Emergence of Vaccine-Induced Escape Mutant of Hepatitis B Virus with Multiple Surface Gene Mutations in a Korean Child

The S protein of hepatitis B virus is the principal component of virus envelope and the primary target of anti-HBs response. Mutants or variants that escape neutralization by anti-HBs have been selected during immunoprophylaxis of HBV after birth and liver transplantation. We investigated a case of a Korean child who was vaccinated at birth against hepatitis B and also given hepatitis B immunoglobulin, but nevertheless later became infected with the virus. Hepatitis B virus-specific deoxyribonucleic acid covering the region of genome encoding the predominant "a" determinant of hepatitis surface antigen was amplified using polymerase chain reaction, and the nucleotide sequence was determined. We present for the first time in Korea the independent emergence of an escape mutant with substitution of arginine for glycine at amino acid 145 and proline for glutamate at amino acid 120 in "a" determinant after immunization.

Key Words : Escape Mutant; Immunoprophylasis; Hepatitis B Virus; Hepatitis B Surface Antigens

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INTRODUCTION

Active vaccination has remained as the most effective way to control hepatitis B virus (HBV). Administration of vaccine immediately after birth is effective in preventing the transmission from a hepatitis B e antigen-positive mother in approximately 70% of cases. This rate may be increased to greater than 90% if the vaccine is accompanied by simultaneous administration of hepatitis B immunoglobulin (HBIG) (1, 2).

The highly antigenic hepatitis B surface antigen (HBsAg) is directly involved in triggering the humoral immune response, which provides protective immunity against HBV infection. Although there are several subtypes of HBV, all share the 24-aa neutralization epitope (the common "a" determinant) in the hepatitis B surface antigen (HBsAg). The common "a" determinant lies between aa 124 and 147 of HBsAg and is believed to have a double-loop conformation. Monoclonal antibody which recognizes the region within this "a" epitope, is capable of neutralizing the infectivity of hepatitis B (3-5).

During the study on immunogenicity and efficacy of hepatitis B vaccines in Italy, a number of individuals who had apparently mounted a successful immune response later became infected with HBV (6, 7). These cases were characterized by the co-existence of anti-HBs, HBsAg and other marker of hepatitis B infection (8, 9). Sequence analysis of the virus from one of these cases revealed a mutation in the nucleotide sequence encoding the "a" epitope involving a substitution of arginine for glycine at amino acid position 145 (10-12). Similar variant has been described in serum sample of liver transplantation patient who was chronically infected with HBV and later treated with monoclonal anti-HBs (12, 13).

The emergence of virus which escapes neutralization by vaccine-induced antibody poses a serious threat to treatment aimed at controlling hepatitis B. Here, we report the appearance of vaccine-induced escape mutant of HBV for the first time in a Korean baby.

CASE REPORT

Patient

Patient was born to an HBsAg, HBeAg-positive mother and was vaccinated immediately after birth and simultaneously given HBIG at contralateral site. At 19 months after birth, he presented elevated liver enzyme level (AST 484 U/L, ALT 427 U/L) and unusual serological marker (HBsAg positive, anti-HBs positive, HBeAg positive). At 21 months, level of liver enzyme remained high (AST

Table 1. Laboratory results from patient and mother

	AST/ALT (IU/L)	HBsAg/Anti-HBs	HBeAg/Anti-HBe	HBV-DNA (pg/mL)	Anti-HCV
Mother	16/27	+/−	+/−	+	−
Patient					
At birth	Unknown	Unknown	Unknown	Unknown	
At 19 months	484/427	+/+	+/+	7.5	−
At 21 months	478/970	−/+	+/+	1.9	
At 23 months	271/680	−/+	−/+	2.1	
At 25 months	35/16	−/+	−/+	−	
At 32 months	29/12	−/+	−/+	−	
At 35 months	36/13	−/+	+/+	−	

478 U/L, ALT 970 U/L) and serum HBsAg disappeared but serum HBV-DNA was positive. At 23 months, liver enzyme level slightly decreased (AST 271 U/L, ALT 680 U/L) and was nearly normalized (AST 35 U/L, ALT 16 U/L) after 25 months (Table 1).

Blood test

Routine biochemical tests were undertaken by standard procedures. Serum HBsAg, anti-HBs and anti-HBe were tested with commercial radioimmunoassay kits (Abbott Laboratories, North Chicago, IL, U.S.A.). HBV DNA was determined quantitatively by liquid hybridization assay (Digene Hybrid Capture System, Diegenes, Beltsville, MD, U.S.A.)

Extraction of HBV DNA, PCR, Cloning and Sequencing

HBV DNA was extracted from 100 μL serum of patient and his mother. After incubation for protein digestion in lysis buffer (0.5% SDS and 100 μg/mL pronase for 1 hr at 37℃), DNA was extracted twice with phenol/chloroform/isoamyl alcohol and precipitated with pure ethanol.

Nucleotide sequence encoding "a" epitope in HBsAg was amplified in two PCR sessions. Each reaction was carried out in 30 cycles of 95℃ 1 min, 58℃ 1 min, 72℃ 2 min, in 1 cycle of 95℃ 5 min and in 1 cycle of 72℃ 7 min. The external primers for the first session were 5'-TCCTGCTGCTATGCCTCATC-3' (BF105, sense, 411-430) and 5'-TTCCAATTACATATCCCATGAAG-TTAAGGGA-3' (BR112, antisense, 895-876). Primers for the second session were 5'-CCAGGAACATCAAC-CA-3' (BF107, sense, 485-504) and 5'-AAGGGAGTA-GCCCCAACGTT-3' (BF109, antisense, 870-851). PCR products were purified with QIAGEN purification kit and ligated into T easy vector with promega PGEM-T easy vector system. The vector was transformed into JM109 competent cells by heat shock method. Cells were grown in LA media broth at 37℃ and harvested. DNA

products were then extracted with DNA mini-preparation method and digested with restriction enzyme *EcoRI*. The final DNA products were directly sequenced with Promega fmol DNA cycle sequencing kit.

Detection of mutations in the "a" epitope of S gene

Fig. 1 shows the nucleotide sequences corresponding to the "a" epitope in S gene from mother and patient. Sample 1 consisted of 5 clones which were obtained from patient at 21 months after birth. Sample 2 showed 6 clones obtained at 23 months. Six of these independent 11 clones (4 of sample 1, 2 of sample 2) contained a point mutation (G to A) at nucleotide position 587 which would result in a substitution of arginine for glycine at amino acid position 145 in the major surface antigen. Other mutations at aa position 120 (substitution of proline for glutamine) and 126 (substitution of isoleucine for threonine) were also observed.

DISCUSSION

The envelope proteins of HBV are the products of the S open-reading frame and span the lipid bilayer of the virus. They are involved in receptor binding, viral assembly and secretion and are important targets for immune-mediated virus elimination. The computer analysis of topogenic element of S protein shows that there are at least three hydrophobic and two hydrophilic domains. Hydrophobic regions are important for anchoring the S protein to the membrane. The second hydrophilic region is exposed on the outer virion surface and is called major hydrophilic region (aa 99 to aa 169) because it contains the major group and subtype-specific antigenic determinant. The major B cell epitope cluster of S gene, which is widely and historically referred to as the "a" determinant (aa 124 to aa 147), is located in the second hydrophilic region. Change of amino acid sequence in this region could result in the alteration of antigen structure
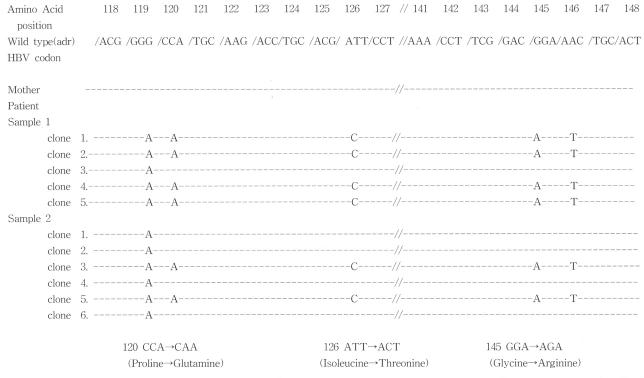
Amino Acid position	118	119	120	121	122	123	124	125	126	127	//	141	142	143	144	145	146	147	148
Wild type(adr) HBV codon	/ACG	/GGG	/CCA	/TGC	/AAG	/ACC	/TGC	/ACG/	ATT/	CCT	//	AAA	/CCT	/TCG	/GAC	/GGA	/AAC	/TGC	/ACT
Mother	---	---	---	---	---	---	---	---	---	---	//	---	---	---	---	---	---	---	---
Patient																			
Sample 1																			
clone 1			A	A					C		//					A	T		
clone 2			A	A					C		//					A	T		
clone 3			A								//								
clone 4			A	A					C		//					A	T		
clone 5			A	A					C		//					A	T		
Sample 2																			
clone 1			A								//								
clone 2			A								//								
clone 3			A	A					C		//					A	T		
clone 4			A								//								
clone 5			A	A					C		//					A	T		
clone 6			A								//								

120 CCA→CAA	126 ATT→ACT	145 GGA→AGA
(Proline→Glutamine)	(Isoleucine→Threonine)	(Glycine→Arginine)

Fig. 1. Amino acid variation across the "a" epitope of S gene in the serum of patient and mother. The most common described variant, G145R, has been found in this case with additional mutations at aa 120 and aa 126.

and the loss of immunoreactivity (3-5).

Vaccine-induced escape mutants of HBV were first found in Italian children who was administered with the hepatitis B vaccine. In the virus, a point mutation (G to A) at nucleotide position 587 which would result in a substitution of arginine for glycine at amino acid position 145 in HBsAg was found. Since then many cases of Gly 145 Arg mutants have been reported world-wide, but there has not been a case of escape mutant of HBV in Korea (6, 7, 9, 10). This is the first reported case in Korea. The patient is a child who was born to chronic HBV hepatitis mother and became infected with hepatitis B virus in spite of vaccination and HBIG after birth. Nucleotide sequencing study showed that the mother was infected with wild type HBV. Among the 11 independent clones of patient, 6 clones contained point mutation (G to A) at nucleotide position that would result in amino acid substitution of arginine for glycine at 145 amino acid position. Additionally, they contained two other substitutions of proline for glutamine at 120 amino acid position and of isoleucine for threonine at 126 amino acid position (10-12).

Based on these results, patient was suspected to be infected with wild type HBV from maternal-fetal transmission. After immunoprophylaxis, escape mutant type HBV could proliferate more easily and selectively under the immune pressure. However, naturally occurring escape mutants have also been reported in chronic HBV carriers (14, 15). In this case, unfortunately we could not test the patient serum obtained just after birth and could not completely exclude the possibility that the patient was infected from a source other than his mother after birth. However, patient's infection period, between birth and initial diagnosis with hepatitis, is very short, being only 19 months. Most of the perinatal and postnatal HBV infection source is mother and during that period, the chance of being exposed to person other than mother is very rare.

Additional mutants associated with Gly 145 Arg included Pro 120 Gln and Ile 126 Thr in this patient. The proline-to-serine or threonine changes at position 120 of HBsAg have been detected in immunized Singapore infants born to HBV carrier mother (16) and chronic hepatitis patients (14), respectively. However Pro 120 Gln detected in our study has not been previously reported. The mutation Pro 120 Ser has been shown to affect the binding of antibodies to the second loop of the "a" determinant (16). Thus, it is conceivable that Pro 120 Gln causes a decreased binding to the "a" determinant-specific monoclonal antibody. A Ile 126 Thr change is invariably associated with Gly 145 Arg in this study. This has been reported in a Chinese baby born to an HBsAg carrier mother (7) and both are found among prototype HBV sequences.

The Arg 145 variant circulated as a stable strain and retained its ability to replicate at high titer for 5 yr (6). Vaccine escape mutants were detected in infants who develop acute or chronic hepatitis despite immunoprophylaxis (11). In this study, however, biochemical and virological remission occurred at age 2 yr, and the number of HBV DNA clones with mutation decreased with recovery. Therefore, these findings highly suggest that host cell-mediated immune response can eliminate vaccine escape mutants.

HBV has a higher intrinsic mutation rate than other DNA viruses, probably because its replication requires an intermediate RNA molecule and reverse transcriptase. Single G145R mutation is by far the most common escape mutant being after immunoprophylaxis and in nature. Screening for HBV mutants in vaccinated carrier in Singapore showed that 42% had some mutations in "a" determinant (9). Study in Taiwan indicated that 22% was infected with mutated HBV after vaccination (10, 12). These results suggest that there may be some geographic differences in the prevalence of HBV mutant. With universal vaccination, it is of concern that HBV surface protein mutants may become dominant strains, infecting even people with protective levels of anti-HBs. Adding of appropriate HBsAg to current vaccine may be a possible solution for preventing infections by escape mutants.

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