CLINICAL—LIVER

Increased Seroprevalence of HBV DNA With Mutations in the S Gene Among Individuals Greater Than 18 Years Old After Complete Vaccination

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BACKGROUND & AIMS: Despite the success of a universal vaccination program against hepatitis B virus (HBV) in Taiwan, a small but substantial proportion of individuals remain infected by mutant viruses that escape the vaccine. We investigated the seroepidemiology and genotypic characteristic of HBV for long periods after neonatal vaccination. **METHODS:** We measured hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), and antibody to hepatitis B surface antigen (anti-HBs) in 1214 serum samples collected throughout Taiwan from individuals 0.6–87.8 years old in 2007. HBV DNA was detected using polymerase chain reaction and sequence analysis in vaccine recipients who tested positive for anti-HBc and/or HBsAg. **RESULTS:** The overall seroprevalence of HBsAg and anti-HBc was significantly lower among individuals born after the initiation of the nationwide vaccination program ($P < .001$). However, we observed increasing seroprevalence of anti-HBc and isolated anti-HBs when subjects were grouped by age: at 10–14, 14–18, to 18–21 years of age, values were 0.4%, 1.9%, and 8.1% ($P = .0135$) and 43.7%, 55.4%, and 59.6% ($P = .0093$), respectively (χ^2 test for trend). A large increase was observed in the percentage of patients who tested positive for HBV DNA at 18–21 years of age (3.0% vs 0.2% [$P = .002$] for all eligible subjects and 5.7% vs 0.3% [$P < .001$] for subjects vaccinated with ≥3 doses). Five of 8 completely vaccinated individuals who were seropositive for HBV DNA carried variants with mutations in the S gene. **CONCLUSIONS: Universal vaccination effectively controls HBV infection in children and adolescents. However, after adolescence, there is a significant increase in the seroprevalence of anti-HBs, anti-HBc, and HBV DNA, indicating that new preventative strategies are needed for adults.**

Keywords: Immunization; Viremia; Resistance Mechanisms; Immune Escape.

Chronic hepatitis B virus (HBV) infection continues to be a major health burden, with more than 350 million chronically infected patients worldwide. Long-term infection leads to severe sequelae such as decompensated cirrhosis and hepatocellular carcinoma in approximately 15%–40% of patients with hepatitis B.[1,2] Epidemiological studies have shown that the age of primary infection is the most important determinant of chronicity in HBV infection.[3] Vertical and perinatal transmission from carrier mothers to unimmunized neonates and horizontal transmission from siblings or caregivers in early childhood are the major routes of infection in hyperendemic regions such as Asia and Africa.[4–6] Therefore, interruption of viral transmission in early life through effective immunization programs is the most cost-effective strategy to prevent primary infection and the subsequent sequelae. Plasma-derived hepatitis B vaccine was approved and became commercially available in 1982, and yeast-derived recombinant vaccine followed in 1986. Taiwan was the first nation to adopt an universal hepatitis B vaccination program in the world, initially to newborns of carrier mothers in July 1984 and then to all newborns since July 1986.[7] According to several seroepidemiological studies, the carrier rate has been effectively reduced from more than 10% to less than 1% to 2% in the postimmunization era.[8–11] Even more impressive is the significantly decreased incidence of hepatocellular carcinoma and fulminant hepatitis in the vaccinees.[12–14] To eliminate HBV infection, the World Health Organization has recommended HBV vaccination to all nations since 1992, and 177 nations have implemented a universal immunization program. The 3-dose coverage rate was approximately 70% globally in 2009.[15]

Despite the success of the HBV vaccination program, breakthrough infections do occur infrequently in vaccines, as reported in Gambia, Alaska, and Taiwan.[16–18] High maternal HBV DNA level, intrauterine infection, emergence of escape mutants, and vaccine hyporesponsiveness are the contributing causes.[19,20] Most of the breakthrough infections are attributed to wild-type HBV, but mutants in the antigenic "*a*" determinant of hepatitis B surface antigen (HBsAg)

Abbreviations used in this paper: CI, confidence interval; COI, cutoff index; nt, nucleotide; PCR, polymerase chain reaction.

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are found in approximately 20% of infected victims.[21–23] The "vaccine- or immune-escape mutant" was first reported in an infant receiving human hepatitis B immunoglobulin and 3 doses of plasma-derived vaccine. The patient acquired an sG145R mutant and presented with serious hepatitis. The same immune-escape mutant was discovered in a liver transplant recipient treated with monoclonal antibody to hepatitis B surface antigen (anti-HBs) for prophylaxis of hepatitis B relapse.[24,25] Similar surface antigen variants were reported in many different geographic areas, raising concern about the transmissibility of these mutants through close intrafamilial contact or blood donation. Some of these mutants were able to evade vaccine-stimulated immunity and avoid detection by commercial HBsAg assays.[26–29] A mathematical model has predicted that the prevalence of these surface mutants possibly took 5 decades over time after universal vaccination.[30] However, the prevalence of escape mutants, after a significant increase in the postimmunization era, remains in stable proportion in quinquennial epidemiological surveys over the past 2 decades in Taiwan.[31] In infected patients, the escape mutant sG145R or others could become the dominant viruses and remain stable over several years.[24,26] Reversion to the wild-type or complete disappearance of the mutants has also been observed.[31] Continuous monitoring of breakthrough infections as well as surveillance of the escape mutants is obligatory for clinicians, especially when the vaccinees have entered their adulthood with an increased possibility of transmission through childbearing, percutaneous exposures, and sexual behaviors. Here, we made use of samples from a large-scale survey for the epidemiology of vaccine-preventable diseases in Taiwan to investigate the seroepidemiology of HBV infection 23 years after the mass immunization program was initiated. We found alarming evidence indicating an increased prevalence of HBV infection by HBsAg mutants in individuals older than 18 years after neonatal vaccination.

Subjects and Methods

Universal Vaccination Program

The universal HBV vaccination program was initiated in Taiwan on July 1, 1984. The vaccination was given to newborns of HBsAg-positive mothers from July 1984 to June 1986. The program was then expanded to all newborns in July 1986. Healthy newborns of highly infectious (hepatitis B e antigen [HBeAg]-positive or high titer of HBsAg) carrier mothers received an additional 0.5 mL of hepatitis B immunoglobulin within 24 hours of birth. The vaccination was introduced to preschool children and susceptible medical personnel from 1987 to 1989 and further extended to cover all elementary school children from 1988 to 1990. The vaccination records of the first graders have been checked at school entrance since 1991, and those who missed any dose were given a catch-up HBV vaccination. Before November 1992, 4 doses of plasma-derived vaccine were applied at 0, 1, 2, and 12 months of age. After November 1992, 3 doses of recombinant vaccine were administered at 0, 1, and 6 months of age.

Serum Samples

A total of 1214 serum samples from various age groups (mean, 12.84 ± 10.84 years [range, 0.58–87.75 years]; 585 male and 629 female subjects) were available from a concurrent epidemiology study for vaccine-preventable diseases in Taiwan in 2007. The subjects were recruited from the northern, central, southern, and eastern parts of Taiwan. The study project was approved by the institutional review board of Chang Gung Memorial Hospital.

Serology Tests

Hepatitis B seromarkers (HBsAg, anti-HBs, and antibodies against hepatitis B core antigen [anti-HBc]) were checked by electrochemiluminescent immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). According to the manufacturer, HBsAg was considered positive if the cutoff index (COI) was >10, equivocal if the COI was between 1 and 10, and negative if the COI was <1; anti-HBs was considered positive if >10 IU/L and negative if <10 IU/L; and anti-HBc was considered positive if the COI was <1 and negative if the COI was >1.

DNA Extraction and Polymerase Chain Reaction

Nucleic acid was extracted from serum samples with positive anti-HBc and/or positive or equivocal HBsAg (n = 65; among them, 5 cases were excluded because they disagreed on HBV DNA test). To isolate HBV DNA, serum (100 μL) was mixed with 300 μL of buffer (13.3 mmol/L Tris-HCl, pH 8.0; 6.7 mmol/L EDTA; 0.67% sodium dodecyl sulfate; 133 mg/μL proteinase K) and incubated at 55°C for 4 hours. Equal volume phenol, phenol-chloroform, and chloroform extractions were performed step by step, and DNA was precipitated with cold ethanol. The precipitate was suspended in 20 μL of TE buffer (10 mmol/L Tris-HCl, pH 8.0; 1 mmol/L EDTA). Polymerase chain reaction (PCR) was performed for 35 cycles in a DNA thermal cycler (Perkin–Elmer Cetus, Norwalk, CT). Nested PCR was performed for detection of HBV DNA. For the initial step of PCR, the primers for the first-round amplification of the pre-S region (pre-S1 and -S2) were PreS-os 5′-CACACGTAGCGCATCATTTTGC-3′ (nucleotides [nt] 2801–2822, sense; nucleotide sequences were numbered according to a reference sequence of GenBank accession number X02763.1, *Eco*RI site as nt 1) and PreS-oa 5′- GAGCAGGGGTCCTAGGAATC-3′ (nt 197–178, antisense) and for the S gene region were S-os 5′-CAGGGGTCTGTATCTTCCTG-3′ (nt 43–62, sense) and S-oa 5′-CCAATTATGTAGCCCATGAAG-3′ (nt 897–876, antisense). The primers for the second-round amplification were PreS-is 5′-ATATTCTTGGGAACAAG AGC-3′ (nt 2832–2850, sense) and PreS-ia 5′-CTGATGTGATGTTCTCCATG-3′ (nt 177–157, antisense) and for the S gene region were S-is 5′-GGTGGCTCCAGTT CAGGAACACGT-3′ (nt 65–88, sense) and S-ia 5′-GGAATAACCCCATCTTTTTG-3′ (nt 870–851, antisense). The primers for the first-round amplification of the basal core promoter to precore regions were PreC-os 5′-GCCTTCTCATCTGCCGGTCCG-3′ (nt 1560–1580, sense) and PreC-oa 5′-GTATGGTGAGGTGAGCAATG-3′ (nt 2061–2042, antisense) and for the second round were PreC-is 5′-CATAAGAGGACTCTTGGACT-3′ (nt 1657–1675, sense) and PreC-ia 5′-AAAGAAGTCAGAAGGCAAAAACGA-3′ (nt 1976–1952, antisense). To avoid PCR-generated mutation, TaKaRa Ex Tag polymerase (Takara Shuzo Co, Shiga, Japan), which was capable of proofreading, was used in the PCR assay. An HBsAg-negative serum sample obtained from healthy subjects and an aliquot of double distilled water were