBV is safe and has been accepted worldwide as a part of the routine immunization program. Although recombinant HBV vaccines are the ones which are used prophylactically, HBV immunoglobulins are also used to offer immediate protection in certain circumstances. There are numerous factors which determine the nature and duration of protection for the HBV vaccination. Certain immunological and clinical phenomena guide the preclinical and clinical development process of HBV vaccines. Regulatory recommendations for such clinical development are specific and comprehensive. Upcoming approaches for improvement of HBV vaccination includes pre-S1 and pre-S2 portions of HBsAg, increasing the antigen dose, accelerated vaccination schedules, alternative vaccination route, use of new adjuvants like immunostimulatory DNA sequences, etc. Therapeutic vaccination is being tried to activate humoral immunity and induce a multifunctional and multispecific T cell response, to counter the major HBV antigens for effective viral control.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualized the article and provided overall guidance (SS), and drafted the article (SD, KR, SKB, MG, and ASX).

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