Introduction

The consequences of HBV infection include acute hepatitis, fulminant hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [1]. In most endemic areas, primary infection occurs mainly during infancy or early childhood [2,3]. Perinatal transmission from highly infectious mothers to their infants is an important route of transmission, which leads to very high rates of chronicity (approaching 90%) in infants of mothers who are seropositive for both hepatitis B surface antigen (HBsAg) and e antigen (HBeAg) [4]. By contrast, the risk of chronic HBV infection among infants of mothers who are seropositive for HBsAg, but not HBeAg, is significantly lower (<1%).

Hepatitis B immunization is an effective way to interrupt HBV transmission. Universal HBV vaccination in infancy is remarkably efficacious in reducing HBV infection, and in Taiwan has led to marked decrease in the incidence of acute, fulminant and chronic hepatitis B, and of hepatocellular carcinoma [5–12]. Despite the success of hepatitis B vaccination, there are still problems that hamper the success of global control and the ultimate eradication of HBV infection and its sequelae. Vaccine-escape mutants might be selected by the host after immunoprophylaxis and contribute to breakthrough HBV infection [13]. Understanding the epidemiology of the emergence of viral mutants should help in designing better strategies of immunoprophylaxis against HBV infection.

Preventing HBV infection by universal immunization

Current HBV prevention strategies include passive immunization with hepatitis B immunoglobulin (HBIG) within 24 h after birth and active immunization with three to four doses of hepatitis B vaccine [12–16]. The World Health Organization (WHO) recommends that all infants should receive the first dose of hepatitis B vaccine at birth [17]. The three-dose vaccination coverage rate in infants has risen to 60% globally, with the highest rates (90%) in North America and the lowest rates in Southeast Asia (20–30%). There are three main strategies of vaccinating infants, variously adopted in different countries depending on their prevalence of HBV infection and the resources available to them (Table 1) [18–20].

HBV epidemiology in Taiwan before and after universal vaccination

The world’s first universal hepatitis B vaccination programme was launched in Taiwan in July 1984 [18]. This
programme combined with HBIG given to infants born to HBeAg-seropositive, HBsAg-positive mothers has reduced the risk of transmission from 70–90% to <10% [12,13]. Five-year HBV seroepidemiological studies have revealed a significant reduction of HBsAg carrier rate in Taiwanese children younger than 15 years from 9.8% in 1984 (before implementation of vaccination) to 1.3% in 1994 and then to 0.5% in 2004 (Table 2) [3,5–7,21]. A long-term prospective study revealed a very low rate of new HBV infection among vaccinated individuals, as evidenced by annual 0.2% seroconversion rates [22].

Breakthrough HBV infection

Breakthrough infection of HBV is defined as ‘having HBV infection despite receiving three or more doses of HBV vaccine’. Before inferring that an individual has breakthrough infection, the vaccination history needs to be carefully validated. A survey conducted in 2004 among children younger than 18 years in Taiwan showed breakthrough HBV infection in 136 of 13,765 (1%) children sampled [7]. Children with such infection were all born after implementation of the universal vaccination programme and had received three or more doses of the HBV vaccine.

The main causes of breakthrough HBV infection include high maternal viral load, intrauterine infection, emergence of vaccine-escape mutants, delayed vaccination, and immunosuppression and hyporesponsiveness to the vaccine. The HBsAg seropositivity rate in mothers of HBsAg-seropositive children born after the launch of universal HBV vaccination was 88.5% [7]. By contrast, the maternal HBsAg seropositivity rate in the pre-HBV vaccination era was between 40 and 50%. Furthermore, the mean maternal HBsAg seropositivity rate in the general population of the vaccinated birth cohorts was 16.7% (data from Taiwan Center for Disease Control, Department of Health). These data suggest that maternal transmission of HBV in the vaccination era is a major factor associated with breakthrough infection [7]. High maternal viraemia has indeed been found to be positively correlated with neonatal HBV infection and vaccination breakthrough [23]. Another risk factor is maternal seropositivity for HBeAg, which is a well-recognized marker of high-level HBV replication [4]. Intrauterine infection is also a contributory factor; one study showed that HBsAg seropositivity could be detected at birth in 2.4% of 665 neonates of HBsAg-positive, HBeAg-positive mothers, with a chronicity rate of 100% during follow-up to 12 months of age [24].

Strategies to further overcome perinatal transmission of HBV include the implementation of hepatitis B vaccination to all newborns <24 h after birth, as

<table>
<thead>
<tr>
<th>Immunization extent</th>
<th>HBV markers screened in mother</th>
<th>Vaccine given to infants</th>
<th>HBIG given to infant</th>
<th>Cost</th>
<th>Efficacy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine + HBIG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>HBsAg, HBeAg</td>
<td>Yes</td>
<td>Infants of HBsAg-positive, HBeAg-positive mothers</td>
<td>Higher</td>
<td>High</td>
<td>[18]</td>
</tr>
<tr>
<td>Type II</td>
<td>HBsAg</td>
<td>Yes</td>
<td>Infants of HBsAg-positive mothers</td>
<td>Highest</td>
<td>High</td>
<td>[19]</td>
</tr>
<tr>
<td>Vaccine only</td>
<td>None</td>
<td>Yes</td>
<td>No</td>
<td>Lower</td>
<td>Modest</td>
<td>[20]</td>
</tr>
</tbody>
</table>

Table 1. Strategies of HBV vaccination

<table>
<thead>
<tr>
<th>Evidence of HBV infection</th>
<th>Before vaccination</th>
<th>5 years*</th>
<th>10 years*</th>
<th>15 years*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surveyed, n</td>
<td>1,200</td>
<td>1,134</td>
<td>1,515</td>
<td>1,357</td>
</tr>
<tr>
<td>HBsAg-positive, %</td>
<td>9.8</td>
<td>4.6</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Seropositive for anti-HBc, %</td>
<td>26.2</td>
<td>14.5</td>
<td>4.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Analysed for HBV DNA, n</td>
<td>148</td>
<td>91</td>
<td>65</td>
<td>71</td>
</tr>
<tr>
<td>Seropositive for HBV DNA, n</td>
<td>103</td>
<td>51</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td>Seropositive for HBV DNA and S gene mutant, %</td>
<td>7.8</td>
<td>19.6</td>
<td>28.1</td>
<td>23.1</td>
</tr>
<tr>
<td>Total surveyed who were seropositive for S gene mutant, %</td>
<td>0.7</td>
<td>0.9</td>
<td>0.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 2. HBV S gene mutants in children <15 years old seropositive for HBsAg or anti-HBc

All children under *5 years old, †10 years old and ‡15 years old were vaccinated. anti-HBc, hepatitis B core antibody; HBsAg, hepatitis B surface antigen.
recommended by the WHO; administration of HBIG within 24 h after birth for neonates of HBsAg-carrier mothers; and administration of antiviral therapy for high-risk pregnant women during last trimester, although whether this is safe and effective requires further confirmation [25,26]. Some risk factors are associated with transplacental leakage of maternal blood, which might occur during threatened spontaneous abortion, pre-term labour, chorionic villus sampling, amniocentesis or assisted delivery (when using, for example, vacuum suctioning and forceps) [27]. Mutations in HBsAg are also associated with breakthrough infection [28–30].

**Hepatitis B S gene mutants before and after universal vaccination**

The ‘a’ determinant region of HBsAg is the major target for the neutralizing antibody produced during natural infection, or following active or passive immunization [31]. Production of antibodies to this determinant mediates cross protection against infection by all HBV subtypes. The ‘a’ determinant was regarded initially as a conformational cluster of epitopes located between residues 120 and 150 of HBsAg. It has been further expanded to include residues 110–160 after the discovery of two more epitope clusters [32]. The major HBsAg B-cell epitopes are located in the hydrophilic, conserved domain between residues 137 and 149 [33]. Vaccine-escape mutants with amino acid changes at the ‘a’ determinant are selected under immune pressure exerted after HBV vaccination, administration of HBIG or both. These mutants have been identified in children with acquired HBV infection despite immunoprophylaxis [34]. They might be transmitted or carried undetected by conventional HBsAg screening tests, particularly in tests using single monoclonal antibodies [35], threatening long-term success of hepatitis B immunization. Their presence also raises the possibility that current vaccines need to be modified. However, licensed recombinant HBV vaccines have been reported to protect against infection with the prototype G145R mutant [36].

In Taiwan, before universal HBV vaccination began, ‘a’ determinant mutants could be found in 7.8% of HBsAg-carrier children [37]. The proportion of ‘a’ determinant mutants among carrier children 5, 10 and 15 years after the launch of the universal vaccination programme was determined to be 19.6%, 28.1% and 23.1%, respectively (Table 3) [38]. The rate was significantly higher in those fully vaccinated compared with those who were not (15/46 versus 15/153; *P* < 0.001). However, the prevalence of S gene mutants in the total population of children surveyed increased in the first 5–10 year period after the launch of the universal HBV vaccination programme and slightly decreased 10–15 years after; this was due to the reduction of the total carrier-children pools [7].

The spectrum of mutations in the ‘a’ determinant region occurred in HBsAg-carrier children has widened (Table 3). Mutations in HBsAg tend to occur more frequently in the region with greater local hydrophilicity (between residues 140 and 149) in vaccinated than in unvaccinated children carrying HBV mutants (12/15 versus 6/15; *P*=0.062). Mutations also appeared more frequently in the 2nd loop of the ‘a’ determinant region than in the first loop [38].

**S gene mutants in vaccinated children with fulminant, acute and chronic hepatitis B**

S gene mutants have been detected as the dominant or only HBV variant in immunized children with acute or fulminant hepatitis B [29]. They can also persist in vaccinated children with chronic HBV infection [29].

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Table 3. HBV S gene mutants in children <15 years old in Taiwan

<table>
<thead>
<tr>
<th>HBV S gene mutants</th>
<th>Before vaccination</th>
<th>After vaccination 5 years</th>
<th>10 years</th>
<th>15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with ‘a’ determinant mutants, n (%)</td>
<td>8 (7.8)</td>
<td>10 (19.6)</td>
<td>9 (28.1)</td>
<td>3 (23.1)</td>
</tr>
<tr>
<td>‘a’ Determinant mutants detected</td>
<td>T126A</td>
<td>T126A</td>
<td>T125A</td>
<td>T131I</td>
</tr>
<tr>
<td>M133L</td>
<td>T126A</td>
<td>T126A+P127T</td>
<td>G145R</td>
<td></td>
</tr>
<tr>
<td>F134L</td>
<td>P127T</td>
<td>T126A+T143M</td>
<td>G145R</td>
<td></td>
</tr>
<tr>
<td>C138S</td>
<td>T129H</td>
<td>T126S</td>
<td>D144H (m)</td>
<td></td>
</tr>
<tr>
<td>T140R</td>
<td>S143W (m)</td>
<td>T140P (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T140L</td>
<td>S143W (m)</td>
<td>T144H(m)+G145R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T143M</td>
<td>G145R</td>
<td>N146S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G145R</td>
<td>C147R (m)+C149 R (m)</td>
<td>T148I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D144A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Derived from [37,38]. m, mixed with wild type.
In some infants, the mutants might not be detected in their mothers, even after cloning and sequencing. In a study of four infants with fulminant hepatitis B and three infants with acute hepatitis B (all <5 months old [29]), one 2-month-old infant was initially found to carry the T126A ‘a’ determinant mutant, to be replaced by G145R 4 days later. Only the wild-type virus was detected in the mother. Such mutants might have pre-existed in the mother as minor strains, and not detected by conventional PCR and cloning. Another infant was infected by wild-type virus, but the mother carried the T126A mutant.

Among 15 children diagnosed before 3 years of age with chronic HBV infection with or without abnormal aminotransferase levels, 5 (33%) were found to carry ‘a’ determinant mutants. Of these, three carried the G145R mutant, one the T126A mutant and the last the Q129R mutant; the other 10 children carried wild-type HBV. None of the family members of children infected with the G145R variant carried that variant, consistent with low transmission potential of this variant [29,38].

**Surveillance for vaccine-escape mutants**

While conducting seroepidemiological surveillance of the vaccinated general population for S gene mutants, it is recommended first to select vaccinated individuals who are seropositive for HBsAg or antibody to hepatitis B core antigen, regardless of their serostatus for antibody to HBsAg (anti-HBs), and check their serum for HBV DNA [37,39]. Sera found to be positive
for HBV DNA are then processed for sequencing to identify ‘a’ determinant mutations (Figure 1). For vaccinated individuals who are seropositive for anti-HBs only, no further analyses need to be performed because the surveillance reward is meagre. The exception is for those who have seroconverted from being HBsAg-seropositive or borderline HBsAg-seropositive to being HBsAg-negative, with positivity for anti-HBs – they might warrant testing. Vaccinated individuals who are seronegative for all HBV markers, which together can account for >95% of the general vaccinated population [7], also need not be tested further.

Aside from periodic surveillance of the seroepidemiology of S gene mutation rates before and after universal vaccination, other population groups that might be surveyed for carriage of S gene mutants include blood donors, children of HBsAg-carrier mothers and people who are at risk of HBV infection (for example, injecting drug users and attendees of sexual transmitted disease clinics).

Implications for future vaccination strategies

Understanding the contexts behind the emergence of S gene mutants post-vaccination will help in designing more efficacious strategies of against HBV infection. A universal HBV immunization programme can result in exerting selection pressure to facilitate the emergence of mutants among those with breakthrough HBV infection. Before 1992, the only vaccine available in Taiwan for children in the national programme was the plasma-derived hepatitis B vaccine. After 1 November 1992, it was replaced by the yeast-derived recombinant vaccine. The prevalence of ‘a’ determinant variants seems to be lower in those who received recombinant vaccine than in those who received plasma-derived HBV vaccine [38]. Theoretically, the plasma-derived vaccine has a more diverse and immunogenic HBsAg than recombinant vaccine; however, the recombinant vaccine elicits a higher anti-HBs titre and probably confers an extended duration of protection because it introduces more antigen [38]. Although a study investigating an HBV vaccine containing pre-S epitopes has not revealed substantial increase in vaccine efficacy against the escape mutant, it is still premature to make inferences about its efficacy [40]. Further studies are needed to compare the efficacy of new vaccines to protect against infection by escape mutants.

The studies conducted in Taiwan have associated universal HBV vaccination in childhood with the increasing prevalence of S gene mutants among HBsAg-positive children 5–10 years after vaccination, to eventually reach a plateau after 15 years. The G145R mutant has invariably been detected in post-vaccination surveys of vaccinated individuals and is the most prevalent mutant (27%); the T126A/S mutant is the next most prevalent mutant (20%). Thus, the G145R and T126A/S mutants account for nearly half of all ‘a’ determinant mutants and could be incorporated into future vaccines. Nonetheless, despite the increase in the proportion of ‘a’ determinant mutants among the children with breakthrough infection, the prevalence rate of children mutants in the general population has not increased. Furthermore, current vaccines are cross-protective against infection by S gene mutants to some degree [36], so a new vaccine is not required immediately.

There are limitations to the Taiwanese data summarized here. Using current methodologies, all the studies of breakthrough HBV infection reported in this review involve the detection of major strains of S gene mutants; infection with minor mutant strains or occult infection might have been overlooked. In addition, with the increasing use of antiviral therapy worldwide, the transmission of polymerase gene mutants needs to be carefully monitored. These mutants might play a potential role in establishing infection later in life, when anti-HBs antibodies have decreased [39]. However, so far in Taiwan, one long-term cohort study and five-yearly seroepidemiological surveys provide evidence to reassure that loss of protective anti-HBs levels does not lead to increased rate of chronic HBV infection in vaccinated children growing up to adolescence [5–7,22].

Conclusions and future prospects

HBV vaccination has increased the rates of detection of HBV S gene, in particular the sG145R and sT126A/S mutants, among vaccinated individuals with breakthrough HBV infection. However, this increase needs to be viewed in conjunction with the substantial decrease in the HBsAg carrier rate in the total number of vaccinated children following the implementation of the universal HBV vaccination programme. Nevertheless, long-term surveillance of and research into S gene-related mutants are needed, especially as the vaccination era now coincides with widespread use of antiviral drugs. As S gene mutants do not seem to be transmitted with ease, they do not need to be integrated into the HBV vaccine as yet. Perinatal transmission of wild-type HBV from highly viraemic mothers accounts for the majority of breakthrough infection; therefore, interrupting mother-to-infant transmission of wild-type and mutant HBV by highly effective antiviral therapy given to infectious mothers is an important approach to consider.

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