

# Horizontal Transmission of Hepatitis B Virus From Mother to Child Due to Immune Escape Despite Immunoprophylaxis

\*†Jamil N. Kanji, ‡Robert E.D. Penner, ‡Elizabeth Giles, §Karin Goodison, ¶Steven R. Martin, ‡Eric Marinier, and ‡Carla Osiowy

## ABSTRACT

Hepatitis B virus (HBV) vaccination starting at birth is approximately 95% effective in preventing mother-to-child transmission to infants born to HBV-infected mothers. A higher risk of transmission is associated with birth to a highly viremic mother, often due to transplacental exposure, while later horizontal transmission is much less common, particularly following complete vaccination. This study reports a case of infection in an older child despite appropriate immunoprophylaxis starting at birth and an apparent protective immune response post-vaccination. Two immune escape mutations within the antigenic determinant of the surface antigen-coding region were observed in the child's dominant HBV sequence, whereas the maternal HBV variant lacked mutations at both sites. Ultra-deep sequencing confirmed the presence of 1 mutation at low levels within the maternal HBV quasispecies population, suggesting early exposure to the child followed by viral evolution resulting in immunoprophylaxis escape and chronic infection.

**Key Words:** next-generation sequencing, quasispecies, vaccine escape

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**D**ue to the extremely high risk of developing chronic hepatitis B virus (HBV) infection in young infants exposed to the

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From the \*Division of Infectious Diseases, Departments of Medicine & Medical Microbiology/Immunology, University of Alberta, the †Provincial Laboratory for Public Health (Microbiology), University of Alberta Hospital, Edmonton, the ‡National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, the §South Zone, Alberta Health Services, and the ¶Division of Pediatric Gastroenterology, Hepatology, & Nutrition, Department of Pediatrics, University of Calgary, Calgary, Alberta, Canada.

Address correspondence and reprint requests to Carla Osiowy, National Microbiology Laboratory, Public Health Agency of Canada, Viral Hepatitis and Bloodborne Pathogens, 1015 Arlington St, Winnipeg, MB, Canada R3E 3R2 (e-mail: carla.osiowy@canada.ca).

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## What Is Known

- Although uncommon, breakthrough infection with hepatitis B virus can occur in infants born to chronically infected mothers despite complete immunoprophylaxis at birth.
- A higher risk of breakthrough infection is associated with maternal hepatitis B e-antigen positivity, high maternal hepatitis B virus DNA levels ( $>7 \log_{10}$  IU/mL) and potentially, surface antigen immune escape mutations such as G145R.

## What Is New

- A rare delayed onset of breakthrough infection in a child due to an immune escape mutant transmitted from the mother occurred despite an apparent vaccine response.

virus, vaccination, and delivery of hepatitis B immunoglobulin (HBIG) within 12 hours of birth are crucial and effective (85%–95%) measures to prevent infection in infants born to mothers chronically infected with HBV. Reports of vaccine breakthrough, or more accurately, “failure to clear infection” (1), in infants or children is infrequent following complete immunoprophylaxis, but has been associated with intrauterine or perinatal exposure to the virus (2) or in rare cases, evolution and expansion of isolates having mutations within the viral genome resulting in escape from neutralization by antibodies elicited to the vaccine or present in immunoglobulin (ie, immune escape; (3)). These immune escape mutations are often transmitted from the mother or they may emerge as de novo mutations (1), although studies have shown that infants born to mothers having HBV immune escape mutations successfully respond to vaccination and are protected against infection (4). A glycine to arginine substitution at amino acid 145 (G145R) within the antigenic determinant of the hepatitis B surface antigen (HBsAg) protein (aa 124–147) is the most common mutation described to result in immune escape following vaccination (5,6). Although still rare, immune escape due to G145R is more commonly associated with HBV reactivation following transplantation or immunosuppressive treatments (7,8). The present study describes a breakthrough HBV infection in a child having HBsAg mutations at amino acids 144 and 145 despite appropriate and complete immunoprophylaxis starting at birth and an apparent vaccine-induced response following completion of vaccination.

## METHODS

### Case Patient

A 5-year-old girl was referred for evaluation of chronic HBV infection. She was the product of a normal pregnancy, born in 2012 via spontaneous vaginal delivery at term with no complications. Her mother was diagnosed with HBV 4 months before delivery based upon a confirmed HBsAg and positive total HBV core antibody (anti-HBc) result, but otherwise had an uncomplicated pregnancy with no gestational diabetes, hypertension, other infections, or delivery complications. The mother's HBV viral load was unknown before and at the time of delivery, and she had no history of antiviral treatment. The patient received HBIG (0.5 mL intramuscular) and HBV vaccine at birth with subsequent HBV vaccine doses at 2 and 6 months of age. Serum samples for this study were collected from the child at 4 years 10 months of age and from the mother at approximately 3.5 and 4.5 years after the birth of the child (samples M1 and M2, respectively).

### HBV DNA Sequencing

Sera (150 µL) were extracted for HBV DNA, amplified and sequenced using primers targeting the HBsAg-coding region as described previously (9). Massively parallel deep amplicon-based sequencing of the HBsAg, Polymerase, and Core coding regions was performed using methods and primer sequences provided in the Supplemental Digital Content (Text, Supplemental Digital Content 1, <http://links.lww.com/MPG/B602> and Tables 1 and 2, Supplemental Digital Content 2, <http://links.lww.com/MPG/B603>). Deep sequencing analysis was performed on the mother-child paired samples to evaluate the quasispecies complexity within each sample and to determine the presence of low-level mutant or wild-type populations within the mother or child, respectively. Quasispecies complexity measures were performed as described in the Supplemental Digital Content (Text, Supplemental Digital Content 1, <http://links.lww.com/MPG/B602>).

## RESULTS

The hepatitis B surface antibody (anti-HBs) titer of the patient was tested approximately 1 month following the third dose of vaccine at 7 months 19 days of age and was found to be 588.8 IU/L with no evidence of circulating HBsAg (anti-HBs value >10–12 IU/L is considered to be protective/immune). At 4 years 10 months of age, the patient was re-tested for hepatitis B serologic markers due to parental anxiety and found to be neutralization-confirmed HBsAg positive. On the same serum draw, she was seropositive for total anti-HBc, HBV e-antigen (HBeAg), and negative for anti-HBc IgM and anti-HBeAg antibody. Her anti-HBs titre was 9.68 IU/L with a detectable HBV viremia quantified at 127,993 IU/mL (5.1 log<sub>10</sub>). Alanine aminotransferase level was within normal range at 26 U/L (normal <33). Repeat serologic testing 3 and 15 months later confirmed these results and established the development of chronic hepatitis B infection.

The patient was up-to-date with her vaccinations and had not received any further HBV vaccine doses. Other than her mother, all other house-hold members were found negative for HBV around the time of her diagnosis. Both the patient and her mother are HBeAg positive and thus deemed to be in the immune tolerant phase of HBV infection with unremarkable abdominal ultrasound findings. The patient's viral load has ranged from 5.1 to 5.3 log<sub>10</sub> IU/mL since diagnosis in 2017 and her mother's has ranged from 8.2 to 8.7 log<sub>10</sub> IU/mL since 2014, with neither treated with antiviral medication nor any abnormality in serum bilirubin, aminotransferases, or alpha-fetoproteins observed.

Based on the acquisition of HBV infection despite an apparent robust immune response at the age of 7 months 19 days and a complete course of immunoprophylaxis starting at birth, sequencing of HBV isolates from both the patient and her mother was undertaken. Both isolates typed as genotype B by direct sequencing and both had identical sequences throughout the partial HBsAg region (364 nucleotides), apart from 2 mutations in the child's sequence resulting in D144E/G145R amino acid substitutions within the HBsAg antigenic determinant. Deep sequence analysis showed that both maternal samples contained extremely low quasispecies population levels having substitutions at either D144 or G145, yet mutations at both sites were not observed in combination on any of the mother's sequence reads (Table 1). Only the more recent maternal sample M2 (4.5 years after birth of child) had a G145R quasispecies population above the experimental error rate. The child's sample also had an extremely low wild type G145 population, but wild type D144 sequence populations were not observed above the experimental error rate.

The number of dominant quasispecies populations among the child's sample was greatly reduced compared to the mother's samples (Table 2). Similarly, complexity measures of incidence (number of haplotypes, polymorphic sites, and unique mutations) and abundance (maximum mutation frequency (Mfm), population nucleotide diversity ( $\pi$ ), and Simpson index ( $H_{Si}$ )) showed that overall, the child's isolate had reduced genetic diversity compared to the maternal isolate at different time periods (Table 3). Although the Mfm and  $\pi$  were reduced among immune targets such as the core and surface antigen regions of the virus, the polymerase-coding region investigated (nt 803–1153) from the child displayed a similar or even higher mutation and population nucleotide diversity as the mother's isolates, suggesting the influence of multiple, and varied evolutionary influences following transmission and infection.

## DISCUSSION

HBV vaccination starting at birth is approximately 95% effective (10). Aside from host factors that may interfere with vaccine response (host genetics, immune status, etc), virological

TABLE 1. Frequency of wild-type or mutant amino acid residue at site 144 and 145 of the hepatitis B surface antigen antigenic determinant following deep sequencing

AA residue	Amino acid	Percentage (of total haplotypes)*		
		M1 (547) <sup>†</sup>	M2 (496) <sup>†</sup>	Child (258)
144	Wild type (D144)	98.92 <sup>§</sup>	99.21	0.38
	Mutant (E144)	0.18	0.00	97.71
	Substitution (G144) <sup>‡</sup>	0.54	0.59	1.15
145	Substitution (other 144) <sup>‡</sup>	0.36	0.20	0.76
	Wild type (G145)	98.92	97.63	1.91
	Mutant (R145)	0.36	1.78	97.71
	Substitution (other 145) <sup>‡</sup>	0.72	0.59	0.38

The total number of haplotypes for each patient sample is given in parenthesis for each column heading. AA = amino acid.

\*Haplotypes indicate the number of representative sequences for each quasispecies population meeting a 0.99 similarity threshold (see Text, Supplemental Digital Content 1 <http://links.lww.com/MPG/B602>). Values >0.60% (calculated error rate) are considered valid.

<sup>†</sup>M1 and M2 samples are from the mother, taken approximately 3.5 and 4.5 years after the birth of the child, respectively.

<sup>‡</sup>Amino acid substitutions observed at the site (not necessarily associated with immune escape); for amino acid 144: H, K, V, Y; for amino acid 145: E, K, S, V.

<sup>§</sup>Values >0.60% (calculated error rate) are considered valid.

TABLE 2. Number of quasispecies populations per patient sample showing the percentage of total hepatitis B surface antigen region deep-sequencing reads per population

Population*	Percentage (of total reads)		
	M1† (6674)	M2† (6271)	Child (5608)
1	37.5	62.3	73.9
2	22.0	13.2	15.7
3	10.3	7.0	—
4	7.5	3.1	—
5	3.8	1.6	—
6	1.9	—	—

The total number of deep sequencing reads for each patient sample is given in parenthesis for each column heading.

\*Quasispecies population having >100 reads (with a population defined as meeting a 0.99 similarity threshold).

†M1 and M2 samples are from the mother, taken approximately 3.5 and 4.5 years after the birth of the child, respectively.

factors, such as maternal HBV DNA levels (>7 log<sub>10</sub> IU/mL; (11)) and HBeAg positivity, can have a significant impact on the risk of chronic infection in infants born to chronically infected mothers. Although reports have suggested the prevalence of HBsAg mutants are increasing, which may compromise the long-term success of vaccination programs (12), it has been repeatedly shown that mutations verified to result in immune escape, such as G145R, remain a rare exceptional cause of vaccine breakthrough, and that over time the HBsAg mutation frequency has not accelerated due to immunization (13). Indeed, the recombinant vaccine was shown to durably protect against challenge with a G145R variant in a vaccinated chimpanzee model (14).

In the present study, an unusual occurrence of late (>7 months of age) infection breakthrough despite complete HBV immunoprophylaxis starting at birth was observed. The breakthrough was likely associated with the presence of immune escape mutations (D144E/G145R) dominant within the child's isolate and the higher risk posed by the mother's HBeAg positivity and presumed high HBV viral load at the time of birth. To reduce the risk of mother to child transmission, patient management guidelines recommend antiviral therapy for women with a viral load >5.3 to 7.3 log<sub>10</sub> IU/mL in the third trimester of pregnancy (2,15). The mother's viral load at the time of birth is unknown; however, due to her immunotolerant phase and consistently high HBV DNA levels post-pregnancy it is assumed that she also had a

high HBV viral load at birth. Amino acids 144 and 145 fall within a conformational epitope in the second loop of the HBsAg antigenic determinant. These amino acids are crucial for immunogenicity via the proper display and structure of the epitope (5,16), with mutations resulting in reduced antibody binding affinity and virion stability (16).

Due to the rapid rate of HBV replication and mutation under the control of an error-prone polymerase (17), a viral quasispecies population results, composed of a multitude of closely related but non-identical viral genomes due to random point mutations. A quasispecies nature allows flexibility among the viral population during periods of selective pressures resulting in selection of adaptive or most fit strains. Immuno-prophylaxis and the vaccine immune response are selective pressures which likely drive nucleotide substitution (18). In this study, an extremely low population level (<2%) of R145 or G145 sequences was observed within the total HBV quasispecies of the mother or child, respectively, as was the lack of a detectable E144 mutant population in the mother. This suggests that quasispecies populations containing R145 were selected in the child following transmission which continued to evolve and expand under immunoprophylaxis-based immune pressure to establish the dominant haplotype iteration observed at the time of sampling. As a specific founder haplotype was not observed among the mother's HBV quasispecies, it possibly indicates that the child's dominant populations were either not transmitted or they became extinct within the mother's HBV quasispecies over the intervening time. The observation of overall reduced genetic diversity among the child's quasispecies compared to the maternal isolate at different time periods is consistent with the evolutionary bottleneck that occurs with HBV transmission (19). Pressure from immunoprophylactic extrinsic and intrinsic forces acts upon the transmitted HBV population to select specific quasispecies genomic variants, such as those able to evade neutralizing or B-cell responses, thus narrowing the number of haplotypes (1). The mutation frequency and level of complexity among genomic regions of the haplotypes differed, such that the polymerase region of the child's isolate was similar or slightly higher than that observed with the mother's isolates. This is likely the result of different evolutionary pressures acting upon the viral genome, such as immune-based pressures or the evolutionary constraints of overlapping open reading frames.

Immune escape mutations have been more frequently observed in vaccinated infants having a late onset of infection (≥6 months after birth), suggesting that the infants were likely non-responders to the vaccine (6). Although post-vaccination testing of the child in this study indicated an apparent vaccine-induced

TABLE 3. Measures of quasispecies complexity and genetic diversity by genomic region (core, polymerase, surface antigen)

	Core			Polymerase			Surface		
	M1*	M2*	Child	M1	M2	Child	M1	M2	Child
Complexity measures of incidence									
Number of haplotypes	591	615	466	398	367	201	547	496	258
Number of polymorphic sites	299	293	288	289	285	237	281	281	240
Number of unique mutations	504	468	425	462	437	313	463	456	336
Complexity measures of abundance†									
Percentage Simpson index (H <sub>Si</sub> )	0.17%	0.16%	0.22%	0.25%	0.27%	0.50%	0.19%	0.21%	0.40%
Percentage maximum mutation frequency (M <sub>fm</sub> )	1.01%	1.00%	0.79%	0.94%	1.01%	1.04%	0.98%	1.07%	0.92%
Percentage population nucleotide diversity (π)	1.84%	1.78%	1.55%	1.85%	2.00%	2.03%	1.84%	1.99%	1.81%

\*M1 and M2 refer to the mother's samples taken at approximately 3.5 and 4.5 years after the birth of the child, respectively.

†Values are expressed as a percentage for simplicity.

immune response (588.8 IU/L), there is the possibility that testing before 9 months of age missed a prolonged HBV incubation, or measurement of the actual vaccine-mediated antibody response was confounded by detection of passive anti-HBs from HBV administered at birth, the latter being rather unlikely. Post-vaccination testing of infants born to infected mothers and vaccinated at birth is recommended at least 1 month following the last dose of vaccine and/or at least 9 months after birth (20). Despite the reduced genomic complexity observed with the child's HBV isolate, the precise time of transmission cannot be determined and may have occurred at any point >7 months of age. The current study underlines the risk, however small, of the failure by some infants to adequately clear infection following birth to a highly viremic mother, despite appropriate immunoprophylaxis and response, due in part to HBV immune escape mutations.

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