EXPERT REVIEWS

Hepatitis B vaccine effectiveness in the face of global HBV genotype diversity

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Recombinant hepatitis B vaccines are of the A2 genotype; one of ten known genotypes whose distribution varies globally. Reports of rare HBV infections in blood donors with an imbalance of non-A2 genotype HBV in vaccinated subjects have raised questions about the cross-protection afforded by HBV-A2 vaccines. Infections in HBV vaccinees were asymptomatic and transient, indicating that vaccination prevented clinical disease. Preclinical data demonstrate cross-reactivity and cross-protection by A2 vaccines against non-A2 HBV genotypes. Substantial improvements in HBV control have been demonstrated in countries with diverse genotype distribution that have introduced universal childhood HBV vaccination programs. Available data show that current HBV-A2 vaccines are highly effective in preventing infections and clinical disease caused by all known HBV genotypes.

Keywords: effectiveness • genotype • hepatitis B • recombinant vaccine • vaccine

The global burden due to HBV

One third of the world's population is estimated to have been infected by HBV [1]. Each year approximately 620,000 individuals die from HBV-related illnesses, including acute fulminant infection, cirrhosis and hepatocellular carcinoma [1,2]. In countries of moderate HBV endemicity (population seroprevalence of HBV surface antigen [HBsAg] between 2 and 7%) or high endemicity (8% or higher of the population HBsAg positive) [1], transmission most frequently occurs either by perinatal transmission or horizontally during early childhood. The outcome of infection is related to the age at which infection occurs, with up to 90% of perinatally acquired infections becoming chronic [3]. By contrast, approximately 30% of infections acquired before the age of 6 years become chronic, and the risk decreases further with increasing age [3]. Approximately 21% of deaths from HBV-related diseases occur in individuals who were infected perinatally and 48% of deaths occur in individuals who were infected during early childhood [2]. Thus, prevention of HBV infection by vaccination is most successful when it targets infants, and when prevention begins with administration of the first dose of HBV vaccine soon after birth. In the most optimal scenario (i.e., administration of birth dose and high vaccine coverage), mathematical modeling suggests that as many as 84% of HBV-related deaths could be prevented by vaccination [2].

Vaccination against HBV began 30 years ago with availability of plasma-derived, inactivated HBsAg vaccines. Soon after, recombinant HBsAg expressed in yeast cells led to the production of vaccines that are currently used in most countries, either alone or in combination with other pediatric vaccine antigens.

Hepatitis B vaccination

Immunization of infants with HBV vaccines was advocated by the WHO [4]. In 1992, the WHO World Health Assembly endorsed the recommendation for the integration of HBV vaccination into national immunization programs in all countries by 1997 [5]. Relatively slow global uptake of vaccination was revitalized by initiatives driven by the Global Alliance for Vaccines and Immunization (GAVI), which was established in 1999 [6]. Development of vaccines combining HBsAg with antigens, such as diphtheria, tetanus and pertussis, already routinely recommended during infancy, facilitated uptake [7]. In 2008, universal childhood HBV vaccination programs existed in 177 countries [8]. Universal infant vaccination against HBV is cost effective in countries of high HBV

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endemicity [9]. Although more costly in countries of low endemicity, the cost–benefit of universal HBV vaccination in these countries compares favorably with other preventative healthcare programs [10–12]. Since the onset of vaccination, significant reductions in morbidity and mortality due to HBV have been recorded, most strikingly in countries of high HBV endemicity [13].

Hepatitis B genetic diversity

HBV is a small dsDNA virus. Classification is into serotypes (and subtypes) based on antigenic B-cell epitopes that map to envelope proteins, or into genotypes according to the entire genomic sequence. There are ten recognized genotypes (A–J). The HBV genome contains four open reading frames, one of which, the *Pre S/S* gene, encodes three coterminal glycoproteins – large (L), middle (M) and small (S) surface protein. The S protein, better known as HBsAg, is the target of the protective humoral immune response in humans induced by vaccination. HBsAg contains a cluster of conformational B-cell epitopes that map to the so-named *a* determinant region (at amino acid positions 121–149), which comprises two loop structures and is common to all serotypes and genotypes of HBV. There are at least four subdeterminants, *d* or *y* and *r* or *w*: *d* or y are determined by variation at amino acid position 122, *r* is determined by variation at position 160 and *w* is determined by variation in up to four amino acid positions [14]. All *d* subtypes have a lysine at position 122, whereas an arginine is present in all *y* subtypes. All *w* subtypes carry a lysine at position 160 and all *r* have an arginine at this position [15]. Based on different potential combinations of the above allelic variations, HBV serotypes can be divided into *adr*, *adw*, *ayr* or *ayw*, with further division into a total of ten serological sub-subtypes. Although this is an apparently complex classification, two simple concepts need to be kept in mind: first, that the *a* determinant region is common to all the different serotypes and genotypes of HBV; and second, that this region is pivotal in conveying protection against HBV infection. Over 90% of human HBsAg-specific serum antibodies and human B-cell lines isolated following HBsAg vaccination are directed toward the *a* determinant [16,17].

The distribution of the known HBV genotypes has recently been reviewed [18,19]. The distribution of the ten known genotypes varies markedly both within and between continents, and is thought to be influenced by immigration (Figure 1) [18]. Genotype A is prevalent in North America, parts of South America, sub-Saharan Africa, northern and western countries in Europe and is also found in Australia. Genotypes B and C are common in Asia and North America. Genotype D is widespread, found commonly in all parts of Europe, North Africa and the Middle East and some parts of South East Asia. Genotype E is found mainly in West Africa, whereas genotype F is prevalent in Central and South America. Genotype G is rarely detected in most regions. Genotype H is found mainly in Central America. Recently, molecular and phylogenetic analyses characterized two new HBV genotypes: the first designated as genotype I, in Vietnam and Laos [20–22], and the second as genotype J in Japan [23]. Some evidence suggests that the severity of clinical disease may be influenced by HBV genotype [24], but the risk of disease progression and development of HCC for individual serotypes appears to be regional [19], and more evaluation is needed before implications of infecting genotype for treatment options become clear.

Do recombinant A2-derived HBV vaccines protect against infection due to other genotypes?

All currently available genetically engineered HBV vaccines are produced with the A2 genotype, serotype *adw*. The humoral immune response following vaccination with recombinant HBV vaccines is largely directed against the common *a* determinant, with a lesser response directed against the *d/y* and *r/w* subdeterminant epitopes [16,17].

The ability of A2-derived HBV vaccines to protect against non-A2 HBV genotypes has recently been brought into question following the publication of a study of 3.7 million blood donors in the USA [25]. In this study, specimens from all blood donations collected in 2008 were screened for HBsAg, anti-HBc (HBV core antibody) and HBV DNA. Nine HBsAg-seronegative individuals were found to have circulating HBV DNA. Six of the nine had been vaccinated with HBV vaccines and four vaccinees had anti-HBs >10 mIU/ml (range: 11–96 mIU/ml). The viral load was very low in all subjects (11–86 IU/ml), and in seven individuals with available follow-up samples, the duration of detectable viremia ranged between 34 and at least 137 days. Although acute infection (positive for anti-HBc IgM) was demonstrated in eight of the nine donors, evidence of clinical infection only developed in the unvaccinated donors. Infections in vaccinated individuals were subclinical and transient. All three unvaccinated donors and one of the six vaccinated donors were infected with the A2 genotype. The genotypes infecting the other five vaccinated individuals were C2, F1, B2, 6D-2A2 and 7D-4A2-1A2/D. The authors concluded that while breakthrough infections with non-A2 genotypes were recorded in HBV vaccinees, HBV vaccination prevented clinical disease. In addition, their findings suggested that the vaccine may be less effective for non-A2 infections. In view of the variability in genotype distribution globally, any gap in the efficacy of A2 vaccines has potentially important implications for the ongoing protection of populations against HBV infection and its consequences.

In order to address the questions raised by the study of US blood donors, we reviewed the available evidence to evaluate the ability of current vaccines to protect against infections and clinical disease caused by non-A2 genotypes. Our approach was first to assess preclinical data on cross-serotype protection by current HBV vaccines, and second to review effectiveness studies of HBV vaccination in different geographic areas where non-A2 genotypes are prevalent.

In vitro *& preclinical evidence in support of cross-genotype protection*

Following vaccination the dominant response is directed towards the *a* determinant, common to all genotypes of HBV. In one analysis of sera from vaccinees receiving three doses of HBV-A2 vaccine, over 90% of HBsAg-specific antibodies were directed to the

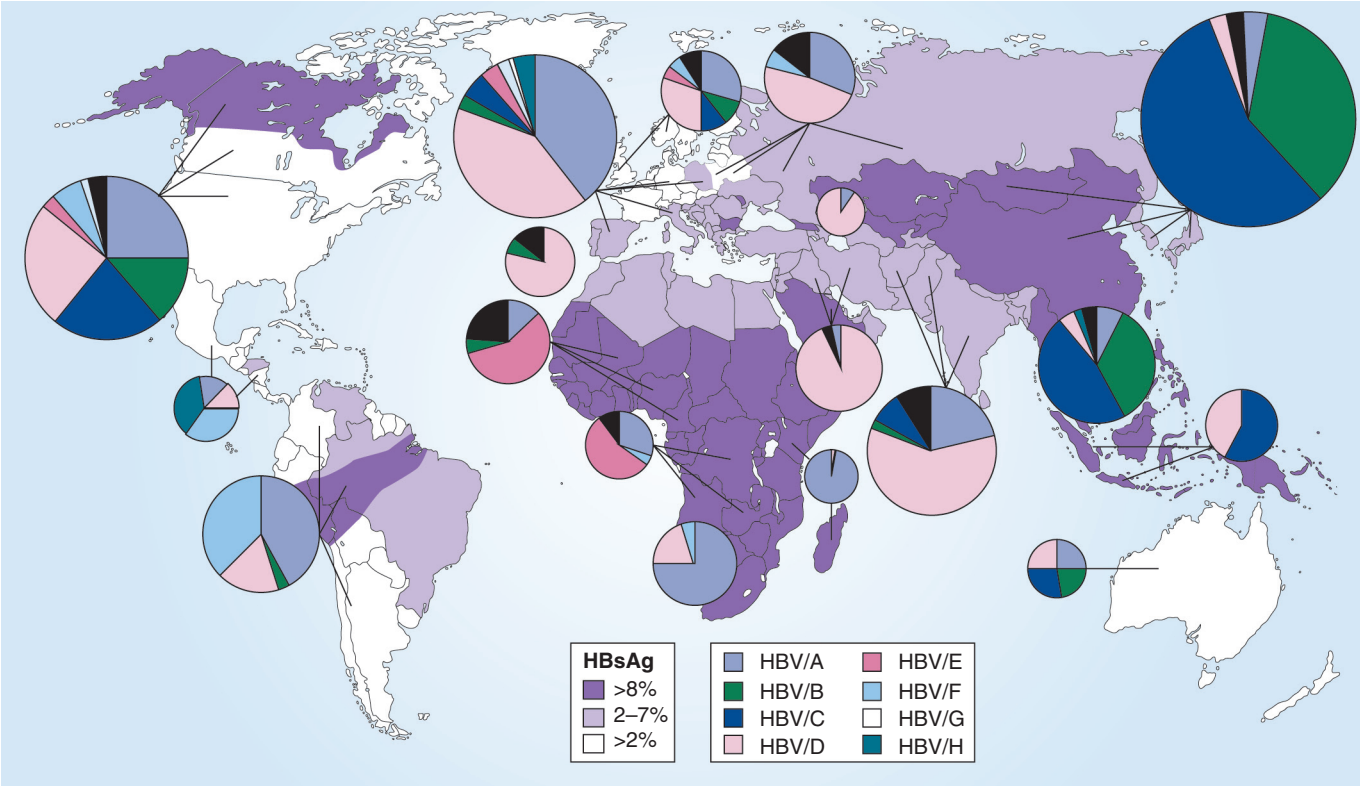

Figure 1. Geographical variation in the prevalence of HBV genotypes A–H. Results of a review of genotyping results from 45,000 HBV-infected individuals over a 10-year period.
HBsAg: HBV surface antigen.
Reproduced with permission from [18].

a determinant, with the remainder being specific for the *d* determinant [16]. 1 month after the first vaccine dose approximately 65% of anti-HBs antibodies have anti-*a* specificity. This increases to more than 90% after the third dose [26]. Similarly, a study investigating antibody specificity in B-cell lines derived from the blood of 34 vaccinated individuals showed that of 222 lines isolated, 97% were found to be *a*-specific, 2% were directed towards the *d* determinant and less than 1% towards *w* [17].

A human monoclonal antibody derived from a subject vaccinated with an HBV vaccine (*adw*) showed equivalent binding activity against *adw*, *ayw* and *adr* viral particles [Leroux-Roels G, Cooreman M, Pauly W *et al.*, Unpublished data]. Similarly, polyclonal sera from five vaccine recipients showed reactivity against viral particles from the same three subtypes, albeit with some donor-to-donor variability in the response profiles [Leroux-Roels G, Cooreman M, Pauly W *et al.*, Unpublished data]. Monoclonal antibodies specific for the *a* determinant are capable of protecting chimpanzees against challenge with diverse serotypes of HBV. A human monoclonal antibody mapping to the *a* determinant neutralized HBV (*adw*) challenge virus and protected animals from infection [27]. In an earlier study, a single *a*-specific mouse monoclonal antibody neutralized challenge with both HBV *adr* and *ayw* serotypes [28]. Cross-serotype protection against serotype *ayw* was demonstrated in chimpanzees vaccinated with a recombinant DNA HBV (*adw*) vaccine. Vaccination conveyed full protection against infection for 1 year, whereas unvaccinated controls rapidly developed signs of hepatitis B infection [29].

Impact of hepatitis B vaccination

Countries that have a distinctive genotype distribution and were early to adopt universal infant HBV immunization include Taiwan (1984), Thailand (1991), China (1992), the Gambia (1990) and South Africa (1995).

Taiwan is a good example of a highly endemic country with a substantial reduction in HBV achieved through universal childhood vaccination. HBV is highly endemic in Taiwan, with chronic carriage evident in as many as 20% of adults [30]. In Taiwan, 80% of infections are due to genotype B and the remaining 20% due to genotype C [31]. HBV vaccination of infants born to HBV carrier mothers was introduced in Taiwan in 1984, and universal vaccination of infants began in 1986 [32]. Plasma-derived HBV vaccines were used until 1991 and recombinant vaccines thereafter. The overall prevalence rate of HBsAg seropositivity in children younger than 12 years of age decreased from 9.8 to 1.3% after 10 years of systematic vaccination of children [33]. In the same children, the overall prevalence rate of HBV core antibody (anti-HBc) decreased from 26 to 4.0% between 1984 and 1994.

Marked reductions in HBsAg seroprevalence and in HCCs in vaccinated cohorts have also been observed in Thailand where

genotypes B and C are also prevalent [34,35]. Moreover, protection against clinical HBV in Thailand has been documented for at least 20 years after the introduction of routine infant vaccination [36]. In China where genotype C and B predominate, a universal childhood vaccination program was implemented in 1992, with pronounced effects due to high vaccine coverage achieved [37]. A recent sero-epidemiological survey reported that in 2006, HBsAg seroprevalence was 2.1% in vaccinated individuals compared to 9.4% in unvaccinated individuals [38]. HBsAg seroprevalence was 1.0% in children born after 1999 [39]. In the Gambia, genotype E accounts for more than half of HBV infections [18]. Since the introduction of routine infant and childhood vaccination in 1986, childhood HBsAg seroprevalence decreased from 10 to 0.6%, with an estimated efficacy of vaccination against HBV carriage of 94% [40]. In highly endemic South Africa where genotypes A1 and D predominate [41], evidence of the impact of routine vaccination was demonstrated by a significant decrease in the incidence of HBV-associated membranous nephropathy (a complication of acute HBV infection) among children at a single hospital in 2000–2001 as compared with incidence during the prevaccination era [42].

Recent case reports of acute [43] and chronic [44] HBV infection with genotype F suggest that current recombinant vaccines may not confer significant protection against infections with HBV genotype F. Genotype F is predominant in indigenous populations in Central and South America. To our knowledge, comparable population-level data on the impact of universal infant vaccination is not available for countries in this region, but several studies have demonstrated a reduction in HBV infection following vaccination with recombinant vaccines in Peru [45], Venezuela [46] and Brazil [47]. Moreover, a recent analysis of the *a* determinant of HBsAg in 21 Chilean patients chronically infected with HBV genotype F revealed that none of their isolates contained changes that would affect binding to vaccination-associated anti-HBs [48].

In Alaskan natives, genotypes A2, B6, C2, D and F1 circulated prior to vaccination [49]. 25 years after onset of universal vaccination, HBsAg seroprevalence in Alaskans less than 20 years of age decreased from 3.2% in 1983–1987 to <1% in 2008 [49]. Moreover, the incidence of HCC in the same age group decreased from three per 100,000 to zero per 100,000 population, with no HCC cases reported since 1999 [49].

Effects of HBV vaccination have also been documented in countries of low HBV endemicity with universal infant immunization programs. In the USA for example, where infant vaccination began in 1991, the incidence of acute hepatitis B decreased from 8.5 per 100,000 population in 1990 to 1.3 per 100,000 population in 2009, with the greatest declines over time observed in children less than 15 years of age [50,101].

Irrespective of the worldwide diversity of HBV genotypes, it is clear that substantial reductions in the disease burden have resulted from long-standing policies of universal childhood HBV vaccination. Because effectiveness studies of HBV vaccination tend to focus on a single country, a mathematical model based on epidemiological data was developed to estimate the global HBV disease burden and vaccination impact [2]. For the year 2000, the model estimated that 620,000 persons died worldwide from HBV-related causes: 94% from cirrhosis and HCC caused by chronic infection, and the remainder from fulminant HBV. During the lifetime of the year 2000 worldwide birth cohort, the model estimated that without vaccination 64.8 million persons would become HBV-infected and 1.4 million persons would die from HBV-related disease. The model estimated that routine infant hepatitis B vaccination, with 90% coverage and the first dose administered at birth, would prevent 84% of global HBV-related deaths.

Expert commentary

Studies in countries of low, medium and high HBV endemicity have shown substantial decreases in HBsAg infection, chronic carriage and HBV-related complications, including HCC, after the introduction of universal infant vaccination against HBV. The success of currently used HBV A2-based vaccines in improving HBV disease control is unqualified, and has been observed equally across regions, regardless of HBV genotype. Thus, the continued success of HBV vaccination will not depend on vaccine genotype, but on the successful implementation of vaccination strategies. To this end, programmatic issues, such as integrity of the cold chain during transport and storage, appropriate vaccination scheduling, including commencement of vaccination at birth, and access of the whole population to vaccination, remain critical to success [1].

As yet, few studies have evaluated HBV genotype distribution before and after implementation of widespread HBV vaccination. In Japan, where genotypes B and C predominate, vaccination has not been associated with a change in the distribution of these genotypes to date [51]. A Taiwanese study that determined the infecting genotype in immunized and unimmunized children showed that immunized infants of mothers with genotype C infection were more likely to develop breakthrough infection than infants born to mothers infected with genotype B [31]. The authors postulated that this might be due to higher viral loads typically associated with genotype C infection in mothers, meaning that exposure to higher genotype C viral loads during the perinatal period is more likely to result in infection than exposure to lower loads of genotype B [31]. This study suggests that immunization may modulate HBV genotype distribution, but that this is unlikely to be due to a failure of vaccination to protect against genotype C, and rather it is most likely a function of viral load at the time of exposure. This hypothesis is supported by data from a prospective study conducted in Taiwanese men in whom carriage of genotype C infection was associated with higher viral load and higher risk of development of HCC [52], and by observations made by Stramer *et al.,* in which index cases (partners of HBV DNA-positive blood donors) all had high viral loads [25].

The importance of occult HBV infections (generally defined as individuals with detectable HBV DNA in whom HBsAg is absent [53]) on an individual level and on a population level is still unclear. Transmission of HBV from individuals with occult infections has been documented [54,55]. Occult HBV infections appear to have minimal clinical effects in healthy individuals, but individuals who are immunosuppressed or who have underlying liver disease

are at higher risk of fulminant hepatitis B or progression to cirrhosis [54,55]. In US blood donors, an imbalance in the occurrence of nongenotype A2 infections in HBV-infected vaccinated blood donors versus nonvaccinated infected blood donors was noted [25]. The A2 vaccine was able to modify the course of infection in all subjects. Further studies are needed to evaluate the risk of HBV transmission in HBV DNA-positive vaccinated individuals.

Preclinical *in vitro* and *in vivo* data predict that HBV A2-based vaccines are likely to provide broad protection against other HBV genotypes in humans. Clinical vaccine effectiveness data in regions where non-A2 genotype are prevalent show that there is a significant benefit for cross-protection against other genotypes of the virus afforded by HBV A2 genotype vaccines. The available data to date suggest that current HBV A2 vaccines are highly effective against all of the known HBV genotypes.

Five-year view

Significant progress has been made in HBV prevention globally. Nevertheless, in some regions the burden of disease from HBV remains high, and continued advancement of prevention through vaccination is needed [19]. In 2009, the WHO estimated that 70% of children aged less than 1 year had received three doses of HBV, although there is substantial variation in coverage across regions [102]. The next 5 years will see continuing efforts by GAVI to promote HBV vaccination in the world's poorest countries, and a progressive increase in the number of countries implementing universal infant HBV immunization programs. Studies assessing the effect of vaccination on genotype distribution will continue to expand our knowledge of the effectiveness of HBV A2 vaccines. The problem of the detection and management of occult HBV infections will continue to be studied and our understanding of the clinical implications of occult infection in vaccinated subjects will improve. The cost–benefit of using expensive nucleic acid testing screening methods to detect rare cases of occult HBV infection in donated blood is poor [56], and facilitating high HBV vaccine coverage in populations is likely to be more effective in preventing clinical HBV. However, screening for occult carriage may be more relevant in tissue or organ donors because transmission of HBV to immunosuppressed organ recipients has been demonstrated [54,55].

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Key issues

- All currently available genetically engineered HBV vaccines use the A2 genotype, serotype *adw*.
- A study showing occult HBV infections in US blood donors demonstrated that none of the unvaccinated donors versus five out of six vaccinated donors had non-A2 HBV infections, raising the question of whether available A2-based HBV vaccines are able to fully protect against infections caused by different HBV genotypes.
- Preclinical *in vivo* and *in vitro* data show cross-reactivity and cross-protection by A2 vaccines against non-A2 HBV genotypes.
- The implementation of universal hepatitis vaccination in most countries has substantially reduced the prevalence of HBV over the last decades, irrespective of genotype distribution.
- Limited available studies undertaken before and after implementation of widespread HBV vaccination have not linked vaccination to changes in genotype distribution.
- Available data suggest that current HBV-A2 vaccines afford cross-protection against non-A2 genotypes and are highly effective in preventing infections and clinical disease caused by all of the known HBV genotypes.
- Ongoing evaluation of potential associations between changes in genotype distribution and vaccination is needed. Further studies to understand the clinical implications of occult infection in vaccinated subjects are needed.

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