Title

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Authors

Stevens, Cladd E
Toy, Pearl
Kamili, Saleem
et al.

Publication Date

2017-11-01

DOI

10.1016/j.biologicals.2017.08.008

Biologicals xxx (2017) 1–17

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Review

Eradicating hepatitis B virus: The critical role of preventing perinatal transmission

Cladd E. Stevens [a, *, 2], Pearl Toy [b], Saleem Kamili [c], Patricia E. Taylor [a, 2], Myron J. Tong [d, 1], Guo-Liang Xia [c], Girish N. Vyas [b]

[a] New York Blood Center, 310 East 67th Street, New York, NY, 10021, United States
[b] University of California School of Medicine, San Francisco, CA, 94143-0134, United States
[c] Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, GA, 30298, United States
[d] Huntington Memorial Hospital, Pasadena, CA, 91105, United States

ARTICLE INFO

Article history:
Received 15 April 2017
Received in revised form
12 August 2017
Accepted 14 August 2017
Available online xxx

This review is dedicated to the memory of the late Robert Palmer Beasley for his role as a mentor, scientist, and leader in the global effort to eradicate hepatitis B.

Keywords:
Chronic HBV infection
Eradication
Escape mutant
Hepatitis B immune globulin
Hepatitis B vaccine
Hepatitis B surface antigen
Mother-to-child transmission
Perinatal HBV infection
Prevention

Abbreviations used:
HBV
hepatitis B virus
HBIG
hepatitis B immunoglobulins
HBsAg
hepatitis B surface antigen
HCC
hepatocellular carcinoma
ccc-HBV DNA
covalently closed circular HBV DNA
anti-HBc
hepatitis B core antibodies
HBeAg

ABSTRACT

Prevention of hepatitis B virus (HBV) transmission from infected mothers to their newborns is critical to HBV control and eventual eradication. Mother-to-child perinatal transmission causes the highest chronic carrier rate (>85%) with a high rate of subsequent chronic liver disease and hepatocellular carcinoma. This risk is reduced by 90% with HBV vaccine given along with hepatitis B immune globulin (HBIG) starting at birth. New analyses of our data from US trials of HBIG and HBV vaccine in high-risk infants revealed better efficacy with yeast-recombinant vaccine than plasma-derived vaccine, especially in preventing late onset infections, with evidence that vaccine prevented transmission of maternal HBV infection with the glycine to arginine mutation in surface antigen codon 145 (sG145R). Most late infections with sG145R were in vaccine non-responders, suggesting escape from HBIG rather than from vaccine-induced antibody. Our findings also help explain survey results from Taiwan following universal childhood immunization implemented in the mid-1980s. We conclude that current vaccines will remain effective against surface antigen mutants. Anti-viral drugs in high-risk pregnant women, in combination with newborn HBIG and vaccine, show promise for eliminating residual breakthrough neonatal infections, critical to meeting WHO 2030 goals and for eradicating HBV.

* Corresponding author. 180 East End Avenue, Apt. 16G, New York, NY, 10128, United States.
E-mail address: cladd.stevens@gmail.com (C.E. Stevens).
[1] Currently at Ronald Reagan UCLA Medical Center, Los Angeles, CA 90024, United States.
[2] Retired.

http://dx.doi.org/10.1016/j.biologicals.2017.08.008

Please cite this article in press as: Stevens CE, et al., Eradicating hepatitis B virus: The critical role of preventing perinatal transmission, Biologicals (2017), http://dx.doi.org/10.1016/j.biologicals.2017.08.008

hepatitis B e antigen
sG145R
mutation of glycine to arginine at amino acid 145 of HBsAg

1. Introduction

In 1972, not long after the discovery of the Australia Antigen and its linkage to type B hepatitis, Palmer Beasley asked a question: "Could hepatitis B virus be eradicated?" [1]. Even then the short answer was yes. It was already known that, like smallpox and poliomyelitis, hepatitis B virus (HBV) had no relevant natural non-human reservoir and person-to-person routes of transmission were clearly defined (i.e., parenteral, sexual or from mother-to-child perinatally). Acute infections in healthy adults generally resolved with disappearance of Australia Antigen (the HBV surface antigen, HBsAg) from the blood and emergence of antibody (anti-HBs). Presence of anti-HBs was associated with resistance to reinfection, including from differing serologically-defined subtypes or serotypes. This raised the possibility that neutralizing antibody - either from an immune globulin or vaccine-induced - might protect against HBV infection [2].

The occurrence of chronic HBV infection, however, complicated this prospect. HBV infections are most likely to become chronic if they occur early in life or in people who are immune deficient (in Down's syndrome or patients on dialysis, for example). The highest rate of chronicity is in newborn infants who acquire HBV *in utero* or around the time of birth (perinatally) from their infected mothers [3,4]. Nearly all such newborns become chronic carriers. The carrier rate drops for infections acquired later in childhood (e.g., 23% among pre-school children in Taiwan) and is <5% among healthy adults [5–9].

Chronic HBV infection, even if asymptomatic for decades, can result in eventual death from cirrhosis and hepatocellular carcinoma (HCC) [10–16]. Mother-to-child transmission of HBV has been associated with an increased risk of HCC [15]. Consequently, in Asian and African countries with a high incidence of perinatal and early childhood infection, death from cirrhosis or HCC (more than half due to HBV infection) is common, among the top ten causes of death [17]. Thus, from the outset, prevention of chronic infection in children has been a key component of HBV immunization strategies. The premise for eradication is that, if new infections are prevented, HBV would eventually disappear by attrition as older chronic carriers die off or their infections are cured with anti-viral drugs. An early estimate predicted eradication in 200 years, provided immune-escape mutants did not emerge and replace wild-type virus [18].

Hepatitis B vaccines are composed of HBsAg particles either harvested from pooled plasma of chronic HBV carriers or made by recombinant technology in organisms (predominantly yeast) or cells [19]. HBV vaccines from plasma were purified by physical and chemical means (including proteases) and inactivated (generally with formalin or heat) to kill any residual HBV or other infectious agents that might have been present in the source plasma. Purification removed most, if not all, of two surface proteins present in whole virus particles, pre-S1 and pre-S2. Thus, they consisted almost entirely of the S-protein which includes the "a" determinant common to all BHV strains and two epitopes that define mutually exclusive variants designated "*d/y*" and "*w/r*" and result in four distinct HBsAg serotype combinations, *adr*, *adw*, *ayr* and *ayw* (with some additional subtypes). Plasma-derived vaccines reflect the serotype and genotype of the donor pool (predominantly subtype *adw*, genotype A2 in US vaccines). Plasma from persons who have recovered from infection and have high titers of anti-HBs is also the source of polyclonal hepatitis B immune globulin (HBIG) for passive immunoprophylaxis.

Recombinant vaccines made by yeast or mammalian cells with HBV S-gene inserts mitigated safety concerns of plasma-derived vaccines and, therefore, did not require inactivation [19]. Yeast recombinant vaccines, however, required extraction and were not glycosylated. Most recombinant vaccines available also have been composed only of the S-protein, serotype *adw*, genotype A. Some scientists advocate including pre-S1 and pre-S2 to enhance immunogenicity especially since pre-S1 is now recognized as the HBV-ligand for a hepatocyte HBV-receptor, sodium taurocholate cotransporting polypeptide (NTCP), a component of the enterohepatic circulation of bile salts [20–22].

HBV vaccines have proved to be immunogenic (inducing only anti-HBs), safe and efficacious in high-risk adults and effective in preventing perinatal transmission from mother to child, setting the stage for control and possible eradication of the virus. Although considerable progress has been made as we approach four decades since HBIG and the first HBV vaccines became available, Beasley's question remains relevant. The incidence of HBV infection has declined in much of the world but the virus is not yet close to being eradicated, especially in parts of Asia and Africa. Now the question is not *if* eradication is possible but *how* can we make it happen sooner rather than eventually?

In July 2016, the World Health Organization (WHO) released its new "Global Health Sector Strategy on Viral Hepatitis, 2016–2021" outlining a plan to minimize transmission of all hepatitis viruses (A, B, C, D and E) and their disease burden [23]. The goals for 2020 are a 30% reduction in new HBV infections, and an HBsAg prevalence in children of no more than 1.0% and, by 2030, a 90% reduction in new infections with a prevalence in children of no more than 0.1%. The specific goal for prevention of mother-to-child-transmission is to improve provision of a birth dose of vaccine from the 2015 worldwide baseline of 39% to 50% by 2020 and 90% by 2030 with "hepatitis B virus testing" of pregnant women and "development of new interventions … based on antiviral treatment." [23,24]. The National Academies of Sciences, Engineering and Medicine, sponsored by the U.S. Centers for Disease Control and Prevention (CDC), has developed a plan for the United States. Its Phase One Report on feasibility and barriers was published in June 2016 and Phase Two Report on strategies to eliminate hepatitis B in March 2017 [25,26]. Both reports establish challenging short-term goals and also make clear the difficulties in achieving complete eradication.

Our review provides a synthesis of evidence for eradication of HBV, summarizing critical components including: pre-vaccine era global epidemiology of HBV; importance of childhood infections, especially those acquired perinatally from infected mothers; role of viral load in transmission; efficacy of immunoprophylaxis (passive by immune globulin, active by vaccine and passive-active); potential problem of immune-escape mutants; role of anti-viral drugs; durability of immunity; and effectiveness, thus far, of efforts to control HBV using the national universal HBV immunization program in Taiwan as a model. We also present new analyses of data from our own studies of perinatal transmission, focusing on the impact of the time of onset of infection in the infant and the relevance of "escape" mutants to the prospect of long-term vaccine efficacy. This paper provides support for the view that prevention of

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perinatal HBV transmission is the single most urgent goal for prevention strategies because of its high frequency in hyperendemic areas, its role in perpetuating infections from generation to generation and as a source of infection for other children, and its life-threating sequelae, cirrhosis and HCC. Reaching WHO goals for 2030 and eradication will be possible only if *in utero* and mother-to-child transmission of HBV are prevented. The greatest obstacle is the complexity of providing optimum immunoprophylaxis on a global scale starting at birth, including use of HBIG and/or treatment of mothers with antiviral drugs during pregnancy.

2. Global HBV infection and disease patterns

Sensitive, reliable assays to detect HBV markers became available in the 1970s and rapidly became critical tools for defining the epidemiology of HBV worldwide. HBsAg, present on the surface of whole virus as well as in circulating sub-viral particles, was the primary serological marker of HBV infection and induced an anti-HBs response that appeared as acute infection resolved and HBsAg disappeared. HBV-core capsid structural proteins in the whole virus particle also induced an antibody response (anti-HBc) which appeared early during the acute phase of infection, remained positive in chronic carriers but also persisted after resolution. Thus, in the vaccine era, anti-HBc positivity (usually with anti-HBs) in the absence of HBsAg became a marker of past HBV infection. Anti-HBs alone became evidence of response to vaccine. Hepatitis B e antigen (HBeAg), a non-particulate, soluble protein dimer derived from the same open reading frame in the HBV genome as the pre-core/core proteins, was present early in acute infection and persisted for prolonged periods in some chronic carriers. As a secreted pre-core/core derivative, HBeAg was associated with high viral replication, and, therefore, with a high circulating viral load and infectivity [27,28].

In the pre-vaccine era, HBsAg prevalence rates among adults ranged from <2% in Europe and North America to as high as 15–20% in much of Asia and Africa [29]. Most HBsAg-positive people in these prevalence surveys were chronic carriers. New infections in Europe and North America mainly occurred in adults through parenteral or sexual exposure whereas most infections in hyperendemic areas were acquired perinatally from infected mothers or in childhood from other sources. The prevalence of HBeAg among HBsAg-positive individuals paralleled that of HBsAg, low in Europe and North America and high in Africa and Asia also reflecting regional risks of perinatal and childhood infection [30].

Endemic areas also have the highest incidence of death from cirrhosis and HCC. Indeed, the concordance between HBsAg prevalence and HCC was one of the first lines of evidence that HBV caused liver cancer [13]. A causal relationship was further supported with prospective studies, notably Palmer Beasley's study of 22,544 male government workers in Taiwan, which showed that HBV infection was present long before HCC developed [14]. HCC incidence during follow up among the men who were healthy but HBsAg-positive when they enrolled was 495/100,000/year, 98 times higher than the incidence in HBsAg-negative men [31]. The incidence rose with age, reaching more than 900/100,000/year in 60 to 69-year-old men. These data predicted a life-time HCC risk of 30–40% for male chronic HBV carriers in Taiwan. Among, HBeAg positivity and high viral load in HBV carriers increased the risk of cirrhosis and HCC [32].

3. Mother-to-child perinatal transmission of HBV

Mother-to-child transmission of “serum” hepatitis was first documented by Stokes and colleagues in 1954 [33]. Following the diagnosis of clinical hepatitis in a newborn infant, volunteers inoculated with serum samples from either the infant or its mother subsequently developed clinical hepatitis. In the early 1970s, Schweitzer and colleagues describe HBV transmission to infants from mothers who had symptomatic acute hepatitis B during their third trimester or within two months of delivery, but not when hepatitis occurred earlier or later [34,35]. All ten infants in his report who were infected became chronic carriers. One who was HBsAg positive at birth may have had an *in-utero* infection. Another demonstrated unequivocal evidence that the virus was acquired around the time of birth (i.e., peri-natal rather than post-natal). The infant was given up for adoption and had no contact with the mother after birth. He was HBsAg negative one week after birth but was positive when retested at three months.

Infants born to HBV carrier mothers also proved to be at risk. In our studies of infants born to HBsAg positive mothers in Taiwan 35–40% became infected and 85–95% of these became chronic carriers [3,4]. If such infants remained chronically infected into adulthood, perinatal transmission would account for about one-third of chronic infections in Taiwan and would be an important source of the virus for other children. Most infants who became infected were HBsAg negative at birth but tested positive at 1–3 months of age, a time frame that fits the known incubation period for HBV if transmission occurred mostly during delivery (Fig. 1A) [3]. Thus, from studies of both acute hepatitis B and chronic carriers, the risk to the infant seemed related to presence of HBV in the mother around the time of delivery rather than earlier in gestation. Presumably, infants acquire HBV by leakage of mother's blood across the placenta (especially common during labor and delivery) or from contact with mother's blood during delivery through skin abrasions or mucous membranes or by swallowing maternal blood or iatrogenically from medical procedures during pregnancy or delivery [36–38].

Maturation of fetal hepatocytes, including development of the NTCP HBV-receptor has also been proposed as an explanation for the gap between birth and the onset of antigenemia [21]. Expression of NTCP mRNA was low in the fetus at weeks 16–20 gestation (only 1.8% of the level in adults in one study) [39]. While NTCP protein expression in newborn hepatocytes is comparable to that of adults, no data is available on expression from the mid-gestation to birth [40]. Regardless of the mechanism (perhaps both are at play), the gap between birth and development of antigenemia provides an opportunity for immunoprophylaxis started at birth to protect the infant even though it would be given post-exposure.

4. Viral load and risk of transmission

Infectivity has been most clearly documented in perinatally acquired infections and in parenteral exposures, both circumstances in which the time of exposure and source are known. With perinatal transmission, the mother's viral load, measured indirectly by HBsAg titer (Fig. 1B) or HBeAg positivity (Fig. 1C) and directly by quantification of HBV-DNA levels (Fig. 1D), correlate with the infant's risk of being infected perinatally and of becoming a chronic carrier [3,41–45]. Similarly, in a study of accidental parenteral exposure, Alter and colleagues found nearly complete concordance between detection of either HBV-specific DNA polymerase or HBeAg in needle stick sources and subsequent risk of HBV infection in the healthcare workers who had been stuck [46].

5. Hepatitis B immune globulin (passive prophylaxis)

Following the precedent of standard immune globulin for passive prophylaxis against hepatitis A, the earliest attempts at HBV-immunoprophylaxis used immune globulin derived from donors with high titers of anti-HBs to produce polyclonal hepatitis B

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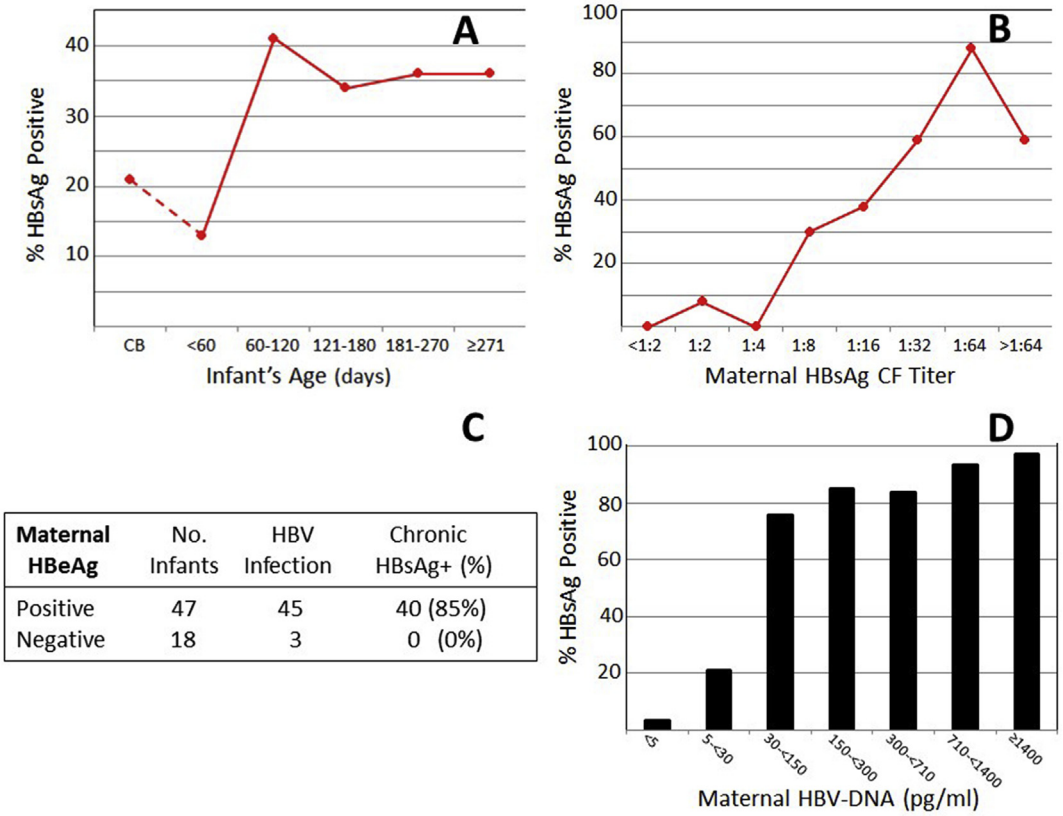

Maternal HBeAg	No. Infants	HBV Infection	Chronic HBsAg+ (%)
Positive	47	45	40 (85%)
Negative	18	3	0 (0%)

Fig. 1. Hepatitis B virus (HBV) infection in infants born to HBsAg positive mothers in Taiwan. Panel A: Time HBsAg was first detected. The peak HBsAg positivity at 61–120 days fits the incubation period for HBV and is evidence of transmission around the time of birth ("perinatal") for most infections. Panels B–D: Associations between infant HBsAg chronic carrier rate and various measures of maternal HBV viral load during pregnancy. Panel B - by maternal HBsAg complement fixation (CF) titer. Panel C - by maternal HBeAg status. Panel D - by maternal HBV DNA level (pg/ml = picograms/milliliter). Based on data from Ref. [3, 43–45].

immune globulin (HBIG) for passive immunoprophylaxis. Krugman's studies in the late 1960s made two fundamental observations regarding the effect of HBIG [47,48]. When given four hours after experimental inoculation of HBV, HBIG produced a decrease in the incidence of antigenemia compared to controls (4/10 vs. 25/25, respectively). Additionally, the incubation period for the time to detection of antigenemia was prolonged to 91–141 days from the 27–70 days observed among controls.

Randomized, controlled trials of HBIG for accidental parenteral exposures also showed delays in the onset of infection but efficacy was ambiguous. Partial efficacy was observed in two U.S. studies of medical staff when HBIG was given within seven days of a needle stick or other identified hospital exposure. Grady and colleagues showed efficacy of HBIG in preventing hepatitis B in the first four months after exposure but an increased incidence during the following five months. They estimated that 80% of these late cases were due to a prolonged incubation period (4–8 months after exposure vs. 1–3 months in controls) with 20% probably due to a second exposure [49]. In another needle stick study, Seeff and colleagues reported efficacy of HBIG prophylaxis without late onset infections but the number of hepatitis B cases in their study was small [50]. In another trial among medical staff in hemodialysis units where there was a high continuous risk of infection, an HBIG regimen of two doses given four months apart showed an average four-month delay in the occurrence of HBV infections in HBIG recipients followed by a "catch up" after the second dose to match the incidence in controls [51]. These studies also showed little impact on the frequency of clinical disease among those who became infected. Thus, while HBIG alone may be effective if given within hours of a known exposure, protection is transient (as expected with passive immunization) and, therefore, its use is considered impractical when there is an ongoing risk of infection.

Trials of HBIG in newborn infants, however, painted a different picture [52–54]. In a placebo-controlled trial among high-risk infants born to HBeAg/HBsAg positive women in Taiwan, for example, single- and three-dose regimens (the first dose given to most infants within one hour of birth) also produced a delay in the onset of infection (evidence of an immunological effect as per Krugman's studies) but negligible impact on the overall incidence of HBV infection (Fig. 2). However, the goal of immunoprophylaxis in newborn infants is to prevent chronic HBV infection and, in association with the delayed onset, infants given HBIG were substantially less likely than controls to become chronic carriers - 54% in infants given a single dose of HBIG and only 26% with three doses compared to 92% in controls (Fig. 2) [53]. As expected with passive immunoprophylaxis, infants who avoided early infection remained susceptible and some became infected after their first year, probably through further contact with their HBV-carrier mothers or possibly from other infected family members [55].

The reduced carrier rate seen in late onset infections with HBIG alone encouraged speculation that a combination of HBIG and vaccine would prove even more effective, with the HBIG-induced delay providing more time for development of active immune response to vaccine that might prevent later onset infection. Chronic infections that occurred despite HBIG, especially those manifest at birth or within the first months of life (Fig. 2) were a

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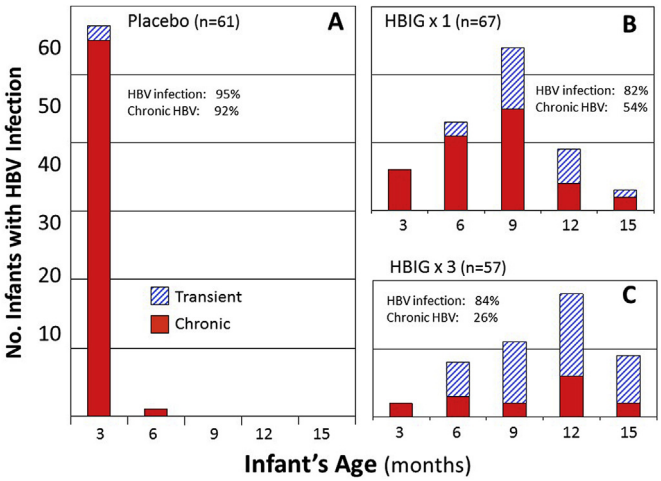

Fig. 2. Efficacy of hepatitis B immune globulin (HBIG) against perinatal HBV infection in a placebo-controlled trial in infants born to HBsAg positive mothers in Taiwan. Infants were inoculated at birth and 3 and 6 months with placebo or HBIG. The placebo group (Panel A) got three injections of placebo. The HBIG X 1 group (Panel B) was given a single dose of HBIG at birth and placebo at 3 and 6 months. The HBIG X 3 group (Panel C) got three injections of HBIG, one at birth, 3 and 6 months. The figure shows the infant's age at the time HBsAg was first detected and whether the HBV infection became chronic (solid red bars) or was transient (blue hatched bars). The immunological effect of antibody in HBIG is shown by the delay in onset of HBV infection and the reduced rate of chronic infections with onset after three months of age. Based on data from Refs. [52–54].

concern, however. If acquired *in utero* weeks before delivery, the virus may have already entered fetal hepatocytes and, therefore, might not be accessible to immunoprophylaxis started at birth.

6. Hepatitis B vaccine immune response, efficacy and subtype cross-protection

Plasma-derived HBV vaccines proved to be safe and immunogenic [56–63]. Vaccines were generally given in 2–3 initial doses a month apart followed by a booster dose at six months or later. Among healthy adults who received the full vaccine course, 90–95% developed anti-HBs levels generally recognized as protective (≥10.0 milli-International Units/milli-liter). Recipient factors such as age, underlying disease (i.e., renal failure, chemotherapy, immune deficiency) and obesity predicted a poor antibody response [64–70]. Failures also occurred when vaccine was mishandled (not refrigerated, mixed poorly before withdrawal from the vial or given subcutaneously rather than intramuscularly). Thus, some initial non-responders develop protective anti-HBs levels when given additional doses [71]. Genetic associations with non-responsiveness to both plasma-derived and recombinant vaccine (commonly an excess human leukocyte antigen DRB1*07:01) have also been reported and encouraged efforts to improve HBV vaccine immunogenicity by, for example, incorporating other viral antigens such as the pre-S1 and pre-S2 protein or using more effective adjuvants [72–77].

Efficacy trials in the early 1980s evaluated plasma-derived vaccines in adults at risk of acquiring HBV through sexual or parenteral exposures [56–63]. In one trial among high-risk homosexual men in New York City, for example, the vaccine was 81% effective in preventing HBV infection and 92% effective in preventing clinical hepatitis B [56–58]. Most HBV infections in vaccine recipients occurred in the first three months after enrollment. Presumably these cases were already incubating the virus at enrollment or were exposed soon after. Among 451 fully vaccinated men who remained uninfected through their immunization period, 4.7% were non-responders (sample to negative control ratio, <2.1) and another 2.0% had a weak anti-HBs response (peak S/N < 10.0). Nine fully immunized men subsequently had an HBV infection. Seven occurred among 21 non-responders (33%), one among nine weak responders (11%) and one in 421 responders (0.23%). During the same time frame, the infection rate in placebo recipients was 21.6%, higher than the incidence in vaccine responders ($p < 0.001$) but not significantly different from the rates in non- and weak-responders. Although not prevented, these HBV infections in vaccine non-responders tended to be milder than those in placebo recipients. Only one had clinical hepatitis (14%) compared to 55% of cases in the placebo group ($p < 0.05$). This observation suggests at least partial protection, possibly from antibody below detectable levels and/or vaccine-induced cellular immunity. None of the HBV infections in non-responders became chronic. Thus, vaccine non-response *per se* did not identify individuals who were predisposed to chronic infection.

Efficacy trials among medical staff in dialysis centers (predominantly women under 40 years old) reported 94–97% protective levels of antibody following vaccination and similar efficacy to that observed among homosexual men [60–62]. Importantly, a multicenter U.S. efficacy trial with monovalent subtype *adw* vaccine documented subtype cross-protection [60]. Among participating dialysis staff 81% of infections were subtype *ay* (*r/w* epitopes not identified). Protective efficacy was 85% against *ay* HBV infection and 91% for clinical hepatitis due to *ay* virus, comparable to efficacy observed in homosexual men who had predominantly *ad* infection.

Efficacy trials in dialysis patients gave less convincing results. Only 50–75% of fully vaccinated dialysis patients developed protective antibody levels and efficacy was consequently compromised [62,69,70]. In fact, the U.S. trial failed to show efficacy, in part because of poor antibody responses but also because the incidence of HBV infections in placebo recipients declined rapidly during the trial due to implementation of infection control measures made possible by the trial itself (i.e., monthly testing for HBsAg and isolation of infected patients) [70]. Nevertheless, there was evidence that vaccine responders were at least partially protected. Fifteen of 279 non-responders (5.7%) had an HBV infection compared to six in 283 responders (2.1% $p < 0.05$).

The high rate of chronic infection in infants raised questions about their ability to mount an effective immune response against HBV. Indeed, chronicity has been attributed to immune tolerance induced by exposure to soluble HBeAg, a hypothesis now in dispute [78]. In fact, newborns respond well to HBV vaccines, including those born to HBeAg-positive HBV-carrier mothers. In one early study in Taiwan, 91% of low-risk infants given a first dose of vaccine at 4–8 days after birth, and a second at one month, reached protective anti-HBs levels by three months of age [79]. Following a booster at six months, all had protective levels. In a large recent study in Hubei, China, 90–94% of infants born to HBsAg-negative mothers and started on HBV vaccine at birth had protective antibody levels when tested 7–15 months later [80].

HBV vaccine alone proved to be partially protective in high-risk newborns. When vaccine was started at one week after birth in a study in Taiwan, only 23% became chronic carriers [81]. Even when begun one month after birth, there was evidence of efficacy, albeit lower - 40% of infants became carriers. Similar data were reported from the Hubei study in which 17% of infants born to HBeAg/HBsAg positive mothers became carriers following vaccination begun at birth [80]. Thus, vaccine started soon after birth is about as effective as the three-dose regimen of HBIG, with the added benefit of long-term protection. These observations are reassuring for countries that still cannot afford or manage the logistics to identify HBsAg-positive pregnant women for targeted immunization strategies but can implement universal immunization within days of birth.

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7. Combined hepatitis B immune globulin and vaccine efficacy against perinatal HBV infection

Given the limits of efficacy with HBIG or vaccine alone in preventing perinatal HBV infection, using both was an obvious alternative approach to explore [82–84]. We addressed this issue in a series of four trials carried out in 1981 through 1993 among high-risk newborn infants in New York City, San Francisco and Los Angeles [85–87]. The studies were approved by local Institutional Review Boards; parental permission with signed informed consent was obtained from the mothers. Because HBIG and HBV vaccines were already licensed when these studies began, they were neither randomized nor placebo controlled. Efficacy was estimated by comparison to historical controls, infants born to HBeAg/HBsAg positive mothers among whom the risk of chronic HBV infection without immunoprophylaxis had been consistently 85% or higher.

During these trials, we screened more than 40,000 pregnant women for HBsAg and HBeAg to identify high-risk infants. Most of these women were ethnic Asians born in Asia. HBsAg prevalence was 8.8% overall, highest among Chinese women from China, Hong Kong, or Taiwan and Southeast Asians from Cambodia, Laos, or Vietnam (11% in each group). About one-third of the HBsAg positive women were also HBeAg positive, decreasing with age from nearly 50% among women <25 years old to less than 20% among women 35 years and older [87].

These trials first used a plasma-derived vaccine (Heptavax B™) in 20 and 10 micro-gram (μg) doses and, when it became available for study, a yeast-recombinant vaccine (Recombivax-HB™) in 5 and 2.5 μg doses (both manufactured by Merck and Company). Table 1 shows the four trials by vaccine used, dosage and when they were conducted (sequentially with some overlap). A single dose of HBIG was given to the infants soon after birth (72% within six hours). The schedule of vaccine injections was varied to address concerns noted above about immune responsiveness in newborns (Table 1). Follow up blood samples were scheduled to be taken from study infants at birth, one month, the time of each vaccine injection and at three-month intervals from 6 to 18 months. Of 1350 infants enrolled, 1068 completed the minimum nine months of follow up.

Anti-HBs levels were measured in samples taken from infants not yet infected with HBV and expressed semi-quantitatively as the S/N ratio. Quantitatively accurate peak anti-HBs titers were measured in milli-International Units per milliliter (mIU/ml) on serial dilutions of the first post-booster sample. Generally, 1.0 S/N = 2.0 mIU within the low S/N range (2.2 in our study for S/N < 100). At one month after birth, there was no significant difference in anti-HBs level by the vaccine (geometric mean S/N = 52.3 for plasma-derived and 54.6 for yeast-recombinant vaccine, p = 0.453) or vaccine dose (data not shown). There was also no difference among plasma-derived vaccine recipients related to the timing of the first dose (geometric mean S/N for those given their first vaccine dose at birth = 50.9 and for those at one month = 56.5. Both observations suggest that antibody detected at one month derived primarily from HBIG. Pre-booster anti-HBs was undetectable in 0.7% of infants and low (<10 S/N) in 10.4%. Pre- and post-booster antibody levels were associated with vaccine given and its dose (Table 2). Following the booster, a protective level (≥10 MIU/ml) was achieved in nearly all infants. Peak geometric mean titer, however, was significantly lower in recombinant vaccine recipients and lowest of all in the 2.5 μg recombinant vaccine group.

Infants were considered to have had an HBV infection if HBsAg was detected and persisted in subsequent specimens or by detection of anti-HBc beyond 12 months of life when passive maternal anti-HBc should no longer be present. HBsAg detected in the birth sample was not considered definitive evidence of an HBV infection (as also reported by others) [88]. Among 774 birth samples tested, 128 were HBsAg positive but only 29% of these had a confirmed HBV infection on follow up (54% if the HBsAg S/N ≥ 20). Thus, some birth samples may have been contaminated with maternal blood or HBsAg positivity may represent leakage of maternal subviral particles across the placenta. Whatever the explanation, HBsAg positivity at birth is not proof of an established *in utero* infection with viral replication in the fetus [88].

Among study infants, 122 (11.4%) had an HBV infection documented during follow up, substantially lower than the 82–84% reported in previous studies among infants who received HBIG alone [52–54]. Twenty-nine infections were transient (24 detected only by persistent anti-HBc positivity beyond 12 months of age). Ninety-three infections became chronic for a cumulative incidence of 8.8% (95% Confidence Interval = 7.0–10.6), an efficacy of 90% compared to the historical incidence of 85% without immunoprophylaxis. The infection rate was also significantly lower than that seen with HBIG alone in the Taiwan trial (54% for one dose and 26% for the three dose regimens, p < 0.001) or reported for vaccine alone (17–25%). These kinds of data formed the basis for recommending the combination of HBIG and vaccine as the most effective immunoprophylaxis for preventing chronic HBV infection in high-risk newborns [89].

We examined the time of onset in breakthrough chronic infections for evidence of immunological effects (i.e., a prolonged incubation period, assuming acquisition primarily at birth) and to assess whether some may have been established *in utero*. The onset of antigenemia was clear within a 1–3-month interval (the time between the last negative and first positive specimen) for 89 of the chronic infections. Three others were HBsAg negative at 3–6 months and positive when next tested after a 6–8-month interval. One infant, a recombinant vaccine recipient, remained negative through 3 months, was then lost to follow up until five years later when he was found to be persistently HBsAg positive. Thus, all

Table 1
Sequential series of four HBIG/HBV vaccine efficacy trials in 1068 infants born to HBeAg/HBsAg positive women in the United States from 1982 to 1993 [85–87] using plasma-derived and yeast-recombinant vaccines (Heptavax B™ and Recombivax-HB™, respectively) at differing doses. All infants received a dose of hepatitis B immune globulin (HBIG) at birth. The time of the first dose of vaccine was given either at birth or one month of age. The trials were carried out sequentially (with partial overlap as seen in the month and year of each study).

Study Group	Vaccine	Dose (micrograms)	Schedule (months)	No. of Infants	Month/Year of Study
A	Plasma-derived	20	0, 1, 6	45	
	Plasma-derived	20	1, 2, 6	124	11/81-6/85
B	Plasma-derived	10	0, 1, 6	55	
	Plasma-derived	10	1, 2, 6	109	7/83-10/87
C	Yeast-recombinant	5	0, 1, 6	574	
	Yeast-recombinant	5	0, 2, 6	33	8/84-10/93
D	Yeast-recombinant	2.5	0, 2, 4, 15	51	
	Yeast-recombinant	2.5	0, 2, 6	77	1/91-1/93

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Table 2
Antibody to hepatitis B surface antigen (anti-HBs) levels pre- and post-booster in U.S. HBIG/vaccine trial infants by vaccine and dose given*.

Vaccine-Dose (micrograms)	Pre-booster			Peak Post-booster				
	No. Tested	% >10 S/N	Median S/N	No. Tested	% >10 S/N	Median S/N	% >10 mIU/ml	GMT mIU/ml
P-20	124	98.4%	139.2	129	99.2%	220.5	100%	947.4[a]
P-10	126	96.0%	98.2	133	100%	197.0	100%	1s010.3[b]
R-5	441	90.9%	53.8	507	99.8%	127.7	99.3%	246.2[c]
R-2.5	93	69.9%	24.4	91	89.0%	53.3	97.8%	110.1[d]

*Excludes patients who had a hepatitis B virus (HBV) infection.
p values for GMTs.
a vs. b: 0.781.
a or b vs. c: < 0.001.
c vs. d: < 0.001.
P = plasma-derived vaccine (Heptavax B™).
R = yeast-recombinant vaccine (Recombivax HB™).
S/N = anti-HBs level as sample to negative control ratio.
mIU/ml = anti-HBs titer in milli-International Units per milliliter.
GMT = geometric mean titer.

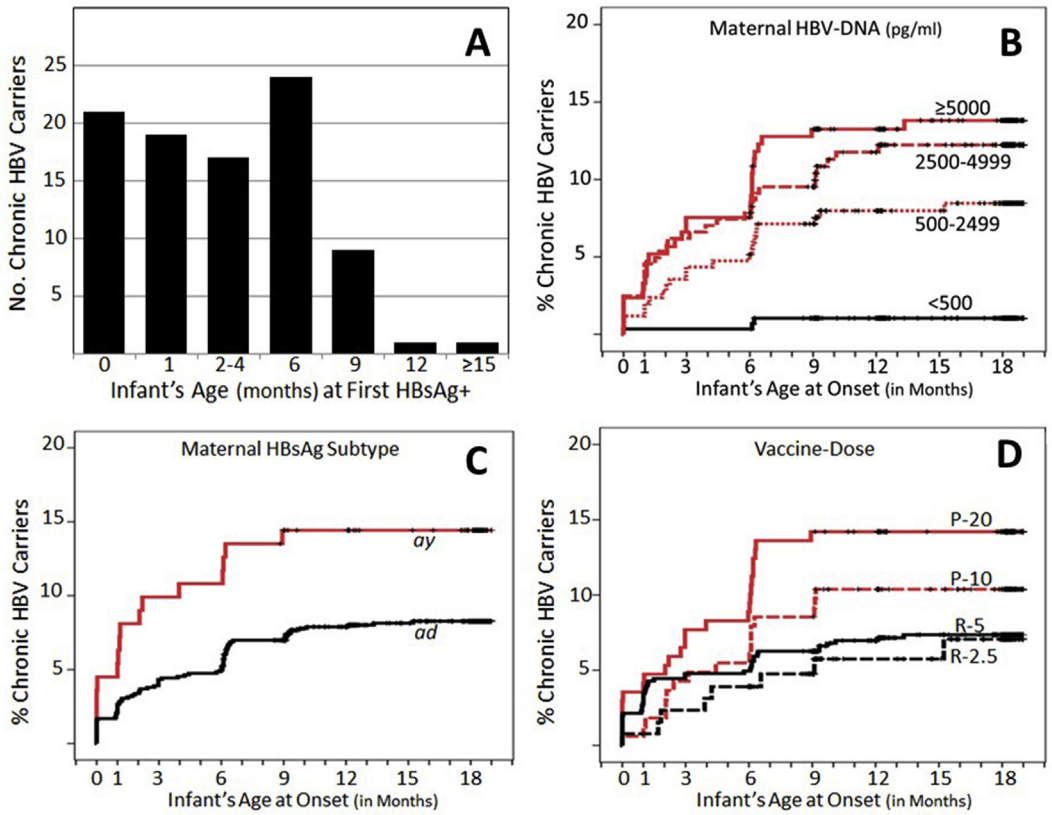

Fig. 3. Efficacy in U.S. trials of hepatitis B immune globulin (HBIG) and HBV vaccine against chronic HBV infection among high-risk newborn infants born to HBeAg/HBsAg-positive mothers. Panel A shows the infant's age at the time HBsAg was first detected. Overall incidence = 8.8%, 95% confidence interval = 7.0–10.6% (Kaplan-Meier analysis). Panels B, C and D, respectively, show the incidence of chronic infection in the infants by the maternal HBV-DNA level (picograms/ml), maternal HBsAg subtype, and vaccine and dose administered. The differences incidence between doses of plasma-derived vaccine and between doses of the yeast recombinant vaccine were not significant (see Table 3). Based on data from Refs. [85–87].

chronic infections could be classified as having an onset by one month of age, at 2-4 months, or first detected at ≥ six months.

Compared to HBIG alone, the addition of vaccine had little impact on the incidence of chronic HBV infections with onset in the first 2–4 months of life (Figs. 3A and 4A-B). Forty chronically infected infants were first HBsAg positive at birth or by one month: 21 were HBsAg positive at birth and remained positive thereafter, 18 were not tested at birth but were positive at one month and one was HBsAg negative at birth and first positive at one month. None of these infants were anti-HBs positive during follow up. An additional 17 infants were HBsAg negative at one month but were positive when next tested at 2–4 months. Thus, there were a total of 57 chronic carrier infants in our studies who were first HBsAg positive during the first 2–4 months of life for an incidence of 5.3% (95% CI = 3.9–6.7), not significantly different from the 6.5% rate observed with HBIG alone at three months (95% CI = 2.1–10.9) in the Taiwan studies (Fig. 2) [52–54]. Perhaps some, if not all, of these infections were already established in hepatocytes prior to delivery.

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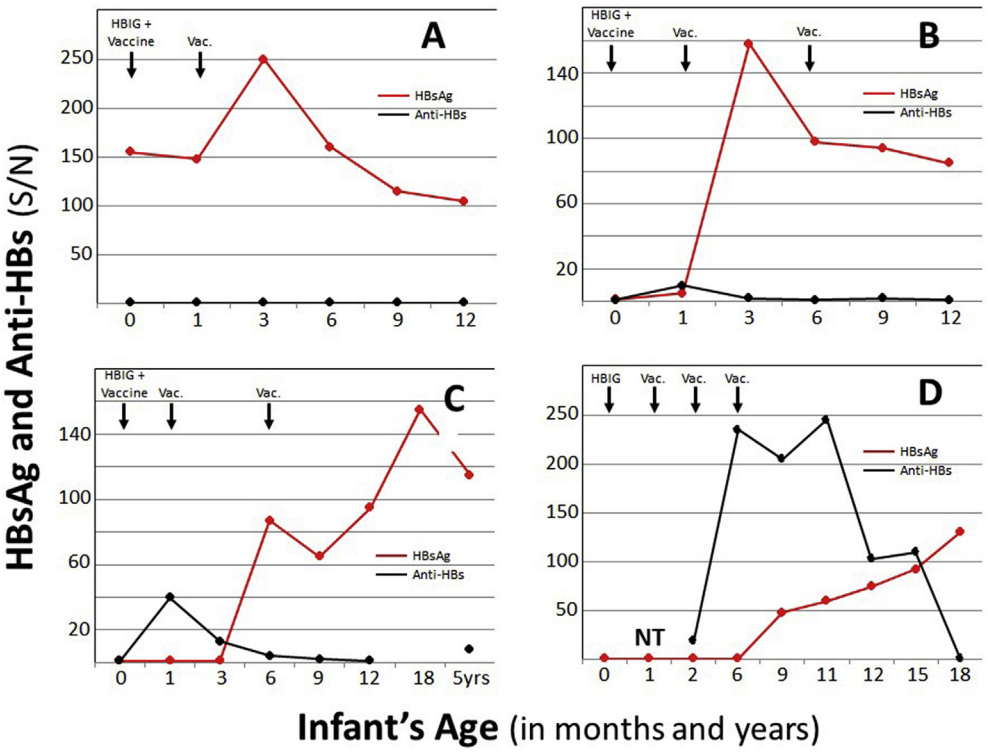

Fig. 4. Breakthrough chronic HBV infections in high-risk Infants from the U.S. HBIG/vaccine trials illustrating typical patterns of infection (anti-HBs levels and the onset of antigenemia). Panel A: An infant with a possible *in utero* infection (strongly HBsAg positive at birth and during subsequent follow up). No anti-HBs detected. Panel B: An infant with onset at one month (weakly HBsAg positive at one month with low level of anti-HBs and strongly HBsAg positive thereafter). Panel C: A late onset infection in an infant who failed to respond to HBV vaccine (i.e., waning anti-HBs level after one month of age with HBsAg first detected at six months). Panel D: An infant who had an active immune response to vaccine (rising ant-HBs levels) but, nevertheless, had a late onset infection with persistence of antibody. Based on data from Refs. [85–87].

Most chronic infections with onset after one month had low or waning antibody levels prior to the onset of infection suggesting that they were vaccine non-responders (Fig. 4C). Infants with onset of antigenemia at 2–4 months, for example, had a mean anti-HBs level at one month that was about half the level in infants who did not become infected (mean S/N = 33 and 65, respectively, $p = 0.004$). Seven of 11 infants who became antigenemic after six months were tested for anti-HBs at six months. Five (71%) had no detectable antibody or low levels (<20 S/N) at six months compared to 17.6% in uninfected infants ($p < 0.001$). Two infants had high antibody titers at six months and remained positive after they became antigenemic (Fig. 4D). These two infants are the only ones who had a definite antibody response to vaccine but, nevertheless, became chronic HBV carriers.

As reported in other studies, the incidence of chronic HBV infections was associated with maternal viral load during pregnancy (tested under code by Alfred M. Prince, New York Blood Center. 1 pg/ml = approximately 2.83×10^5 copies/ml and 5 copies = 1 International Unit) (Table 3 and Fig. 3B). Maternal viral load, however, was not associated with the time antigenemia was first detected in the infant. Mean HBV-DNA in mothers of carrier infants with onset of 0–1 month, 2–4 months and ≥6 months was 4756, 4651 and 4559 pg/ml, respectively ($p = 0.733$, Kruskal-Wallis test). This lack of association implicates infant rather than maternal factors determining the occurrence of late onset infections (e.g., an HBIG-induced prolonged incubation period coupled with the infant's failure to respond to the vaccine).

The incidence of chronic infection tended to be higher for *ay* subtype infections, not quite significant on multivariate analysis (Table 3). The excess in *ay* infections, however, was due almost entirely to cases with onset by one month of age - i.e., most likely already established *in utero* and, therefore, not relevant to vaccine efficacy (Fig. 3C). When these early infections were excluded, the incidence after one month was 5.2% for *ad* and 6.9% for *ay*, hazard ratio (HR) = 1.26 (95% CI = 0.56–2.80, $p = 0.577$ by multivariate Cox regression with variables included as shown in Table 3).

Unexpectedly, given the lower anti-HBs levels induced, recombinant vaccine recipients had a lower incidence of chronic infection than did recipients of plasma-derived vaccine, (Table 3 and Fig. 3D). This difference, however, was due primarily to infections with onset at six months or later. Before six months, the hazard ratio for recombinant vaccine recipients was 0.80 (95% CI = 0.49–1.38, $p = 0.420$ by multivariate Cox regression). In contrast, the HR for later infections for recombinant vaccine was 0.44 (95% CI = 0.23–0.86, $p = 0.015$).

Route of infant delivery and vaccination schedule were not associated with the incidence of chronic infection (Table 3). Time of HBIG administration also was not associated with the incidence, although there was a trend toward a higher incidence with a longer interval. When included in the multivariate analysis, year of entry into the studies had no independent association with incidence (HR = 1.06, 95% C.I. = 0.95–1.19, $p = 0.308$).

HBV-DNA level was measured in 90 of the chronic carrier infants (three had insufficient samples) and was associated with the time of onset of infection (testing by Omana Nainan, U.S. Centers for Disease Control and Prevention). Median HBV-DNA level was 8.97 $\log_{10}$ copies per ml (range = 4.36–10.40). Among infants with onset within one month of birth HBV-DNA level averaged one log higher than those with a later onset (Table 4). High HBV-DNA levels in these early onset infections might portend a higher risk of cirrhosis and HCC later in life and a higher risk of transmission to HBV-susceptible contacts, including future children of girls who

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remain infected.

In summary, our trials of HBIG and vaccine identify the occurrence of very early onset infections that were probably established *in utero* and are probably not amenable to immunoprophylaxis started at birth. Most infections with antigenemia first detected after one month, on the other hand, seem to reflect vaccine non-responsiveness. The recombinant vaccine was more efficacious in our studies than the plasma-derived vaccine, especially in preventing late onset infections. As noted above, the two vaccines differed in their source and manufacturing method and this may have affected their efficacy. The yeast recombinant vaccine, for example, had a single antigenic structure which may have induced an antibody response that was more focused thereby achieving a greater neutralizing potential than might be suggested by anti-HBs titer alone (William J. Rutter, personal communication). Vaccines that are more broadly immunogenic or produce a more rapid immune response might be better at preventing late- and even early-onset infections. Preliminary data on one such recombinant vaccine that includes pre-S1 and pre-S2 proteins in addition to the S protein (Sci-B-Vac™, licensed in Israel and several Asian countries) may fit this need and is currently being evaluated in high-risk infants [19,77].

8. Immunoprophylaxis "escape" mutants

A possible "vaccine-induced escape mutant," was first reported in 1990 in an Italian child born to an HBeAg/HBsAg positive mother (subtype *ayw*) [90]. The infant had been given HBIG at birth and one month and plasma-derived vaccine at 3, 4 and 9 months of age. When tested at 11 months she was positive for both anti-HBs and HBsAg. Only antigen persisted on subsequent follow up. DNA sequencing identified a glycine to arginine mutation at amino acid 145 of the surface antigen *a* determinant (designated sG145R). This case raised the possibility that immunologically selected mutants might undermine the potential for HBV eradication if they replace wild-type virus [18]. One publication predicted that it would take fifty years to detect mutant emergence at a level that would threaten control efforts [91].

There was evidence, however, that current vaccines would remain effective. In a chimpanzee challenge study, Ogata and colleagues at the U.S. National Institutes of Health (NIH) gave recombinant vaccine from Merck to two animals and a recombinant from SmithKline Beecham to two others (both monovalent subtype *adw*) [92]. The four immunized animals and two controls were then inoculated with dilutions of serum from the Italian infant who harbored predominantly the sG145R mutant along with wild-type virus. All four immunized animals were protected while both controls became infected. Pre-challenge samples from all four immunized animals found anti-HBs that was reactive against both wild-type and sG145R mutant virus. Another experimental study in chimpanzees further documented the infectivity and pathogenetic potential of pure sG145R mutant generated *in vitro* without admixture of the wild-type virus [93]. Several population surveys after implementation of universal immunoprophylaxis, however, have found this mutant and others [94–96]. Serial surveys in Taiwan, for example, found surface gene mutants, predominantly sG145R, with an increasing proportion of mutants among the chronic HBsAg carriers in first few surveys. The prevalence in more recent cohorts has plateaued, however, in association with the switch from plasma-derived to recombinant vaccine in the 1990s [97–100].

To explore the mutant virus issue, we tested sera from 92 of the infants in our trials who became HBV carriers (one infant not tested). Mutants were identified in the polymerase chain reaction (PCR) product corresponding to amino acids 86–179 of the surface antigen *a* determinant. Three methods were used: direct sequencing (DS), sequence-specific solid-phase PCR (SP-PCR) and amplification of single DNA molecules ("clones") from end-point dilutions (limiting dilution cloning-PCR, LDC-PCR) (samples tested under code by Omana Nainan, CDC) [101–103]. Direct sequencing detected sG145R when it constituted 25% or more in a mixture with wild-type HBV in a standardized concentration of total HBV-DNA. SP-PCR, on the other hand, detected this mutant in concentrations as low as 1% and LDC-PCR as low as a 0.1%. Thus, the combination of these assays provided a semi-quantitative measure of mutant concentration (sG145R detected by direct sequencing $\geq$25%, by SP-PCR but not by direct sequencing 1.0% to <25% and by LDC-PCR alone 0.1% to <1.0%).

A total of 78 mutations were found in samples from 43 infants (47% of infants tested), 26 detected by direct sequencing, 17 by SP-PCR but not by direct sequencing and 35 by LDC-PCR alone. Infants with mutants also had wild-type HBV. Twenty-three infants had more than one mutation. The most common mutation was sG145R, found in 31 infants (34% of the carrier infants tested). There were 15 mutations at amino acid 144 (aspartic acid to alanine, glutamic acid, or glycine) detected in 12 infants (13%), 8 of whom also had sG145R. There were 8 mutations at amino acid 126 (isoleucine or threonine to alanine, asparagine or serine) in 8 infants and 5 at amino acid 120 (proline to serine, alanine or threonine) in 4 infants. sG145R was the only mutant with a sufficient number to permit analyses by individual mutant.

Detection of mutant virus was more frequent when the onset of infection was $\geq$6 months, an association that was most apparent for the sG145R mutation (Table 5). As noted above, most infants with late infections were probably vaccine non-responders (Fig. 4C). Thus, mutant selection in these cases was most likely by the passive antibody given in HBIG rather than by vaccine-induced antibody. One of the two vaccine responders who became chronically infected (subtype *ay*) had several mutations, predominantly sG145R. The other had only wild-type virus (subtype *ad*). Twelve infants with onset of infection within one month of birth also had mutant virus detected, nine with sG145R. If these infections were truly established *in utero*, as we suspect, they would be naturally occurring rather than reflecting "escape" from either HBIG or vaccine-induced antibody.

Infections in infants with mutants, on average, had lower HBV-DNA levels than did infections with only wild-type virus, mean 8.03 and 8.94 $\log_{10}$ copies/ml, respectively ($p < 0.001$). This association was only significant for late onset infections with sG145R (Table 5). Moreover, eleven of 19 late onset infections with sG145R (58%) were detected by direct sequencing and, therefore, constituted at least 25% of virus present. Only two were detected by LDC-PCR alone. In contrast, sG145R in all nine early onset infections was detected only by LDC-PCR ($p < 0.001$) and would probably have gone undetected by less sensitive assays.

To assess vaccine efficacy against mutant virus, we used three endpoints based on mutant presence: infections with only wild-type, infections with other mutants but not sG145R and infections with sG145R. In estimating the incidence of sG145R ($n = 31$), all other infections were censored at their time of onset. For infections with other mutants but not sG145R ($n = 12$) and those with only wild-type ($n = 49$), the other infections were similarly censored. As with all infections combined, the incidence of both sG145R and non-sG145R infections were associated with maternal viral load and HBV subtype (data not shown). The incidence of non-sG145R infections was not related to vaccine given (HR for wild type and other mutants combined = 0.97, 95% CI = 0.56–1.71, $p = 0.926$) (Fig. 5A and B). In contrast, the incidence of sG145R infections (Fig. 5C) was significantly lower in recombinant vaccine recipients than in those given plasma-derived vaccine

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Table 3
Analysis of Incidence of chronic hepatitis B virus (HBV) infection in U.S. HBIG/Vaccine trial infants during 18 months of follow-up by factors potentially related to immunoprophylaxis failure. Hazard ratios and p values estimated by univariate and multivariate Cox regression. The multivariate analysis included only variables with p values < 0.100 in the univariate analysis.

Group	No.in the study	No. with chronic HBV	Incidence % (95% C.I.)	Univariate hazard ratio (95% C.I.)	p value	Multivariate hazard ratio (95% C.I.)	p value
Maternal HBV-DNA Level (picograms/ml. 1.0 pg/ml = approximately 2.83 × 105 copies/ml):							
<500	289	3	1.0% (0.0–2.2)	1.00 (Reference)		1.00 (Reference)	
500–2499	253	21	8.5% (4.9–12.1)	8.27 (2.47–27.7)	0. 001	7.86 (2.34–26.4)	0.001
2500–4999	242	29	12.2% (8.0–16.4)	12.20 (3.72–40.0)	<0. 001	11.63 (3.54–38.2)	<0.001
≥5000	212	29	13.8% (9.0–18.6)	13.99 (4.26–45.9)	<0.001	13.15 (4.00–43.2)	<0.001
Not Tested	72	11	15.3% (6.9–23.7)	16.16 (4.51–57.9)	<0.001	14.64 (4.07–52.7)	<0.001
Maternal HBsAg Subtype:							
ad	947	77	8.3% (6.5–10.1)	1.00 (Reference)		1.00 (Reference)	
ay	111	16	14.4% (7.8–21.0)	1.85 (1.08–3.16)	0.026	1.62 (0.90–2.82)	0.087
Not Tested	10	0	0.0% (N.A.)	0.00 (0.00-»>)	0.966	0.00 (0.00-»>)	0.966
Mother's Ethnicity:							
Chinese	701	60	8.7% (6.5–10.9)	1.00 (Reference)			
Southeast Asia	257	25	9.8% (6.0–13.6)	1.14 (0.71–1.81)	0.594		
Other/Unknown	110	8	6.3% (1.3–11.3)	0.84 (0.40–1.76)	0.642		
Study City:							
New York	563	49	8.8% (6.4–11.2)	1.00 (Reference)			
San Francisco	349	31	9.0% (6.0–12.0)	1.00 (0.64–1.57)	0.997		
Los Angeles	156	13	8.3% (3.9–12.7)	0.93 (0.51–1.72)	0.819		
Infant's Route of Delivery:							
Vaginal	926	85	9.3% (7.3–11.3)	1.00 (Reference)			
C-Section	128	7	5.6% (1.4–9.8)	0.58 (0.27–1.25)	0.164		
Not Recorded	14	1	7.1% (0.0–20.9)	0.80 (0.11–5.76)	0.827		
Time (after birth) HBIG Administered:							
0-5 h	622	45	7.3% (5.1–9.5)	1.00 (Reference)		1.00 (Reference)	
6-11 h	177	16	9.1% (4.7–13.5)	1.27 (0.72–2.25)	0.408	1.16 (0.66-2.07)	0.605
≥12 h	133	15	11.4% (5.8–17.0)	1.57 (0.87–2.81)	0.132	1.25 (0.69–2.27)	0.459
Not Recorded	136	17	13.0% (7.0–19.0)	1.81 (1.04–3.16)	0.038	1.90 (1.07–3.38)	0.028
Vaccine:							
Plasma-derived	333	41	12.3% (8.7–15.9)	1.00 (Reference)		1.00 (Reference)	
Recombinant	735	52	7.3% (5.3–9.3)	0.57 (0.38–0.86)	0.007	0.60 (0.39–0.93)	0.022
Dose of Plasma-derived Vaccine (in micrograms):							
20	169	24	14.2% (8.8–19.6)	1.00 (Reference)			
10	164	17	10.4% (5.6–15.2)	0.71 (0.38–1.32)	0.276		
Dose of Recombinant Vaccine (in micrograms):							
5	607	44	7.4% (5.2–9.6)	1.00 (Reference)			
2.5	128	8	7.1% (2.1–12.1)	0.90 (0.42–1.91)	0.776		
Vaccine Schedule (infant's age in months for first two doses):							
0 and 1	674	56	8.4% (6.2–10.6)	1.00 (Reference)			
0 and 2	161	10	6.9% (2.5–11.3)	0.77 (0.39–1.51)	0.446		
1 and 2	233	27	11.6% (7.4–15.8)	1.40 (0.89–2.22	0.149		

P = plasma-derived vaccine (Heptavax B™).
R = yeast recombinant vaccine (Recombivax-HB™).
C.I. = Confidence Interval.

(HR = 0.22, 95% CI = 0.10–0.48, p < 0.001 by multivariate Cox regression as in Table 3).

We also looked for mutations among study mothers in a case-control study. Although we had tested these women for HBsAg in the 1980s and 1990s, their HBV infections were probably acquired in childhood in Asia during the 1960s and 1970s when HBIG and hepatitis B vaccines were not available. Thus, HBsAg mutants found in these women should represent their occurrence in nature without immune selection by HBIG or HBV vaccine. Case mothers were those whose infants became chronic HBV carriers. Controls were selected (2:1) from among mothers whose infants did not become carriers, matched to cases by the vaccine given to the infant (56.5% and 55.4%, respectively, received recombinant vaccine), the mother's HBV subtype (16.3% and 17.4%, respectively, were *ay*) and maternal HBV-DNA level (mean of 4347 and 4537 pg/ml, respectively). Because of our matching criteria, control mothers were not representative of women who were not selected for this study or of HBeAg/HBsAg positive Asian women in general.

Sequencing of the S-gene of HBV-DNA for this study allowed us to assign HBV genotype to these case-control mothers. As expected for an Asian population, genotypes B (48.6%) and C (44.6%) predominated. Most infections with *ay* subtype were found in HBV genotype B (89.1%) and most of these were from Vietnam, Cambodia or Laos [104,105]. Genotype prevalence was not significantly different between case and control mothers (43.6% vs. 49.2% were genotype C, respectively, p = 0.639), suggesting no relationship between genotype and vaccine efficacy. Because our case and control mothers were matched by HBV-DNA level, this finding is not incompatible with the increased prevalence of HBV genotype C observed in Taiwan among vaccine recipients with breakthrough perinatal infections thought to be explained by the higher HBV-DNA levels in genotype C infections [106].

Among the 276 mothers tested, 115 mutations were detected in 88 women (31%). The prevalence of HBV mutant virus did not differ between case and control mothers (in 34.8% and 30.4%, respectively, p = 0.495). sG145R, found in 25 mothers, was their most common mutation. Four different mutations were detected at amino acid 126 in 32 mothers, most commonly isoleucine to serine (16) and isoleucine or threonine to asparagine (14). Ten mothers had mutations at amino acid 120, five each from proline to serine or

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Table 4
HBV-DNA level (log_{10} of copies/ml) in hepatitis B virus (HBV) carrier infants from the U.S. HBIG/vaccine trials and the relationship to the infant's age at onset of HBsAg antigenemia. Three carrier infants were not tested.

Infant Age at Onset (months)	No. in Study	HBV-DNA (log_{10} copies/ml) in HBV Carrier Infants						
		4.0–7.9		8.0–8.9		≥9.0		Mean
		No.	%	No.	%	No.	%	Log_{10}.
0–1	40	2	5.0%	8	20.0%	30	75.0%	9.0[a]
2–4	16	4	25.0%	7	43.8%	5	31.3%	8.1 [b]
≥6	34	15	44.1%	10	29.4%	9	26.5%	8.2[c]

p values for means.
a vs. b: p = 0.056.
a vs. c: p = 0.001.
b vs. c: p = 0.861.

Table 5
HBV-DNA mutants in the HBsAg "*a*" determinant among infants with chronic HBV infection from the U.S. HBIG/vaccine trials: Relationship of infant's HBV-DNA level (log_{10} copies/ml) and age at onset of antigenemia. HBV-DNA level was not determined in three infants (two in the sG145R group).

Infant Age at Onset (months)	sG145R		Other Mutant (not sG145R)		Wild-type	
	No. (%)	Mean Log_{10} HBV-DNA	No. (%)	Mean Log_{10} HBV-DNA	No. (%)	Mean Log_{10} HBV-DNA
0–1	9 (31.0%)	9.2[a]	3 (25.0%)	7.9	28 (58.3%)	9.1[c]
2–4	2 (6.9%)	6.4	3 (25.0%)	6.1	11 (22.4%)	8.9
≥6	18 (62.1%)	7.8 [b]	6 (50.0%)	8.5	10 (20.4%)	8.6 [d]

p values for means.
a vs. b: p < 0.001.
c vs. d: p = 0.284.
b vs. d: p = 0.098.
sG145R = mutant HBV with glycine to arginine mutation at HBsAg amino acid 145.

threonine. Only two had an amino acid 144 mutation (both aspartic acid to glycine).

sG145R was the only mutant found in sufficient numbers in both mothers and infants to permit further analyses related to transmission and immune selection. sG145R was found in both mother and infant of nine mother-infant pairs. The onset of antigenemia was at six months or later in all nine infants and sG145R mutant was detected by direct sequencing (≥25% of virus present) in eight infants. In contrast, sG145R was a very minor component (<1%) in seven of the mothers and was not detected by direct sequencing in any of them (p < 0.001). The differences in sG145R concentration between infants and their respective mothers may reflect more efficient neutralization of wild-type virus, leaving a higher residual proportion of mutant virus to infect infant hepatocytes and replicate.

The case-control study also provided evidence of the efficacy of HBIG and vaccine against sG145R. sG145R was found in 12 control mothers (two by direct sequencing) whose infants, by definition, did not become infected with either wild-type or mutant virus. Case and control mothers who had sG145R did not differ in their HBV-DNA level (mean = 4558 and 4643 pg/ml, respectively, p = 0.964). Maternal viral load, therefore, did not explain why control mothers did not transmit HBV to their infants. However, all 12 of these uninfected infants had a persistent or rising anti-HBs level at 6–9 months. More likely, therefore, active immune response to vaccination was protective in these infants against both wild type and mutant virus. Similar failures to transmit the sG145R mutant were recently reported from Japan [107].

In summary, most chronic HBV infections with sG145R among infants were present either prior to immunoprophylaxis (the early onset cases) or reflected the infant's failure to develop an active antibody response to the vaccine. Immune selection was unlikely in the first instance or was by HBIG rather vaccine-induced antibody in the later. Most infants who had an active immune response to vaccine were protected from infection by both wild-type and sG145R mutant. Taken together, this suggests that the better apparent efficacy of the yeast-recombinant vaccine against sG145R mutant infections may be due simply to better prevention of late onset infections. Our findings may help explain two observations in the Taiwan surveillance studies (see section 12): a lower viral load in breakthrough infections having mutant virus and a plateau in mutant prevalence among HBsAg carriers since introduction of yeast-recombinant vaccine [98–100].

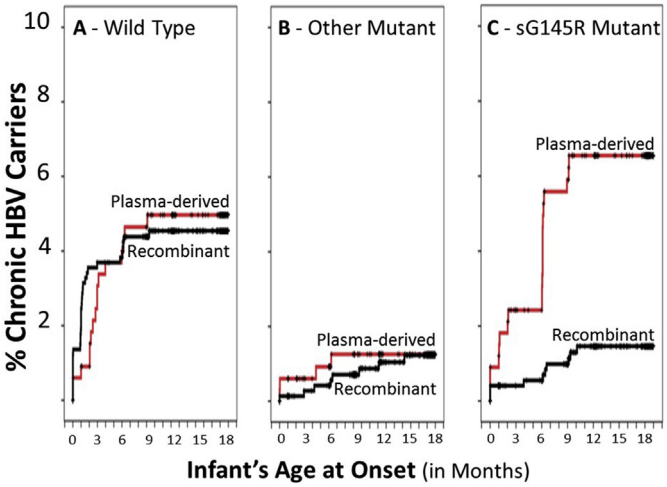

Fig. 5. Comparison of plasma-derived and yeast-recombinant HBV vaccine efficacy (U.S. HBIG/vaccine trials) against chronic HBV infections with only wild-type virus (panel A), with mutant virus but not sG145R (panel B) and with sG145R mutant (glycine to arginine substitution at surface antigen amino acid 145) (panel C). The incidence of sG145R infections as significantly lower among yeast recombinant vaccine recipients compared to plasma-derived vaccine (hazard ratio = 0.22, p < 0.001).

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9. Role of anti-viral drugs

Anti-viral drugs are now commonly used to treat chronic HBV infections with high viral loads to reduce the risk of progression to cirrhosis, liver failure and HCC [108]. Most currently available anti-viral drugs target steps in HBV replication but have no effect on intra-nuclear covalently closed circular DNA (HBV-cccDNA) and, thus, do not clear this viral intermediate [78,109]. HBV clearance with current drugs is, therefore, rare. New methods such as CRISPR/Cas genome editing that could attack HBV-cccDNA may prove effective in this regard and, if appropriate for otherwise healthy young women, might be considered as a way to clear infection before pregnancy and thereby prevent perinatal HBV infections.

Temporary use of anti-viral drugs during the third trimester and early weeks post-partum is being evaluated to achieve a transient decrease in maternal HBV viral load and prevent residual HBV infections in high-risk infants that occur despite immunoprophylaxis. Most studies to date have used tenofovir disoproxil fumarate because of extensive experience with this drug in preventing mother-to-child transmission of human immunodeficiency virus (HIV) and its apparent safety for the fetus [110–114]. One controlled, open-label but not randomized study among HBeAg/HBsAg positive (mean baseline HBV-DNA = 8.2 $\log_{10}$ IU/mL) pregnant women in Taiwan, for example, found an average four $\log_{10}$ drop in maternal HBV-DNA level while the women were on tenofovir [113]. All infants were given HBIG and a yeast-recombinant vaccine starting at birth. Only 1.5% of 65 infants in the tenofovir group had become chronic carriers by six months compared to 10.7% in controls ($p = 0.048$). Significance was lost at 12 months, however, when one more infection was found in the tenofovir group ($p = 0.142$). More recently, Pan and colleagues reported on a multi-center controlled, randomized open-label trial in China of tenofovir in high-risk pregnancies (HBeAg/HBsAg positive mothers with mean baseline viral load = 8.0–8.2 $\log_{10}$ IU/mL) [114]. Mothers were given tenofovir starting at 30–32 weeks gestation through four weeks post-partum. Study infants received HBIG at birth and one month and recombinant HBV vaccine at birth, one and six months and were followed to seven months of age. None of 92 infants born to tenofovir-treated mothers who completed treatment and follow up became chronic carriers compared to 6.8% (6/88) of control infants ($p = 0.01$).

These anti-viral trial results are most encouraging but questions remain. *What about prevention of late onset infections?* In our HBIG/vaccine trials maternal viral load was equally important as a risk factor for both early and late onset infections. Thus, we might expect that a reduced viral load during pregnancy and shortly thereafter would reduce the risk of both early and late onset infections as seen with HBIG/vaccine prophylaxis. But, follow up to at least 12 months, and ideally beyond, is needed to document efficacy against late onset infections as the Taiwanese tenofovir trial demonstrates [113]. *Do anti-viral drugs given to the mother during pregnancy affect the infant's immune response to HBV vaccines?* Infants who do not respond to vaccine remain susceptible and can acquire HBV from their mothers later in life [55]. Thus, mothers whose high viral loads rebound after anti-viral treatment stops could again be a source of HBV for their susceptible offspring. Little information is available to date on anti-HBs levels in infants from anti-viral trials to address this issue. One recent publication from France on tenofovir with HBIG and vaccine prophylaxis reported a "vaccine-type response" among infants tested at 24 months but the number of infants was small and no data was given [115]. *What kind of HBV mutants "escape" both anti-viral treatment and passive-active immunoprophylaxis?* Larger studies of longer duration will be needed to define the circumstances of such treatment failures. The ongoing CDC-sponsored randomized, double-blind, placebo-controlled trial in Thailand with infant follow up to 12 months should provide some answers to the above questions [116]. *Finally, how effective would anti-viral treatment of mothers be if their infants are given only HBV-vaccine without HBIG?* An answer to this question would be especially useful for areas where HBIG is not affordable or getting prophylaxis to infants at birth remains difficult. Screening pregnant women would still be required, however, as for any targeted therapy.

10. Duration of vaccine-induced immunity and long-term need for boosters

Follow up of immunized adults, children and newborns shows that anti-HBs titers wane with time and often drop below peak levels defined as a protective following primary immunization ($\geq$10 mIU/ml) suggesting that such people may be susceptible again to HBV. There is evidence, however, that immune memory generally persists in dormant memory B lymphocytes previously sensitized to HBsAg and capable of a rapid anamnestic response. Protective levels of antibody usually reappear within days, of a single booster dose of vaccine [117–121]. Indeed, adults immunized as children who have low but detectable anti-HBs (<10 mIU/ml) generally have anamnestic responses with a single booster dose [119,121]. In one study in Taiwan, however, only 20% of students who had had no detectable anti-HBs when they were 18–23 years old (even though records of documented neonatal vaccination) responded to a single booster dose [122]. After three doses 99% achieved a level $\geq$10 mIU/ml, a pattern resembling primary immunization responses. Rather than losing memory, perhaps these students had a problem with their initial immunization series.

Importantly, acute symptomatic hepatitis B and chronic infections are rare in people who have been fully vaccinated as neonates even though asymptomatic infections (evidenced by anti-HBc positivity) do occur in vaccine recipients [123–125]. Thus, the CDC and WHO do not currently recommend routine boosters [126,127]. Nevertheless, special groups may need boosters or even full revaccination, especially those who were likely to have had a poor response to their initial series such as infants born prematurely, patients on hemodialysis, immunocompromised persons (including those who are HIV-infected) and people with an ongoing risk (i.e., health care personnel and sex or needle-sharing partners of HBsAg-positive persons) [122,126–130]. Some authors also propose use of a booster dose for young adults in endemic areas as they enter years when they may acquire new risks such as exposure through sexual activity [122].

11. Status of US and global HBV control

After the first HBV vaccines were licensed in 1982, the CDC recommended vaccination for people at high risk, in addition to the standard infection-control measures already in place [131]. In 1992, the WHO recommended that all countries develop hepatitis B control programs and added hepatitis B vaccine to their Expanded Program on Immunization (EPI) for universal childhood vaccination [132]. Further recommendations from the CDC and the WHO followed. Recommendations in the U.S., for example, increased to vaccine coverage of all infants in 1994, all children 18 years old or younger in 2000 and all newborns in 2007 (for current recommendations see: https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm# and http://www.uptodate.com/contents/hepatitis-b-virus-vaccination).

In the United States, as vaccine strategies evolved, the incidence of acute hepatitis B declined dramatically from a peak of 26,116 reported cases in 1985 to around 3000 in 2010–2015 (Fig. 6) [133,134]. This decline is attributable, in part, to

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immunoprophylaxis but also to improved infection control in health care settings and changes in risk behaviors, a side benefit of parallel efforts to control the epidemic of HIV. Recently, however, outbreaks of acute hepatitis B have been recognized in the United States in association with the new epidemic of injection drug use and with assisted living, outpatient surgical and medical procedures (especially those related to diabetic care) reflecting a breakdown in traditional infection control measures as well as the failure of vaccine programs to reach people with newly acquired risks [135]. In fact, many people with acute hepatitis B deny having any recognized risk factors. In 2015, for example, 52% of cases with risk factor information reported no risk [134]. Such cases may be difficult to prevent unless vaccine coverage is truly universal.

In the United States, the National Health and Nutrition Examination Survey (NHANES) found a stable HBsAg prevalence of 0.3–0.4% in periodic surveys since 1988, despite broad application of universal vaccination in infants and older children [136]. This was due, in large part, to immigration of HBV carriers from endemic areas. HBsAg prevalence was 3.1% among Asian-Americans, for example, 93% of whom were foreign-born, compared with only 0.10% for non-Hispanic whites. NHANES also demonstrated substantial progress among 6 to 19-year-olds whose prevalence declined from 0.2% in 1988–1994 to only 0.03% in 2007–2012, below the 2020 WHO goal.

Worldwide, HBsAg prevalence has declined modestly (estimated at 3.6% in 2010) but, consequent to population growth, the total number of people infected has increased to a current estimate of about 250 million [29]. Sub-Saharan Africa, where some countries still do not have effective HBV-control programs, continues to have the highest prevalence (9.1% overall), followed by Asia (5.3%) and the Middle East (3.0%) [137].

12. The Taiwan model

While HBsAg prevalence remains high in many parts of the world, Taiwan has had a highly effective universal newborn immunization program since July 1984 and has already met WHO 2020 goals. Taiwan elected to begin with a targeted immunization strategy focused on infants born to HBsAg positive women, providing them with HBIG and vaccine starting at birth. In mid-1986, HBV vaccination became universal for all newborns and, recognizing the ongoing risk during childhood, included a temporary catch-up phase for all pre-school and older children.

Serial surveys of HBV-marker prevalence every five years have documented the program's impact and provided a model to the world on “how to do it” [96–100,138–140]. Each successive 5-year cohort has shown a persistently low HBsAg prevalence among those born after the national HBV control program was implemented (Fig. 7). In 2014, only 0.2% of children <9 years-old were HBsAg positive compared to 6.7% of adults 30 and older (born before the vaccine era). Most chronic HBV infections in the vaccine cohort were apparently breakthrough infections acquired perinatally despite immunoprophylaxis [106]. The most recent survey identified 17 carriers who had had been fully vaccinated in infancy: 12 had family history information and 10 of these (83%) had an HBsAg-positive mother [140]. Taiwanese scientists have also documented a dramatic decline in the incidence of HBV-related diseases among children, including HCC, definitive evidence that the vaccine could prevent HCC [141–144].

Thirty years into Taiwan's national immunization program, there has not been any apparent loss in vaccine efficacy attributable to emergence of escape mutants. As noted above, HBV mutants (predominantly sG145R) have been found in vaccine recipients in Taiwan but is no longer increasing in prevalence. The question relevant now to eradication is whether the next generation of infants born to vaccinated women who had breakthrough mutant infections can mount effective immune responses to vaccine and will be protected. In the coming decade, most babies born in Taiwan will have parents who were born in the vaccine era, providing a possible opportunity to answer this question. We predict that they will be protected, based on data from our U.S. trials, and that mutant virus will not replace the wild-type. Thus, current vaccines should remain effective and, with a low HBsAg prevalence in childbearing women in the vaccinated cohort, the new generation of infants in Taiwan should easily meet the WHO 2030 goal.

13. Conclusions

Transmission from mother to infant *in utero* or perinatally still accounts for a major portion of chronic HBV infections in the world and produces a highly infectious source of virus for others, especially for other children. Prevention of these infections is the key to controlling HBV, meeting WHO 2030 goal (an HBsAg prevalence in children of no more than 0.1%) and finally eradicating the virus. With the new WHO and National Academies of Sciences, Engineering and Medicine/CDC plans, the will is building and, if implemented, should move the world substantially closer to eradication [23–26,145]. Unfortunately, 90% birth dose coverage with vaccine is not by itself sufficient. In countries with a 10% prevalence of HBsAg, for example, and one-third HBeAg positivity in HBsAg positive childbearing women, at least 2.8% of the next generation would be chronically infected from perinatal transmission alone with no intervention in place (assumes 85% of infants become chronically infected). Vaccine alone started at or within a week of birth, with 70–80% efficacy and 90% coverage, predicts a residual carrier rate in children of 0.8–1.0% due to perinatal transmission. Even a near ideal immunization program like Taiwan's has taken more than a generation to get close to the 2030 goal. Meeting the WHO goals over the next decade, therefore, will require an even more efficacious approach that identifies HBsAg positive pregnant women and provides optimum prophylaxis to their newborn children (vaccine with HBIG or with maternal anti-

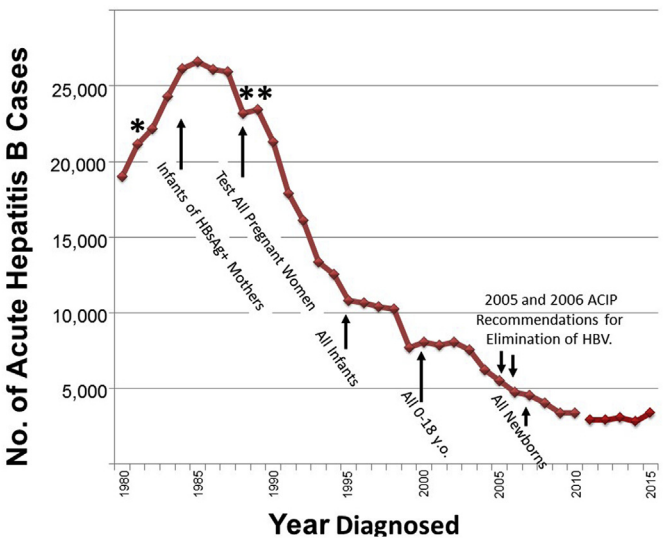

Fig. 6. Annual incidence of acute hepatitis B reported to the Centers for Disease Control and Prevention (CDC) in the United States from 1980 to 2015. * indicates when plasma-derived vaccine was licensed in the U.S. and ** indicates licensure of yeast-recombinant vaccine. The arrows indicate when the CDC Advisory Committee on Immunization Practices (ACIP) added major new recommendations for immunoprophylaxis against HBV. Based on data from Refs. [133,134].

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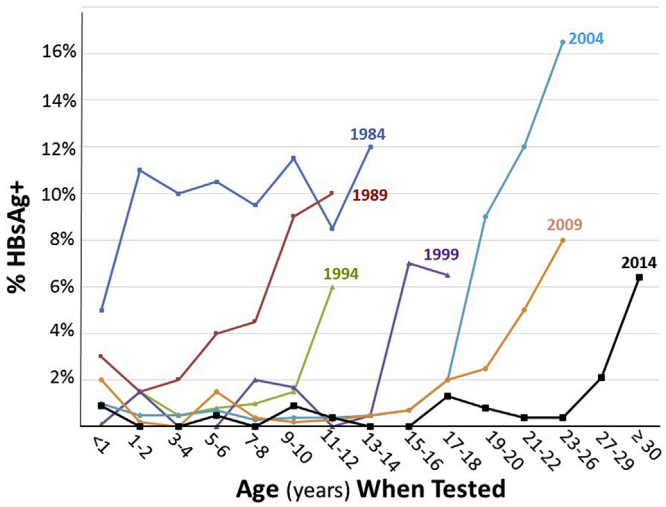

Fig. 7. HBsAg surveillance studies carried out in Taiwan every five years since 1984 by year of survey and participant age at time of testing. The immunization program started in mid-1984 with hepatitis B immune globulin and vaccine for infants of HBsAg positive mothers and vaccine for all infants (with catch-up immunization of older children) starting in July 1986. The 1984 survey was carried out prior to implementation of universal immunization against HBV. The higher HBsAg prevalence among older individuals in later surveys reflects HBV infections in those born before the immunization program began and who, therefore, were unprotected at birth and throughout childhood. The low prevalence in younger age groups shows the impact of Taiwan's universal HBV immunoprophylaxis program. Thus, each successive survey reflects aging of the vaccinated cohort. Based on data from Ref. [140].

viral drug treatment or all three). Moreover, such strategies need to be extended to the poorest endemic areas. Nothing less can ensure that WHO goals will be accomplished the worldwide on time and accelerate the effort to eradicate HBV.

Co-author roles

CES lead the original multi-center US studies of HBIG and vaccine against perinatal transmission of HBV in collaboration with PET. PT and GNV were collaborators at UCSF and MJT was the collaborator in Los Angeles. SK's laboratory collaborated on HBV mutant studies. GLX carried out genotype assignments. This review article was written primarily by CES with the help of each co-author.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Funding

The US HBIG/vaccine studies were funded by Merck and Company. Merck played no role in the conduct, analyses, publications or this manuscript. Costs of HBsAg mutant studies were covered by the US Centers for Disease Control and Prevention under the US Department of Health and Human Services.

Conflicts of interest

The authors declare no conflict of interest. Girish N. Vyas is the Chief Executive Officer of Thera Biol, Inc (www.therabiol.com), a company devoted to research and development of immune prophylaxis against infectious diseases.

Acknowledgements

Several colleagues were consulted on a presentation of much of the material in this paper given in 2015 at University of San Francisco General Hospital Grand Rounds. We gratefully acknowledge their input: Harvey J. Alter, MD, Mei-Hwei Chang, MD, Jules Dienstag, MD, F. Blaine Hollinger, MD, Lu-Yu Hwang, MD, MPH, Ronald J. Prineas, MD, PhD, William J. Rutter, PhD and John Ward, MD, MPH. John Ward also reviewed and commented on this paper.

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