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MINIREVIEWS

Induced immunity against hepatitis B virus

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Abstract

Prevention of hepatitis B virus (HBV) infection with its consequent development of HBV chronic liver disease and hepatocellular carcinoma is a global mandatory goal. Fortunately, safe and effective HBV vaccines are currently available. Universal hepatitis B surface antigen HBV vaccination coverage is almost done. Growing knowledge based upon monitoring and surveillance of HBV vaccination programs has accumulated and the policy of booster vaccination has been evaluated. This review article provides an overview of the natural history of HBV infection, immune responses and the future of HBV infection. It also summarizes the updated sources, types and uses of HBV vaccines, whether in the preclinical phase or in the post-field vaccination.

Key words: Hepatitis B surface antigen; Hepatitis B virus vaccines; Immunological memory; Hepatitis B virus; Booster and therapeutic vaccination

Core tip: Worldwide, it is estimated that more than 2 billion people have been infected with hepatitis B virus (HBV). Of these, approximately 240 million are chronically infected and at risk of serious illness or death from development of cirrhosis and subsequent progression to hepatocellular carcinoma (HCC), estimated to cause one million deaths each year worldwide. Prevention and control of HBV infection can therefore make a significant contribution to community health and to saving lives by preventing HCC. This review concerns the major advances in the field of HBV over the last few decades which have resulted in understanding the natural history of HBV infection and the development of effective vaccines against the virus. In the era of universal HBV vaccination coverage, the current growing body of knowledge regarding monitoring and surveillance of HBV vaccination programs and the policy of booster vaccination, several issues have to be evaluated regarding the vaccination policy and booster doses. In addition, it is worth evaluating vaccine-escape viral mutants, long-term protection and the therapeutic use of HBV vaccine as a promising new strategy for controlling chronic HBV infection.

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INTRODUCTION

A serum antigen named Australia antigen was detected in sera from Australian aborigines[1]. Later it was found in sera from Down's syndrome, leukemia and hepatitis patients. It was identified as a marker of post-transfusion viral hepatitis and given the letter hepatitis B virus (HBV) to replace the previous term of serum hepatitis. Data accumulated to document that HBV has 7-9 genotypes with more or less defined geographical distribution[2]. The "a" component of hepatitis B surface antigen (HBsAg) is shared by all genotypes; however, infection by one genotype does not prevent subsequent infection by other types. Moreover, the antiviral effectiveness is related to HBV genotype and genotype D prevalent in the Middle Eastern countries, which is the most resistant to antivirals.

The virus morphology visualized by electron microscopy was described by Dane *et al*[3] and the complete HBV virion was named Dane particle, synonymous with HBV. The immunogen of HBV is a subviral unit of envelope proteins named HBsAg. During HBV replication, excess of the subviral components are produced. The hepatitis B core antigen (HBcAg) is restricted to the hepatocytes and to the full virion. Modified HBcAg, named hepatitis B e antigen (HBeAg), is released into the circulation. Moreover, excess HBsAg is released into the circulation in different morphological forms.

According to the 2010 Global Disease Burden estimates[4,5], both HBV and HCV caused 1.4 million deaths from acute infection, chronic infection, cirrhosis and hepatocellular carcinoma (HCC). It was reported that 240 million people are chronically infected with HBV and are at risk of serious illness or death from development of cirrhosis and subsequent progression to HCC. Later data showed that approximately 780000 persons die each year from hepatitis B infection[6].

The first anti HBV infectivity vaccine was HBsAg vaccine, which was produced by recombinant-DNA technology using yeasts. The r-DNA HBsAg vaccine produced robust immunity against the HBV infectivity and consequently prevented all the post HBV infection complications, including HCC. Prevention of HBV hepatitis and HCC is a significant contribution to global health and productivity. Progress in knowledge of HBV within the last few decades resulted in the understanding of the natural history of HBV infection, the development of sensitive assays for screening of blood donors for safe blood donation and monitoring of antiviral drugs, viral suppression and clearance[7], plus manufacturing improved r-DNA HBsAg vaccines. This progress paves the way for worldwide future elimination of HBV.

Natural history of HBV infection

Severity of HBV liver diseases shows great individual variations[8]. The outcome of infection and the pathogenesis of hepatic disease are determined by host factors and viral factors that play an essential role in virus clearance and persistence. It has been difficult to fully elucidate such factors on an experimental basis because of the restricted host range of HBV to man and chimpanzees[9]. Clinical research showed that there is a relationship between HBV genotype, natural history of infection and response to specific HBV antiviral treatment[10]. Both antibodies and cytotoxic T cells directed to different HBV antigens play an important role for decreasing viral load and clearing HBV-infected hepatocytes from the liver[11]. On the other hand, very weak or functionally impaired virus-specific immune responses have an essential role in the persistence of HBV infection[12]. Most primary infections of adults (70%-90%), whether symptomatic or not, ends up with efficient control of infection, with virus clearance from blood, liver and extrahepatic cells supporting HBV replication. The risk for chronicity decreases with increasing age at the time of exposure to HBV infection. HBV infection in nonimmune young children is mainly asymptomatic. Unlike adults who mostly do not develop chronic infection, neonates and infants who acquire HBV infection from their mothers at birth are most susceptible to the establishment of chronic HBV infection[13-15]. Currently, it is clearly emphasized that there are extrahepatic cells that support HBV replication and survival for several years or lifelong. These cells cause HBV infection of transplanted liver and rejection of the transplant.

HBV infected adults showed no clinically evident liver disease or mild acute hepatitis that terminated without long-term sequelae with development of lasting immunity to re-infection[16,17]. The other patients (10%-30%) who do not succeed in clearing the virus progress to chronic infection with continuous HBV replication. Host immune response plays an essential role in HBV-related hepatocyte damage because the virus itself is not cytolytic. The balance between host immune response and HBV replication in hepatocytes and in extrahepatic host cells is dynamic[18]. Fortunately, 70%-90% chronic HBV infected patients are asymptomatic without life-threatening effects on liver cells, while 10%-30% of patients develop liver cirrhosis[19] with consequent hepatic insufficiency and portal hypertension that make liver cirrhosis one of the most frightening consequences of chronic HBV infection[8]. Development of HCC is also a catastrophic result of chronic HBV infection with a lot of evidence supporting an association between HBV replication and the risk of development of HCC[18,20]. The HBx protein is a potent transactivator that activates host genes, including oncogenes[21].

Occult HBV infection

Occult HBV (OHBV) infection is a potential transmission source of post transfusion or organ transplantation HBV infection. To investigate the properties of hepatitis B surface antibody (HBsAb) in OHBV infection and its

affinity to different serotypes of HBsAg, Zhang *et al*[22] conducted long-term follow-up in 2 HBsAb positive patients with occult HBV infection where the HBsAb subtype was determined by performing neutralization experiments with different serotypes of HBsAg. They showed that the HBsAbs are mainly specific for common epitopes among different serotypes of HBsAg and are probably different than those produced by vaccine inoculation.

Immune response

Experimental infection of chimpanzees does not produce the same events as in human infection, which greatly limits the tools to study the natural life cycle of HBV in humans. Few well documented human HBV infections have shown that the virus itself is not cytolytic and the hepatolysis is immune-mediated. It was also shown that the HBsAg level varied with a different clinical or virological status. A low baseline level of HBsAg is associated with advanced liver fibrosis in HBeAg positive CHB patients[23]. Experimental studies demonstrated that acute HBV infected animal models developed silent innate immunity[21] and HBV does not immediately begin to replicate efficiently after inoculation[9,24], as it does in acutely infected humans. Following infection and up to 4-7 wk later, neither HBV antigens nor HBV-DNA are detectable in serum or liver[24-26]. The absence of early symptoms in HBV-infected patients is an indirect indication of the defective type I interferon (IFN) production during the early phase of HBV infection when exponential virus replication is going on[9].

The immunological events in the early phase of HBV replication mainly influence the differences in the adaptive immune response to HBV that characterizes resolved and chronic HBV infections[26]. Coordinated activation of the different branches of adaptive immunity is necessary for effective viral control[9]. Researchers showed that macrophages have an essential role in modulating HBV clearance, chronic hepatitis and the developed tissue damage, with M1-like macrophages promoting HBV clearance and M2-like macrophages impairing Th1 immune response and promoting tissue fibrosis/remodeling/wound healing[27]. Full recovery with elimination of HBV infected hepatocytes depends on active $CD8^+$ cytotoxic T lymphocytes. Following HBV infection, early immunological response failed to be activated as it is delayed until the exponential phase of replication[28]. In acute, self-limited hepatitis B infection, strong antiviral T-cell response is detected in peripheral blood. It includes both $CD4^+$ helper and $CD8^+$ cytotoxic T lymphocytes reactive with epitopes of multiple HBV antigens within the HBV core, polymerase and envelope proteins[8]. Virus-specific $CD8^+$ T cells have an essential role in HBV clearance[26]. Resolution of human HBV infection was believed to be solely dependent upon contact cytolysis of virus infected hepatocytes by $CD8^+$ cytotoxic T lymphocytes. However, researches on HBV transgenic mice and HBV-infected chimpanzees showed that T cell control of HBV replication is also influenced by cytokine-mediated noncytolytic mechanisms. $CD8^+$ lymphocytes produce different cytokines, including IFN-γ and tumor necrosis factor-α, that lead to inactivation of HBV in infected hepatocytes[29]. The cytolytic and noncytolytic action of virus-specific $CD8^+$ lymphocytes in its reaction with HBV-producing host cells adds up in acquired immune reactions against HBV infected hepatocytes. However, the noncytolytic mechanisms retain the more effective role[29].

Chronic HBV infected patients have impaired immune response to HBV[30,31]. In chronic HBV carriers, virus-specific T-cell responses are mainly attenuated; however, antibody responses are vigorous and sustained but free anti-HBs antibodies are undetectable because of excess circulating HBsAg[8]. Also, toll-like receptor (TLR) signaling in murine nonparenchymal liver cells (NPCs) is suppressed in the presence of HBsAg. This has been shown when peripheral blood mononuclear cells (PBMCs) from HBV infected patients and controls were stimulated by TLR ligands in the presence or absence of autologous serum. The expression of both TLR-mediated cytokine [*e.g.*, interleukin-6 (IL-6) and IL-10] and TLR3-induced IFN in PBMCs of HBV infected patients demonstrated a significant increase compared to the healthy volunteers, denoting a negative correlation between HBsAg and TLR3-mediated IFN-γ levels[32].

Six viral epitopes that are reactive with autologous HLA-A2 domains on cytotoxic T lymphocytes(CTL) were identified by Rehermann *et al*[33]. These epitopes are present in the highly conserved reverse transcriptase and RNase H domains of the viral polymerase protein. In acute HBV infected patients, the CTL response to polymerase is polyclonal, multi-specific and is mediated by $CD8^+$ T cells, but it is undetectable in chronic HBV infected patients or in healthy blood donors[33]. CTL responses against polymerase, core and envelope epitopes were identified up to one year following complete clinical recovery and seroconversion, indicating either the persistence of viral replication or the presence of long lasting memory CTL despite the absence of the viral antigens. It was shown that wild type viral DNA and RNA can persist indefinitely in trace amounts in serum and PBMC following complete clinical and serological recovery, despite a concomitant, vigorous and sustained polyclonal CTL response[33]. In order to explain the persistence of HBV, the authors further emphasized that the virus may retreat into immunologically privileged places from where it can seed the circulation and reach CTL inaccessible tissues, thereby maintaining the CTL response in apparently cured individuals and thus prolonging the liver disease in chronic HBV hepatitis patients[33]. In a large prospective clinic-based cohort of Asian chronic HBV patients, Desmond *et al*[34] identified significant associations between HLA types and HBV sequence variation at 41 sites within the HBV genome.

HBV VACCINES

Vaccination is the most effective measure to decrease the

worldwide HBV incidence and its complications, including liver cirrhosis and HCC. Worldwide, immunization has been an essential strategy for many countries to decrease the burden of HBV infection[5]. Economically, vaccination is an attractive option, both in terms of cost-effectiveness and benefit-cost ratios when compared with other health care interventions[35]. Commercial HBV vaccine supplies have been available for thirty years. HBV vaccine was the first vaccine against a chronic disease, the first vaccine to protect from a sexually transmitted infection and the first vaccine against a cancer[36]. The choice of a vaccine type and a schedule for doses and route of vaccination varies between countries. An ideal HBV vaccine schedule should protect against infection in infancy when the risk of becoming a chronic HBV carrier is high and in adolescence with common, high risk behaviors such as sex and drug abuse[37]. The CDC Advisory Committee on Immunization Practices (ACIP) recommends that all children should receive a birth dose of HBV vaccine and complete the vaccine series by 6-18 mo of age. It also recommends that older children and adolescents who did not previously receive the HBV vaccine should be vaccinated[38]. These policies were implemented by several countries with medium to high endemicity of HBV infection.

History of HBV vaccine development

The first HBV vaccine was prepared from the plasma of asymptomatic carriers of HBV in the form of purified inactivated HBsAg particles[39]. Later on, the r-HBsAg vaccines that contain the major (s) small protein spanning the hydrophilic amino acids 124-149 as the dominant immunogenic epitope were developed. HBV vaccinations induce neutralizing antibodies (anti-HBs) that are directed mainly towards the "a" determinant of HBsAg in all HBV genotypes from A to H[40]. The r-HBsAg vaccine elicits active synthesis of anti-HBs and prolonged immunological memory which provides continuous protection[41]. Persistent memory for 5 years or more is recognized from large, fast increases in anti-HBs level after booster vaccination, even in those who showed undetectable anti-HBs as measured by the available commercial kits. Using an *in vitro* enzyme linked immunosorbent assay (spot-ELISA), it was shown that the number of memory B lymphocytes able to induce anti-HBs does not decrease with decline of the anti-HBs level[41]. It was found that the value of immune memory and of the following secondary immune response can be estimated by the antibody response after primary vaccination. Both dose and structure of vaccine antigen influence the primary antibody response as well as the development of immune memory[42].

To increase the efficacy and prolong the duration of protection against HBV, second generation r-HBsAg vaccines, including the middle (pre-S_2) and the large (pre-S_1) proteins in HBV vaccine, were recognized. The immunogenicity of r-HBs 20 nm particles secreted by transfected Chinese hamster ovary (CHO) cells was compared with yeast-derived r-HBsAg vaccines[43]. The CHO-derived vaccine contains the small hepatitis B surface antigen (HBs protein) as the major component, together with pre-S_2 and pre-S_1 antigens, induced an augmented anti-HBs response in mice when compared with mice receiving the already used yeast-derived vaccines[43].

The current widely used r-HBsAg vaccines are a viral subunit produced by yeast that has been transfected with a plasmid that contains the *S* gene (codes for HBsAg) either as a single preparation or in combined form. Commercial r-HBsAg single antigen vaccines are Recombivax-HB (Merck) and Engerix-B (Glaxo). There are other approved combined vaccines against both HAV and HBV (Twinrix-Glaxo)[44]. Other formulations for infants are tetra or penta vaccines against diphtheria, tetanus, pertussis (whooping cough) and HBV for tetra vaccine and with the addition of injectable inactivated Salk polio (IPN) for the penta vaccine[45]. Combined Hepatitis B-Haemophilus influenzae type b (Hib) conjugate vaccine cannot be given before age of six weeks or after the age of seventy-one months[15]. Hexavalent combined vaccine, including diphtheria, tetanus, pertussis, HBV, Haemophilus b (Hib) and the three IPV serotypes antigens, is considered the most suitable combination vaccine for routine immunization[35,46]. Combined vaccines aid in improving compliance and simplifying complex pediatric immunization schedules, reduce storage requirements, reduce handling, costs of immunization programs and minimize the number of injections required, thereby reducing distress for infants and parents[46,47]. Their development is restricted because of their technical manufacturing complexity. High technical complexity increases production costs and therefore many manufacturers target them as premium products for developed countries[46].

Formulating of different types of HBV vaccine

Prophylactic vaccines: The need for HBV immunoprophylaxis was recognized as early as the late 1970s. The plasma derived vaccine was licensed in 1981. The vaccine contained a purified HBsAg single component that was obtained from sera of HBsAg carriers. Plasma derived HBV vaccines have been demonstrated to be highly immunogenic, efficacious and safe[48,49]. The long-term effect of plasma-derived HBV vaccine which was given to children born in 1986 was observed in a recent Chinese study. The vaccine showed a good long-term protective effect and there was no need for boosting the immunization 23 years later[50]. However, the use of this vaccine was dropped due to concern regarding the safety of a human blood-derived products, the inconsistency of a source of raw viral particles and the availability of new recombinant vaccines produced from yeast transfected with vector plasmid with DNA sequence coding for the production of soluble r-HBsAg proteins that are easily purified from the yeast proteins[45].

Yeast-derived recombinant hepatitis B vaccine contains a gene for the HBV surface antigen (S) which has been cloned into yeast cells that are cultured on

a wide scale to amplify the recombinant DNA coding for producing a large amount of the specified antigen (HBsAg) which is further purified, concentrated and combined with adjuvant to be ready for vaccination. Alum is included as an adjuvant in all licensed HBV vaccines[45]. HBsAg prevalence decreased dramatically after the implementation of yeast-derived r-HBsAg vaccine for 12 years for children in HBV-endemic areas in China, with no need for booster immunization[51]. Billions of doses of r-HBsAg vaccines have been administered worldwide, with a high record of immunogenicity and safety[44].

Years ago, several preclinical studies were conducted to evaluate the potential use of synthetic preS analogues for hepatitis B vaccination[52]; however, global adoption of these experimental vaccines was not achieved.

HBV DNA vaccines have been shown to be useful for both prophylaxis and treatment of HBV infection. It is one of the most effective ways to elicit protective immunity against infections. Preclinical studies in animal models, including mouse, chimpanzee, duck and woodchuck, were developed to evaluate the ability of DNA vaccines targeting hepadnaviral proteins to induce sustained strong immune responses in naïve animals and to enhance virus clearance and break immune tolerance in chronic HBV carriers[12]. HBV-DNA based vaccine is a plasmid DNA encoding the hepatitis HBVsAg that was shown to be immunologically effective, safe and well tolerated in chronic HBV carriers and non-responders to routinely used HBV vaccines[53,54]. The protective immunogenicity of a particle-mediated HBV-DNA vaccine in subjects who have responded suboptimally to conventional vaccination was evident[54]. In comparison with antigen-based HBV vaccines, plasmid DNA vaccine against HBV was evaluated in chimpanzees and protective anti-HBs antibody response was attained together with a strong anamnestic response achieved one year later[55]. It was well recognized that HBV DNA vaccine induces strong antigens that stimulate both humoral and cellular immunities that can be promoted by a joining of DNA that expresses IL-2 or IL-12 epitopes[56]. HBV-DNA based vaccines stimulated $CD8^+$CTL cells. Among the recently studied adjuvants, layered double hydroxide (LDH) nanoparticles as well as core-shell structure SiO_2@LDH nanoparticles were effectively proven adjuvants. Experimentally, SiO_2@LDH nanoparticles taken in by macrophages caused a higher dose-dependent expression of IFN-γ, IL-6, CD86 and MHC Ⅱ. Furthermore, *in vivo* immunization of BALB/c mice indicated that HBV DNA vaccine loaded-SiO_2@LDH nanoparticles not only induced increased serum antibody response compared to naked DNA vaccine and plain nanoparticles, but also obviously promoted T-cell proliferation and skewed T helper to Th1 polarization[57]; however, these experimental adjuvenated HBV DNA vaccines have not been adopted for humans so far.

Early trials to make use of genetically engineered plants as an alternative way for developing an HBV inexpensive and edible vaccine showed preservation of epitopes of HBsAg of both B- and T-cells when the antigen is expressed in a transgenic plant[58]. Also, HBVsAg encoding DNA gene was previously introduced into *Agrobacterium tumerifacience* LBA4404 that was used to get transgenic lupin (*Lupinus luteus L.*) and transgenic lettuce (*Lactuca sativa L.*) cv. Burpee Bibb expressing envelope surface protein. It was found that mice that were fed on HBV transgenic lupin tissue developed a significant amount of HBV specific antibodies. Meanwhile, human volunteers fed with transgenic lettuce plants expressing HBVsAg also developed a specific serum-IgG response to plant produced HBsAg[59]. Later on, a new modified HBV pre-S_1 protein gene was constructed and expressed by transgenic tomato plants[60]. The specific antigen expression from transgenic plants was proved by molecular techniques, including polymerase chain reaction (PCR) and reverse transcriptase PCR. Enzyme-linked immunoassays using a monoclonal antibody directed against human serum-derived HBsAg revealed that the highest amount of HBsAg was about 0.02% of the soluble protein in transgenic tomato fruit. The amount of HBsAg in mature fruit was found to be much more than that in small or medium fruit or leaf tissues. The ability of potato-derived HBV major surface antigen (P-HBsAg) that was orally administered to mice in different dosages (0.02 to 30 μg) to induce antibody responses was evaluated[61]. It was shown that all immunized mice developed specific serum IgG and fecal IgA antibodies against P-HBsAg even at low levels (< 5 μg), comparable to development of serum IgG anti-HBs following administration of a 0.5-μg yeast-derived HBsAg. Recently, a plant-derived prototype oral tri-component vaccine against HBV was evaluated for the potential of M/L-HBsAg expression in leaf tissue and the conditions of its processing for a prototype oral vaccine were assessed. Tobacco and lettuce carrying M- or L-HBsAg genes and resistant to the herbicide glufosinate were engineered and integration of the HBV-DNA transgenes was evaluated by PCR and Southern hybridizations[62].

Currently, several experimental trials for developing virus vector HBV vaccines are ongoing. Following a single immunization in mice, a recombinant vesicular stomatitis virus-based vaccine vector expressing the HBV pre-S_2 protein was able to efficiently promote a strong HBs-specific antibody response. It also developed robust CD8 T-cell activation, where response was broader in specificity and greater in magnitude than that acquired by a vaccinia virus-based vaccine vector or by recombinant protein immunization[63]. In addition, it was found that recombinant lentivectors could induce strong HBV HBsAg specific T cell responses and humoral immune responses. The HBS-Fc-lv lentivector could effectively break immune tolerance and elucidate strong HBsAg specific adaptive immune responses in HBsAg transgenic (Tg) mice with low serum level of HBsAg. The induction of HBsAg specific immune responses in TG mice accompanied seroconversion from HBsAg to anti-HBsAb[64]. Recently, Song *et al*[65] investigated the generation of recombinant influenza viruses that had

HBV B cell epitopes in its neuraminidase stalk region as a dual vaccine candidate against both HBV and influenza viruses. They successfully generated a chimeric influenza virus which contained 22 amino acid peptides derived from the surface and pre-surface HBV protein as foreign antigens[65].

Universal childhood immunization in the first year of life with three doses of HBV vaccine is a highly effective way for control and prevention of HBV infection[66]. Prevention of prenatal HBV infection is achieved by active and passive HBV immunization after birth as an intervention for preventing mother-to-child transmission of the HBV infection. A recent Taiwanese study was conducted in 2013 to compare the cost-effectiveness of strategies to control HBV that combine universal vaccination with hepatitis B immunoglobulin (HBIG) for neonates of carrier mothers. It was found that HBIG additional treatment to universal vaccination is likely to be cost-effective, particularly in settings with available healthcare infrastructure. Targeting HBIG in neonates of higher risk HBeAg-positive mothers may be preferred where willingness-to-pay is moderate. However, in very resource-limited settings, universal vaccination only is adequate[67]. CDC recommends vaccination of adults at high risk for infection, including dialysis patients, recipients of certain blood products, healthcare workers, household contacts and sex partners of persons with chronic HBV infection, those with a recent history of multiple sex partners, those with a sexually transmitted disease, IDUs and MSM[16].

Following vaccination, testing for antibodies is not needed for healthy people; however, it should be evaluated following vaccination in hemodialysis patients, those at occupational risk of infection, babies born to HBsAg-positive mothers, those with a family history of HBV carriers and human immunodeficiency virus positive people[66]. Long-term protection against HBV infection depends on the persistence of strong immunological memory. There is no need for boosters in immune competent individuals who have finished their vaccination course properly according to the recommended timelines. This was demonstrated in several studies conducted up to 20 years following the original immunization course. However, a booster dose is recommended for immunocompromised individuals based on serological monitoring[68].

The ACIP[69], the United States government, recommends that all children receive a birth dose of HBV vaccine and complete the vaccine series by 6-18 mo of age. Older children and adolescents who did not previously receive the HBV vaccine have to be vaccinated. A titer of anti-HBs antibodies to HBVsAg $\geqslant$ 10 IU/L is the marker of seroconversion to anti-HBs positivity following vaccination. It was found that the mean antibody level decreased significantly with increasing age[70,71]. Long-term follow-up studies have demonstrated that 10% to 50% of infants with seroprotective levels post third dose of vaccination had low or undetectable levels of antibody 5-15 years later[72]. Long-term protection due to persistent memory is obvious from quick rises in antibody after booster vaccination, even in those who have lost their anti-HBs. Recently, we conducted an Egyptian study aimed at estimating the seroprotection rate and evaluating the immune response to a booster dose in children and adolescents with an age range of 9 mo to 16 years who completed HBV vaccination during infancy. It was concluded from this Egyptian study that, in spite of the significant decline of level of antibodies over time, about half of the studied children have a seroprotective level of antibodies after primary compulsory vaccination. Moreover, the developed anamnestic response among children with a non-seroprotective level confirms immunological memory that can outlast the presence of protective level of antibodies[73]. Werner *et al*[74] found that HBsAg vaccine-induced immunity protects against new infection but does not induce sterilizing immunity in vaccinated healthcare workers with occupational exposure to HBV, as evidenced by detection of HBcore- and polymerase-specific CD8 (+) T cells[74].

According to current CDC recommendations[75-77], all healthcare providers and students should receive a 3 dose series of HBV vaccine followed by assessment of HBsAb to determine vaccination immunogenicity. Revaccination should be provided if indicated. Following revaccination (receiving a total of 6 doses), healthcare providers whose anti-HBs concentration is still not protective (< 10 mIU/mL) should be evaluated for HBsAg and anti-HBc to determine their infection status[54].

When considering offering a booster dose of the vaccine, Fitzsimons *et al*[78] recommended that vaccinees who were not tested for anti-HBs antibody one month following vaccination or those who have undetectable anti-HBs antibodies when tested should be potential recipients of it. Long-term protection is usually evaluated by 4 methods: the anamnestic response following administration of a booster dose, *in vitro* B and T cell activity evaluation, infection rate in vaccinated populations and seroepidemiological studies[68]. Estimation of the incidence of break-through infection (positive anti-HBc), as well as chronic carrier state (positive HBsAg) among previously vaccinated individuals, is used to determine the long-term protection provided by the HBV vaccine[79]. Many factors are related to HBV vaccination non response, including age above 40 years, male gender, impaired vaccine storage conditions, administration of the vaccine in buttocks, infections, obesity, drug abuse, smoking, chronic kidney/liver diseases, human immunodeficiency virus infection, celiac disease, thalassemia, type Ⅰ diabetes mellitus, Down's syndrome and other forms of mental retardation that are characterized by a poorer response than healthy subjects to HBV vaccination[66,80]. It has been shown that development of anti-HBs in hemodialysis patients is associated with gene polymorphisms of interleukins involved in the Th1 system[81]. Also, it was found that the administration of HBV recombinant vaccine by the intradermal route is very effective and could be a more useful strategy and an alternative to

conventional intramuscular vaccine in all non-responder patients[80]. As obesity is considered to be a major cause of decreasing the rate of antibody production by the HBV vaccine, recently it was reported that antibody titers can be raised in vaccinating obese youth by using long needles[82].

Therapeutic HBV vaccine: HBV vaccines have been recently identified as a promising therapeutic strategy for treatment and control HBV infection in HBV carriers and persistently infected patients[39,83]. Clinical trials of its therapeutic use in chronic HBV infection rely on using the conventional sAg based HBV vaccine. Specific treatment by the standard anti-HBV vaccine is effective in decreasing the replication of HBV and inhibiting the immune tolerance to HBsAg protein in about 50% of chronic active HBV patients[84]. However, it is well recognized that monotherapy with HBsAg-based immune therapy cannot lead to sustained control of HBV replication and/or liver damage[85]. New therapy strategies are currently shown to provide potent and durable antiviral immune responses in patients who can maintain long-term control of HBV replication[83]. Recent research concentrates on the clinical use of combined HBsAg- and HBcAg-based vaccines in CHB patients[85].

Using a duck HBV model, therapeutic DNA vaccination was proven to be able to enhance hepadnavirus intrahepatic covalently closed circular DNA clearance[86]. It was shown in humans that HBV DNA vaccination can specifically but transiently activate T-cell responses in some chronic HBV carriers who showed no response to the available HBV antiviral therapies[53]. Obeng-Adjei *et al*[11] evaluated the use of multivalent synthetic plasmids against HBV consensus core (HBc) and surface (HBs) antigens genotypes A and C for their immune potential in animal models and they found that it induced binding antibodies to HBsAg and robust cell-mediated immunity. The same responses to both HBc and HBs antigens were demonstrated by inoculation of HBc-HBs cocktails in mice and non-human primates. Besides the cytotoxic T-lymphocyte activities exhibited by the immunized mice, the vaccine-induced responses were broadly distributed across multiple antigenic epitopes[11]. Immune therapy with HBV-related antigens (HBsAg-based vaccine) has been used in CHB patients as a combination therapy with cytokines, growth factors and antiviral drugs, but proper designs of antigens, types of adjuvant, dose of vaccinations and routes of administration need further analyses for the development of an effective protocol of immune therapy for HBV infection[85]. GS-4774 is a safe and well-tolerated recombinant, heat-killed, yeast-based immunotherapy engineered to express HBV (HBV)-specific antigens and it is a promising therapeutic vaccine for chronic HBV infection[87]. Intensive research is currently concentrated on a better understanding of immune responses in hepatocytes, on mechanisms by which HBV evades innate immunity and on proper selection of patients susceptible to benefit from immune therapy, which could increase the efficacy of therapeutic vaccination[88].

What is the future HBV epidemiology after universal mass vaccination?

In 1992, the World Health Organization recommended that all countries integrate the hepatitis B vaccine into their childhood national immunization programs. By 2012, 181 countries implemented this vaccine in their national immunization program, with the global hepatitis B vaccine coverage estimated at 79%. 119 countries have reported > 90% vaccination coverage with consequent decrease in the prevalence of chronic HBV infection in children born since the r-HBsAg vaccine was included in infant immunization schedules[5]. It is well recognized that the use of highly immunogenic HBV vaccines produces long-lasting immunity. Some of the major challenges facing current HBV vaccines have been their inability to induce both humoral and cellular immunity to multiple antigenic targets and the induction of potent immune responses against the major genotypes of HBV[11].

The efficacy of universal immunization has been demonstrated in many countries, with a prominent decrease in the prevalence of HBV carriage in children. Moreover, HBV vaccination can protect children against fulminant hepatitis and HCC[89]. The success of the vaccination programs has also now been challenged by the discovery of mutant viruses showing amino acid substitutions in HBsAg, which may lead to evasion of vaccine-induced immunity[90]. Induction of immune escape mutants is one of the unwanted effects of the widely used HBV vaccine. It was first described in 1990 by Carman *et al*[91] when they observed the acquiring of HBV infection in 44 contacts of HBV carriers despite passive and active immunization according to the implemented standard schedules. There was partial loss of the common "a" determinant to which the vaccine-induced immunity is mainly directed. Globally, several S mutations that are potentially able to evade neutralizing anti-HBs immune response and thus infect vaccinated individuals have been recognized[40]. Inclusion of $Pres_2$ and $Pre\text{-}S_1$ epitopes may be recommended in order to reduce the emergence of such vaccine escape mutants. Meanwhile, it was shown that selection of pre-S/S mutants may demonstrate a relevant pathobiological and clinical impact. HBV mutants with an antigenically modified surface antigen may be potentially infectious for immune-protected patients and may account for those with occult HBV infection[92]. On the other hand, Romanò *et al*[40] confirmed that the overall effect of vaccine escape mutants is likely to be low and thus does not cause a public health threat or a need to modify the implemented HBV vaccination programs.

The effectiveness of universal hepatitis B vaccination is promising, although the coverage of vaccination varies between countries. The inclusion of r-HBsAg vaccine in the expanded programs of childhood vaccination had a major impact on protection against the disease and its complications and was successful in almost

eliminating childhood chronic HBV infection. However, this recombinant HBV yeast derived vaccine has a number of limitations that justify the development of new HBV vaccines: the need for multiple doses, lack of long-lasting immunity, incomplete protection in all vaccinees where a group of non-responders do exist and it is therapeutically ineffective[45,63].

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