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Case report

Hepatitis B caused by a hepatitis B surface antigen escape mutant

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Amino acid substitutions within the *S* gene involving the major antigenic *a* determinant of the hepatitis B virus (HBV) surface antigen (HBsAg) have been detected in cases of failure of immunization against the virus. Our report showed development of clinical hepatitis in presence of antibody to HBsAg in a healthy individual. A single amino acid substitution (G145R) within the *a* determinant of the HBsAg was determined by sequencing of the isolated HBV strain. Lamivudine treatment efficiently cleared the peripheral HBV DNA, HBsAg, and hepatitis B e antigen. In conclusion, the immune escape mutant in the *S* gene can cause hepatitis despite pre-existing naturally acquired immunity.

Key words: hepatitis B, *a* determinant region, G145R escape mutant

Introduction

Hepatitis B virus (HBV) infection of immunocompetent adults is usually transient and induces the development of neutralizing antibodies and immunity to reinfection. Persistent or chronic infection is more likely to occur following vertical transmission (from mother to child) or horizontal transmission to children or to immunocompromised adults. The immune determinants are not fully understood, but both cellular and humoral immune responses are important.[1] The HBV genome contains three partially overlapping open reading frames (ORF). The C-ORF encodes for the core antigen (HBcAg) and for a pre-core protein, which is co-translationally processed and secreted as hepatitis B e antigen (HBeAg). The S-ORF encodes for surface antigens (HBsAgs), which are called PreS/S and S proteins, and the P-ORF encodes for the viral polymerase (pol). A fourth ORF, X-ORF, encodes for a protein with trans-activating activity. Anti-HBs antibodies are a marker of the resolution of transient HBV infection. These antibodies have also been shown to prevent reinfection, inhibiting the ability of virus particles to bind to receptors on target cells. In chronic HBV infection, anti-HBs antibodies are not generally detected in serum, although it is possible that their presence is masked by the formation of immune complexes with HBsAg particles in the blood stream.[1] The HBsAg consists of several antigenic determinants, and HBV is classified into subtypes (serotypes).[2] All of the serotypes share a common "alpha" (*a*) determinant (amino acids 121–149).[3] The *a* determinant is the primary target for both active and passive immune prophylaxis, and the majority of anti-HBs antibodies that appear after natural infection are directed against the *a* determinant epitope cluster.[4,5] Mutations within the *a* determinant may affect antigenicity, and thus may enable the virus to evade the neutralizing antibodies. A mutation from glycine to arginine at position 145 of the *a* determinant is almost always detected in escape mutant strains.[3,6–11]

The present study documents a rare case of hepatitis B that developed despite the presence of neutralizing antibodies to HBsAg after a past HBV infection. Isolation of a strain with G145R replacement indicates that the hepatitis in our case was caused by an HBV *S* gene mutant.

Case report

A 69-year-old man showed abnormal results on liver function tests during a periodic medical examination on 14 February 2006; the biochemical markers serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were elevated at 89 and 181 IU/l, although he did not have any complaints. Blood tests

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Table 1. Laboratory data on 17 March 2006

Test	Result	Units	Test	Result	Units	Test	Result	Units
WBC	3800	/μl	TP	6.6	g/dl	HBsAg	>250	IU/ml
RBC	436×10^4	/μl	Alb	4.2	g/dl	Anti-HBs	126.7	mIU/ml
Hb	14.4	g/dl	T-Bil	0.8	mg/dl	Anti-HBc	8.20	s/co
Ht	45.5	%	AST	99	IU/l	IgM anti-HBc	0.1	
Plt	18.6×10^4	/μl	ALT	268	IU/l	HBeAg	1240	s/co
			ALP	293	IU/l	Anti-HBe	0.1	%
PT	100	%	γGTP	36	IU/l	HBV DNA	6.5	Log-copies/ml
			LDH	218	IU/l	HBV subtype	adr	
			UA	3.7	mg/dl	HBV genotype (PCR-Invader)	C	
			TChol	243	mg/dl	HBV precore (PCR-ELMA)	Wild	
			TG	77	mg/dl	HBVcore promoter (PCR-ELMA)	Wild	
			Glu	104	mg/dl	Anti-HCV	(−)	
			ICGR15	8	%	HCV RNA	(−)	
			Hyaluronate	50	ng/ml	IgM anti-HA	0.1	
			AFP	6.2	ng/ml	EBV-VCA IgG	160	x
			CRP	<0.25	mg/dl	EBV-VCA IgM	<10	x
			IgG	809	mg/dl	EBV EBNA	40	x
			IgA	214	mg/dl	CMV IgG-EIA	146	
			IgM	92	mg/dl	CMV IgM-EIA	(−)	

RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; Plt, platelet count; PT, prothrombin time; Alb, albumin; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGTP, γ-glutamyl transferase; LDH, lactate dehydrogenase; UA, uric acid; TChol, total cholesterase; TG, triglycerides; Glu, glucose; ICGR15, indocyanine green retention at 15 min; AFP, α-fetoprotein; CRP, C-reactive protein; HBsAg, hepatitis B surface antigen; Anti-HBs, antibodies to HBsAg; Anti-HBc, antibodies to hepatitis B core antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction; ELMA, enzyme-linked minisequence assay; HCV, hepatitis C virus; anti-HA, antibody to hepatitis A; EBV, Epstein-Barr virus; VCA, viral capsid antigen; EBNA, Epstein-Barr nuclear antigen; CMV, cytomegalovirus; EIA, enzyme immunoassay; s/co, specimen to cutoff ratio

at our hospital revealed positive results for HBsAg and anti-HBs antibodies in the blood serum. At the examination on 17 March, the results were as follows (Table 1): AST, 99 IU/l; ALT, 268 IU/l; serum albumin, 4.2 g/dl; total bilirubin, 0.8 mg/dl; platelet count, 18.6×10^4/μl; prothrombin time, 100 %; HBsAg positive (>250 IU/ml, Architect HBsAg QT, Abbott Laboratories, Abbott Park, IL, USA); anti-HBs positive (126.7 mIU/ml, Architect Ausab, Abbott Laboratories); HBeAg positive [1240 s/co (specimen to cutoff ratio), Architect, Abbott Laboratories]; anti-HBe negative (Architect, Abbott Laboratories); HBV DNA, 6.5 log copies/ml; hepatitis B core antibody (anti-HBc) 8.20 s/co (chemiluminescence immunoassay, Architect, Abbott Laboratories); anti-HBc IgM negative; hepatitis C virus (HCV) antibody negative; HCV RNA negative; anti-human immunodeficiency virus antibody negative; and positive findings for past infection with Epstein Barr virus and cytomegalo virus . Histological examination of the liver biopsy specimen obtained on 20 March 2006 showed minimal infiltrate of lymphocytes in the portal tracts in the absence of piecemeal necrosis or fibrosis. The fibrotic score was zero. Lobular inflammation was minimal or absent, and minimal steatosis was observed (Fig. 1). Orcein staining revealed no HBsAg in the hepatocytes.

The clinical course is outlined in Fig. 2. Because the ALT level increased to >600 IU/l on 10 April , anti-viral therapy with lamivudine 100 mg daily as well as glycyrrhizin 100 ml was initiated, and HBV DNA levels decreased immediately. ALT levels decreased gradually, and the dose of glycyrrhizin was first decreased and then discontinued on 1 September 2006. At that time, HBsAg, anti-HBs antibodies, and HBeAg were still positive and the ALT level was 52 IU/l, but the HBV DNA level was <2.6 log copies/ml by Amplicor assay, and 100 copies/ml by in-house real-time polymerase chain reaction (PCR) analysis. ALT levels were slightly elevated in October, but by 10 November 2006, HBsAg and HBeAg had become negative and anti-HBe antibodies had appeared. Thereafter, ALT levels decreased to within the normal range. On 16 March 2007, test results were as follows: AST, 16 IU/l; ALT, 18 IU/l; HBsAg negative; anti-HBs positive; HBeAg negative; ant-HBe antibody positive; and HBV DNA <2.6 log copies/ml (undetectable by real-time PCR). At that time, lamivudine therapy was discontinued.

The patient had no history of blood transfusion or heavy alcohol consumption (his alcohol consumption was approximately 17.5 g/day). No family member was positive for HBsAg, and he had no episodic sexual contacts during the past 10 years. ALT levels maintained within the normal range during annual routine health examinations until 28 December 2004. The serological examination in April 1996 showed that HBsAg (assayed

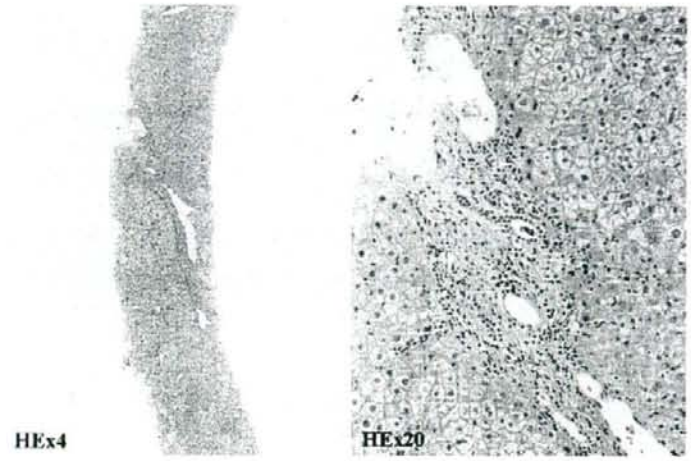

Fig. 1. Histological images of a liver biopsy specimen. Minimal infiltrate of lymphocytes in the portal tracts, in the absence of piecemeal necrosis or fibrosis, was observed. Lobular inflammation was minimal or absent, and minimal steatosis was observed. HE, hematoxylin and eosin staining

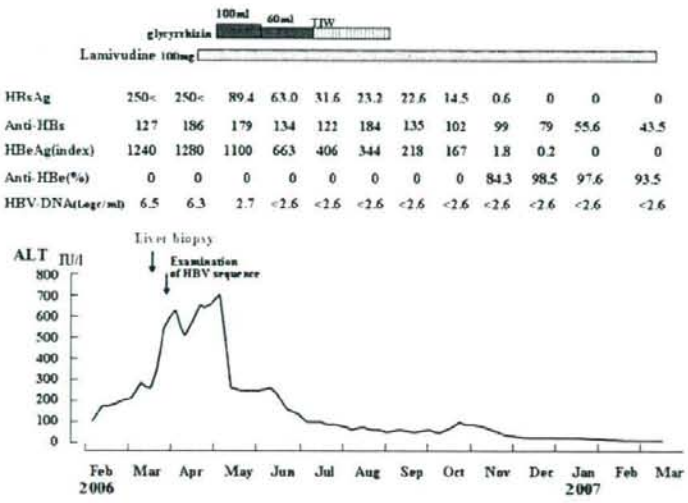

Fig. 2. Clinical course. A 69-year-old man showed abnormal reseults on liver function tests on 14 February 2006. The alanine aminotransferase (*ALT*) level increased to above 600 IU/l on 10 April, and lamivudine 100 mg daily and glycyrrhizin 100 ml were initiated. Hepatitis B virus (*HBV*) DNA levels decreased immediately. Hepatitis B surface antigen (*HBsAg*) and hepatitis B e antigen (*HBeAg*) became negative and antibodies to HBeAg (*anti-HBe*) appeared on 10 November 2006. On 16 March 2007, lamivudine therapy was discontinued because of HBsAg seroconversion and because HBV DNA had decreased to <2.6log copies/ml (undetectable DNA by real-time polymerase chain reaction). *Anti-HBs*, antibodies to HBsAg; TIW, three times per week

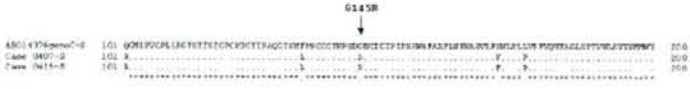

Fig. 3. A single amino acid substitution (G145R) within the *a* determinant of HBsAg was found by sequencing of the isolated HBV strain

by reversed passive hemagglutination) was negative, but anti-HBs antibody (assayed by passive hemagglutination) was positive, although the patient had not been vaccinated against HBV. To elucidate the development of clinical hepatitis in the presence of antibody to HBsAg (anti-HBs antibody) in a healthy individual, possible escape mutants within the *a* determinant of the HBsAg were examined. A single amino acid substitution (G145R) within the *a* determinant of the HBsAg was identified by sequencing of the isolated HBV strain adr (Fig. 3). The HBV genome belonged to genotype C and serotype adr.

Methods

HBsAg and anti-HBs antibody were detected by chemiluminescent enzyme immunoassay (Lumipulse, Fujirebio, Tokyo, Japan). HBV DNA was extracted from 100 µl of serum with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The HBV genome spanning the *S* coding region, including the *a* determinant, was amplified by primer sets described previously.[12] Thereafter, PCR products were directly sequenced with a Prism Big Dye kit (Applied Biosystems, Foster City, CA, USA) in an ABI 3100 DNA automated sequencer.

Discussion

Mutations of the HBV *S* gene have been reported in infants born to carrier mothers despite an adequate anti-HBs response after vaccination[3,6–10,13] and in liver transplant recipients in whom HBV reinfection developed despite prophylaxis with monoclonal or polyclonal anti-HBs (hepatitis B immunoglobulin).[14–16] Furthermore, mutant strains have been reported in patients with acute hepatitis B, fulminant hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma.[15–18]

In this report, we documented acute exacerbation caused by an HBV strain with a single amino acid substitution (G145R) within the *a* determinant of the HBsAg despite pre-existing anti-HBs antibody produced naturally by previous infection. The glycine to arginine substitution at position 145 (G145R) is the most common mutation associated with immune escape in the *S* gene, and this mutant can be transmitted horizontally.[10,11] Experiments in chimpanzee and mouse have demonstrated that the G145R escape mutant is pathogenic.[19,20] In our patient, it was necessary to distinguish whether he had newly contracted the G145R escape mutant or whether he was a carrier of a variant with latent virus replication (occult HBV infection) in whom HBV infection had been reactivated. As he had had no obvious risk factor for several years and the titer of anti-HBc IgM was very low, it is unlikely that he had been newly infected with this escape mutant clone, which is rarely found in Japan. HBV often persists after resolution, and its replication is suppressed by antiviral T cells.[21] As previously reported, HBV *S* gene mutants may be present in HBsAg-negative asymptomatic carriers without any sign of hepatic injury.[18,22] In fact, an G145R escape mutant has appeared in patients with occult HBV infection causing hepatitis B despite the present of anti-HBs antibodies. Reactivation of an occult hepatitis B virus escape mutant in patients with aggressive lymphoma therapy and a- or hypo-gammaglobulinemia has been reported.[23–26] Hence, it is possible that an G145R escape mutant was reactivated in our patient.

To our knowledge, this is a rare case of clinical hepatitis due to a G145R escape mutation in the presence of anti-HBs antibody. It should be noted that even a healthy person with a past history of acute hepatitis B infection can develop hepatitis B infection under such conditions.

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