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# Editorial

## Do we need better hepatitis B vaccines?

Hepatitis caused by hepatitis B or C viruses (HBV, HCV) may lead to chronic liver disease with the severe, life-shortening sequelae of cirrhosis and/or hepatocellular carcinoma (HCC). Chronic viral hepatitis ranks 7 in the list of global causes of death just after lung cancer and before road injuries and AIDS[1]. In 2015, an estimated 257,000,000 (3.5%) people were chronic carriers of hepatitis B surface antigen (HBsAg)[2], and 870,000 annual deaths are calculated to be caused by chronic HBV infection worldwide[2], 145,000 of which are believed to occur in South Asia[1]. India is considered a low-prevalence country for HBV with only 1.46 per cent of HBsAg carriers[3], but due to its large population, this adds up to 17,600,000 persons at risk of chronic liver disease[3]. In addition, there are great regional and ethnic differences with prevalence ranging from 0.1 to 11.7 per cent[4]. Tribes from the Andaman & Nicobar Islands[5] or refugees from Tibet[6] are examples of groups with particularly high HBsAg carrier rates.

In 1992, the World Health Organization (WHO) recommended general childhood vaccination against HBV. The vaccine consists of HBsAg expressed in genetically programmed host cells, typically in yeast cells. In 2015, 184 countries, including India, introduced HBsAg-containing pentavalent or hexavalent vaccines nationwide with three doses given at months 2, 4 and 12. Global coverage with this type of vaccination is relatively good at 84 per cent[2]. However, this approach is only satisfactory for countries with low HBsAg prevalence (<2 %) and which perform HBsAg screening of pregnant women. Newborns of HBsAg-positive or untested mothers should receive an additional birth dose of monovalent hepatitis B vaccine to prevent mother-to-child transmission (MTCT), which virtually always results in chronic HBV infection. Ninety six countries with high HBV prevalence apply this approach, but unfortunately, coverage in this urgent situation is only 39 per cent[2]. In addition to children, vaccination is recommended for persons at an elevated risk for HBV infection, for example, healthcare workers (HCW), but in India, the coverage is suboptimal in this setting[7]. Vaccination with three or four doses in childhood or adolescence virtually always induces protective titres of antibody to HBsAg (anti-HBs) of >10 international units per litre (IU/l), and countries that have introduced vaccination early such as Taiwan[8], Thailand[9] or Gambia[10] have reduced the HBsAg carrier rate from about 10 per cent in unvaccinated young adults to <1 per cent in the vaccinated group of the same age. Authors from Gambia report[10]: "Comparing fully vaccinated vs unvaccinated GHIS *(i.e. study)* participants, current HBV infection was 0.8% (2/255) vs 12.4% (59/475), p<0.0001, suggesting 94% (95% CI 77-99%) vaccine efficacy." Given this excellent efficacy, the major problem left seems to be the still insufficient coverage, particularly in high prevalence areas and high-risk groups[11].

The current hepatitis B vaccines have certain shortcomings[12]. Older persons are not as responsive as children or adolescents, women respond better than men, obesity and smoking reduce the response rate and chronic diseases such as diabetes or kidney disease impair the response rate further. If vaccine recipients have several of these negative factors, the response rate may fall to values <50 per cent. It is known that vaccinated persons who do not reach at least 10 IU/l anti-HBs some weeks after the last dose are not protected against HBV infection and disease. Opinion leaders acknowledge this problem[11] and vaccine producers try to alleviate it by recommending additional doses of vaccine or higher antigen doses or more aggressive adjuvants instead of aluminium compounds[13]. A deficit that is at least as important is the failure rate in newborns from mothers who have very high viraemia with >$10^7$ IU/ml HBV DNA in the serum because in this case the child always develops

chronic infection with a high risk of cirrhosis and HCC. The suggested solution to the problem of mother to child transmission (MTCM) is antiviral therapy of the mothers with tenofovir[11], but for less developed countries, this approach is too demanding, and in its current form, it is still not dependable[14].

An aspect that is completely neglected is the phenomenon of breakthrough infections in individuals who have developed a seemingly sufficient anti-HBs response. Clinical breakthroughs with acute or even chronic hepatitis B are very rare. If these occur, these are typically caused by HBV genotypes different than the vaccine genotypes[15-17]. Recently a breakthrough infection has been reported in children from HBsAg positive mothers who were initially protected by passive/active vaccination for several years but finally became HBsAg positive[18]. Much more frequent are breakthroughs which result in asymptomatic-resolving infection. In the first vaccine trial with newborns from Thailand, the authors wrote: 'During the 20-yr follow up, no individual acquired new chronic HBV infection or clinical hepatitis B disease'. However, in the same publication, it was also reported that 22.8 per cent of the individuals vaccinated as newborns had signs of asymptomatic HBV infection, in fact as many as in the non-vaccinated control group[19]. In a large study on vaccinated newborns from Taiwan, a remarkable reduction of the HBsAg carrier rate from 9.8 to 0.5 per cent was found, and the rate of asymptomatic infections [detectable only by a positive antibody to HBV core antigen, (anti-HBc)] was reduced from 16.4 to 2.4 per cent. Surprisingly, however, those who were vaccinated and had anti-HBc without HBsAg were more often HBV DNA positive in serum than those who had anti-HBc without HBsAg but no vaccination[8]. Frequent asymptomatic breakthrough infections were also reported from Gambia[10]: "Comparing fully vaccinated vs unvaccinated participants, anti-HBc was 27.4% (70/255) vs 56.0% (267/475)". These data suggest that vaccination may mitigate breakthrough HBV infections to a point that frank chronicity does not occur but complete control of HBV release from the liver may be impaired. This situation is known to opinion leaders, but since there is currently no evidence that this may have relevant clinical consequences (except for a few anecdotal cases), this potential weakness is accepted.

A synopsis of seemingly unrelated observations suggests that acceptance of this situation may finally endanger the official goal of the WHO to eliminate viral hepatitis including HBV by 2030[19]. It is generally accepted that HBV may remain in an occult form in the liver in infected persons with or without known hepatitis B disease and even without serological evidence of HBV infection[20]. The experience that occult HBV may reactivate and cause serious or even fatal liver disease in patients under immunosuppression means that occult HBV infections are not negligible[20,21]. Such a reactivation may develop also from an occult infection acquired after partially protective vaccination as exemplified by a remarkable case[22]. A patient who was vaccinated before a long-term stay in Papua New Guinea was obviously infected there by a local HBV strain with subgenotype D4 but did not develop disease or anti-HBc. Years later, he needed treatment of a lymphoma, leading to complete suppression of B cell immunity, and after further two years, he developed massive HBV levels with this local strain which in addition showed several escape mutations of HBsAg. This case highlights two problems of hepatitis B vaccination that are principally recognized, but currently considered less important by the responsible authorities: HBV genotypes and escape mutants.

The concept of the current vaccines was developed by the discoverer of HBsAg, Baruch Blumberg in 1970 without the real knowledge of HBV and its biology. A convincing field trial proved the efficacy of the approach; however[12] soon after its discovery, it was noted that HBsAg was genetically variable, but experimental immunizations[23,24] and field studies[25] showed that a vaccine containing only one HBsAg subtype was able to induce cross-protection to heterologous subtypes. These results, however, were obtained soon after an optimal immunization when the individuals had anti-HBs titres around 1000 IU/l. In the early 1980s, researchers in the USA succeeded in producing the major (small) HBsAg protein (SHBs) in large amounts using genetically transformed yeast cells. Due to the history of discovery, the genotype of the 'recombinant' hepatitis B vaccine was A2 which predominates in the USA[24]. Much later, an accidental observation in blood donors of the American Red Cross showed that protection is not equally successful against heterologous HBV genotypes[26]. The study was aimed at identifying blood donors who were recently infected with HBV using serology (HBsAg and anti-HBc) and nucleic acid amplification techniques for HBV DNA. Of

the 21 non-vaccinated infected donors, 17 had HBV genotype A2 (which is predominant in the USA) whereas five of seven infected vaccinated donors with moderate and presumably protective levels <100 IU/l of anti-HBs had non-A genotypes which was a significant difference. None of the donors with anti-HBs titres >100 IU/l showed signs of recent HBV infection. The breakthrough infections in the vaccinated donors were mostly detectable only by assay of HBV DNA, were clinically asymptomatic and did not show persistence of HBsAg[26]. The finding raised attention, but the reaction of the pharmaceutical industry was that no change of the genotype in the vaccines would be necessary because there were no relevant clinical events[27]. However, it should not be forgotten that probably >99 per cent of HBsAg carriers worldwide have non-A2 genotypes[28] and that this large majority should receive optimal protection.

Interestingly, most breakthrough infections in the US blood donors were not caused by vaccine escape mutants in the antigen loop of HBsAg. The first escape mutation, G145R in the HBsAg antigen loop, had been described in 1990 in a MTCT case[29]. However, escape mutants are not the major cause of breakthrough in vaccinated newborns from highly infectious mothers and they do not often evolve in successfully vaccinated individuals. Escape mutants are often detectable in healthy non-vaccinated persons (*e.g.*, blood donors) with occult, HBsAg-negative and HBV DNA-positive infection. Such persons are relatively rare and their viraemia is usually very low and the virus can be only transmitted by blood or liver donation[30]. This does not mean that escape mutants are not important. Escape mutants are probably much more frequent than would appear, but in most cases, they are hidden in the livers of the two billion individuals[2] who have been exposed to HBV worldwide and are undetectable in the blood. These escape mutants emerge and grow up to high levels of viraemia when persons experience suppression of B-cell immunity by antibody therapy against lymphoma[20,21]. In contrast to vaccine-selected escape mutants, these naturally selected variants usually have several relevant mutations in the 70 amino acids long HBs antigen loop. In one case, 16 mutations were identified and consequently the HBsAg was negative in all serological assays[12]. It is very unlikely that the current vaccines protect against such heavily mutated variants in view of the fact that one mutation like G145R is sufficient to allow for escape in the MTCT setting[29]. The incomplete protection by the current vaccines contributes to the already existing large pool of escape mutants, because in this setting, low-level replication of HBV occurs in the presence of anti-HBs. The reactivated subgenotype D4 strain of the breakthrough infection mentioned above did not only contain seven subgenotype-related differences in the HBs antigen loop but also five additional mutations which were obviously selected before reactivation[22].

It has been emphasized that booster doses are probably not necessary after a complete course of vaccination with sufficient short-term anti-HBs response of >10 or 100 IU/l. However, experience from many countries shows that protective levels wane within 15 years after childhood vaccination to low or undetectable levels and the persistence of protection later in life is not yet foreseeable. In a recent presentation on young HCW vaccinated as newborns in Thailand[31], it was reported: '… at age 17-19 yr, 81.4 per cent were anti-HBs negative or titre <10 mIU/ml. The young adults whom firstly demonstrated anti-HBs negative or titre <10 mIU/ml required at least 2-dose course of HB vaccination to achieve protective levels of anti-HBs'. It remains to be seen whether all the vaccinated persons with low or absent anti-HBs would develop persistent occult HBV infection after exposure, but it appears likely that elimination of HBV would not be achievable with this level of immunity, and booster doses may be inevitable at least for persons at an increased risk. However, more immunogenic and optimally designed vaccines with long-lasting protection would be the better solution.

How could hepatitis B vaccines be improved?

*(i)* The easiest, currently most obvious way to improve the protective capacity would probably be adaptation of the HBV subgenotype pattern of the HBsAg vaccine to the regional prevalence. India has predominantly genotype D, but also a significant prevalence of genotype C and A1 and only a few imported cases of A2. Some countries have developed vaccines with subgenotypes different from A2, for example, South Korea with C or Russia with D. China uses vaccines with A2 although genotypes C and B predominate there.

*(ii)* Expression of HBsAg in yeast cells, introduced in the 1980s[24], was a great step forward

concerning biological safety, availability and cost, but in terms of immunogenicity, it was partially a step backward because the protective epitopes of HBsAg are all conformational, whereas the yeast-derived HBsAg is to a large part misfolded[12,32]. Vaccines have been developed which were expressed in mammalian cells and were more immunogenic and could induce satisfactory anti-HBs levels more rapidly and with two doses, whereas the yeast-derived HBsAg needed longer time and three or four doses[12].

*(iii)* At the time when the current vaccines were designed, the attachment and entry of HBV was not understood, and the SHBs protein was the only well-characterized component of HBsAg. The middle-sized and large proteins (LHBs) of the HBV envelope were unknown. The preS1 domain of LHBs was later shown to be essential for HBV infectivity[33], and preS1 antibodies were found to be virus neutralizing[32]. PreS1 antigen has the advantage that it is located mainly on complete HBV particles, whereas the SHBs protein is mainly present in non-infectious subviral particles which distract the protective anti-HBs from its intended target. Furthermore, variability of preS1 is much lower than that of the HBs antigen loop and escape mutants are not known[12]. Monoclonal preS1 antibodies have been isolated from donors previously exposed to HBV and have been found to block the attachment of HBV to hepatocytes which is a marker for potential protectiveness[34]. One of the preS1-containing vaccines expressed in mammalian cells and with genotype A2 and D (Hepacare from Medeva) was successfully tested and already licensed in the European Union but never brought to the market[12]. Another similar vaccine (Sci-B-Vac from SciVac, Israel) is available in some countries but normally not used[35]. Most recently, a conjugate of preS and allergy-preventing peptides was shown to induce HBV-neutralizing anti-preS1 antibodies. However, it is unlikely that this construct will become a widely used hepatitis B vaccine[36].

*(iv)* A disadvantage of the preS1 antigen is that it is only weakly immunogenic in its natural context as is the HBsAg itself. The current vaccines need three doses of 10-40 µg HBsAg for a satisfactory result. A highly immunogenic vaccine like that against hepatitis A virus needs only two doses of 10-40 ng-killed HAV particles. A very immunogenic structure of HBV is the core particle[37]. Its major epitope is located on the tips of viral spikes. The non-protective B-cell epitope of the core particle can be replaced by epitopes of choice, for example, by preS1 peptides carrying neutralizing epitopes. Using these chimeric particles as immunogen, mice developed very high titres of neutralizing preS1 antibodies[32]. This approach has the additional advantage that the T-cell epitopes of the core particles would strengthen the anti-HBV immune response. The bacterial RNA that is packaged nonspecifically within the core particles may act as an inducer of innate immunity and as an adjuvant[37].

There are many other promising ideas related to how immunity against HBV can be induced in a more dependable and efficacious way. However, decision makers in the pharmaceutical industry and health authorities should consider first the possibility that better hepatitis B vaccines may solve well-known problems such as incomplete vaccinations due to a lacking 3rd or 4th dose, non- or low response in the elderly or immunocompromised, need for booster doses and breakthroughs. Further, preS-containing vaccines may mitigate the threat caused by already existing natural and future vaccine-selected escape mutants. This would initially cause expenses for research, development and field studies, but may save much money in the long term and may contribute to the eventual elimination of HBV infections.

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