Front. Med. China 2009, 3(3): 277–283
DOI 10.1007/s11684-009-0047-0

RESEARCH ARTICLE

Preparation, identification, and clinical application of anti-HBs monoclonal antibody that binds both wild-type and immune escape mutant HBsAgs

Fanghe LI (✉), BM, Chunyan ZHANG, MS, Jinghua LIU, MS, Xiaoyan ZHANG, MS, Bing YAN, Bo ZHANG, MS, Yongguo HUANG, MS, Jingsong GONG, BM, Yan CHEN, BM

Experimental Center of Medical Sciences, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Abstract Using a standard cellular fusion technique and indirect enzyme-linked immunosorbent assay (ELISA), a hybridoma cell line strain secreting anti-HBs monoclonal antibody (mAb) (defined G6 mAb) was obtained. The cells grew and secreted mAb stably. Antibody titers in the culture supernatant and ascites were 2.048×10^6 and 4.096×10^6, respectively. By applying the anti-HBs G6 mAb and horseradish peroxidase (HRP)-labeled goat anti-HBs antibody, we developed a sandwich ELISA (defined G6m ELISA) for detecting both wild-type and immune escape mutant HBsAgs (IEM HBsAg). The assay was performed to detect 17 species of genome recombinant expression HBsAg, including two wild-type species and 15 IEM HBsAg species, which varied in the "a" determinant, in a group of patients infected with hepatitis B virus (HBV). The patients previously had a lower ELISA detection signal [(absorbance of patients/absorbance of normal people (P/N): 1.0–4.5)]. The results demonstrated that the sensitivity of this assay to wild-type HBsAg was no less than 0.125 μg/L; 12 of 15 IEM HBsAg species (P/N $\geqslant$ 2.5) were positive for G6 mAb. Of the positive IEM HBsAg species, two had a low absorbance value at 450 nm (A_{450}), one had an intermediate A_{450} value and nine had a high A_{450} value, which was 7.55%(mean), 59.4% and 92.1%–109.4% of the wild-type A_{450} value, respectively. The two species with low A_{450} value and the three negative species mutated at the bases 120–124 in the first loop of the HBV "a" determinant. Using the G6 ELISA and two commercial ELISA kits (A and B), 177 patients were tested. The G6 ELISA had a significantly higher detection rate than either commercial ELISAs (19.21% *vs* 14.89% and 6.21%, respectively; $P < 0.01$, $P < 0.05$, respectively).

Keywords hepatitis B virus; gene variation; hepatitis B surface antigen; immune escape; enzyme-linked immunosorbent assay

1 Introduction

The ongoing epidemic of hepatitis B virus (HBV) is one of the most serious public health issues in the world [1,2]. As the virus polymerase enzyme lacks proofreading activity, it is likely to have a much higher mutation frequency than the polymerases of other DNA viruses [3,4]. When mutations develop in some specific gene regions, such as the "a" determinant, a shift can occur in the expressed hepatitis B surface antigen (HBsAg), which can result in an HBV immune escape mutant strain (HBV IEMS) [1,4]. Many researchers have demonstrated that the immune escape mutant of HBV (IEM HBV) is similar to wild-type HBV in both pathogenesis and infectivity [5–7]. Because the mutant virus cannot be neutralized by the protective antibodies already circulating in the body and most commercial kits cannot detect it, it is possible that clinical misdiagnoses can be given to infectious HBV patients and both blood donor screening and serum epidemiology investigations become unreliable. As a result, this problem has attracted much attention [6,8–10]. Our group has begun to investigate this phenomenon that goes back to the last century. A new monoclonal antibody (mAb)-producing cell line (anti-HBs G6 mAb) has demonstrated a strong capacity for combining with mass IEM HBsAg. In this study, we primarily evaluated the binding ability of this mAb with the HBV mutant in the "a" determinant.

Received December 2, 2008; accepted February 6, 2009

E-mail: fhli@tjh.tjmu.edu.cn

2 Materials and methods

2.1 Materials

BALB/c mice were provided by the Experimental Animal Center of the Hubei Center of Disease Control and Prevention, Wuhan, China. HBV Dane particles, genome recombinant expression HBsAg and $Al(OH)_3$ gel were obtained from Wuhan Institute of Biologic Products, China [11,12]. Sp2/0 cells were obtained from the China Center for Type Culture Collection, Wuhan, China. RPMI1640 culture medium was purchased from Gibco Corporation, USA. Goat anti-mouse IgG and horseradish peroxidase (HRP)-labeled goat anti-mouse IgG were purchased from the American Pierce Corporation (USA). HRP-labeled goat anti-HBs and the commercial HBsAg enzyme-linked immunosorbent assay (ELISA) kits were procured from market. Anti-HBs A11 mAb and anti-HBs Mt mAb were purchased from the Wuhan Institute of Biologic Products, China [13]. G145R-mutant HBsAg was prepared by our group [14,15]. The highly concentrated HBsAg quality control blood serum was obtained from market. The other expression products of wild-type HBV S protein and recombined IEM HBV S protein were collected from the Division of the Clinical Immunology Laboratory, Tongji Hospital, China [16].

2.2 Subjects

The enrolled included 177 patients (96 males) with an average age of 27.9 ± 15.3 years, who were previously examined using G145R HBsAg kits which were collected from the Clinical Laboratory in our hospital from September to November 2006. The detection values [absorbance of patients/absorbance of normal people (P/N)] ranged from 1.0 to 5.0, when common commercial ELISA kits were used for screening. The control group included 52 college freshmen who took the physical examination during the 2006 school year. The students had received the hepatitis B virus vaccine no longer than one year previously, and were positive for anti-hepatitis B surface antibody (anti-HBs) but were negative for hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), anti-hepatitis B e antigen (anti-HBe), anti-hepatitis B core antigen (anti-HBc) and HBV DNA. All physical and biochemical examination indices were normal.

2.3 Methods

2.3.1 Preparation of anti-HBs G6 mAb

Anti-HBs G6 mAb was prepared as previously reported by Qian *et al* [17]. Briefly, BALB/c mice were immunized routinely with the HBV Dane particle fraction extracted from the sera of infected patients [11]. Three days following the last immunization, the mice were killed and the spleen cells were isolated. Cells were mixed with SP2/0 cells at a ratio of 1∶10. Anti-HBs hybridoma lines were prepared by cell fusion and limited dilution screening. Ascites was induced by pristane. Total protein content in ascites was determined at 280 nm using an ultraviolet-visible spectrophotometer. Bovine serum albumin (BSA) was used as a standard for protein quantification. Anti-HBs G6 mAb was purified by using caprylic acid-ammonium sulfate fractionation.

2.3.2 G6 ELISA development

The G6 ELISA (Dual-antibody sandwich ELISA) was developed by our group using anti-HBs G6 mAb and HRP-labeled goat-anti-HBs IgG according to the following procedures: (1) anti-HBs G6 mAb was diluted in carbonate buffer (pH 9.6), distributed into microwell plates, and incubated at 4°C overnight; (2) the coated microwell plates were washed 5 times with phosphate buffered saline (PBS)-Tween; (3) the microwell plates were blocked with 20 g/L BSA (150 μL/well) and incubated at 37°C for 2 h, then the liquid was discarded; (4) undetected and contrast samples were added (100 μL/well), allowed to react at 37°C for 30 min, and washed as above (2); (5) goat anti-HBs-HRP IgG (100 μL) was added to each well, incubated at 37°C for 30 min and then washed; (6) tMB-H_2O was added to each well and incubated at 37°C for 15 min. The reaction was terminated by the addition of 50 μL H_2SO_4 (2 mol/L) to each well. The samples were measured using a microplate reader at A_{450}; (7) A_{450} values were confirmed with the positive criterion at P/N ⩾ 2.1.

2.3.3 Neutralization assay

The neutralization assay was developed by our laboratory [18]. Anti-HBs G6 mAb was added to rG145R HBsAg and r-wHBsAg, and incubated at room temperature for 30 min. The mixtures were then transferred into a microwell plate coated with Mt mAb or A11 mAb, incubated for 30 min, and washed three times with PBS-Tween. HRP-labeled goat anti-HBs IgG was added and wells were incubated and washed as above. TMB-H_2O was added for incubation and the A_{450} value was measured as it was for the G6 ELISA. The inhibiting rate was calculated by comparing the above A_{450} value with that of the non-neutralization wells.

2.3.4 Other routine HBsAg ELISA trials

The examinations with the commonly-used commercial HBsAg ELISA kits were performed according to the manufacturer's instructions.

2.3.5 Data analysis

All statistical analyses were performed using SPSS13.0 software. A $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 Determination of mAb

A hybridoma line, G6B3F1, which has a high secreting capacity for anti-HBs IgG6 mAb, was selected from 376 hybridomas prepared by routine cell fusion. The ascites of anti-HBs IgG6 mAb was induced by pristane. Anti-HBs IgG6 mAb IgG was purified using caprylic acid-ammonium sulfate fractionation. Cell culture supernatants, ascites and purified antibodies (anti-HBs G6 mAb IgG) were examined using an ultraviolet-visible spectrophotometer and sandwich ELISA. The titers of anti-HBs in the supernatant, ascites and purified products detected by ELISA were 2.048×10^6, 4.096×10^6 and 1.024×10^6, respectively. The protein concentrations of ascites and purified antibodies were 11.2×3.16 and 3.60 g/L, respectively.

3.2 G6m ELISA sensitivity

The A_{450} values of serial diluted quality-control HBsAg-positive serum were detected by G6m ELISA, and compared with those of the recombinant G145R mutant HBsAg expression product in the condensed culture supernatant (Fig. 1). The color intensities and the slopes of the linear dilution curves were very similar (0.716 and 0.729, respectively). The control serum was still positive (P/N value = 4.5), with the concentration of HBsAg being 0.125 μg/L.

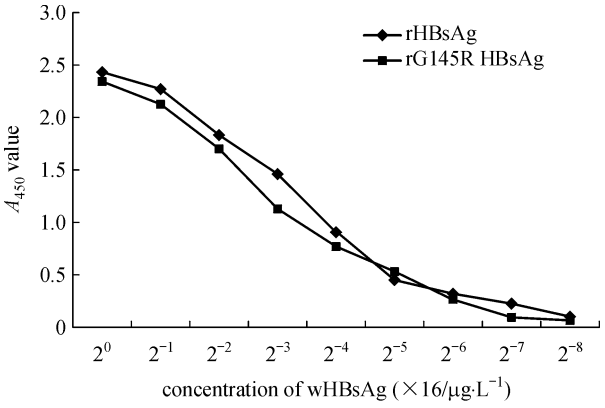

Fig. 1 Comparison of the reactivity between wild-type hepatitis B surface antigen (wHBsAg) and G145R mutant HBsAg in the G6 ELISA

3.3 Binding characteristics of mutant antigens

The G6-ELISA was used to examine the genome recombinant expression variants (15 samples) which had mutations in the "a" determinant. The results were compared with those of the wild-type and non-"a" determinant mutants (Table 1). The G6-mAb reacted

Table 1 The reactivity of anti-HBs G6 mAb to genome recombinant HBsAgs that varied in the "a" determinant

plasmid number	mutation	detection results		
		absorbance	P/N**	percent/%*
Arg145yw	G145R	2.683	44.75	99.2
AP3.1	D144A	2.793	46.55	109.44
1056sp	S143L/P120S	2.512	43.50	96.5
91-4696	D99N/122N/T123/G145R	2.641	44.02	97.6
C126	I126T/S143T	2.736	45.6	101.12
SA7	M133T	2.710	45.16	100.1
SA4	M133T/Y161F	2.490	41.50	92.02
SA6	Q129R/G130N/A166V	2.729	46.5	100.9
129K	G129L	1.607	26.78	59.40
129X35	G129H	0.041	0.68	1.51
BA3.2	T123N/C124R	0.067	1.12	2.50
122/A34	R122A	−0.001	0.00	0.00
BA3.4	T123N	0.261	4.30	9.6
M5	Y100S/T118V/R122K/M133I/Y134N/P142S/S143L/G145K	0.148	2.50	5.50
HK188	L98V/Q101R	2.683	44.72	99.20
Gly Dw	no mutation	2.729	45.50	100.9
Gly Dr	no mutation	2.683	44.72	99.20

*: The average A_{450} of the Gly Dr and Gly Dw mutants was 100%; **: the A_{450} of the non-transfection supernatant control was 0.06. P/N: absorbance of patients/absorbance of normal people.

with 12 of 15 IEM HBsAgs, 9 of which had a coloring intensity that was 90% stronger than recombinant wild-type HBsAg (r-wHBsAg).

Twelve recombinant IEM HBV S proteins with "a" determinant mutations were detected by 5 different commercial ELISA kits in which the tracing antibodies were replaced with HRP-labeled goat anti-HBs. The average coloration rate of each protein was calculated by using the homochronous detecting signal of two recombinant wild genome expression products as baseline. The results were compared with those from G6m-ELISA (Table 2).

3.4 Analysis of anti-HBs G6 mAb antigen binding sites

Differences in the antigen binding sites of anti-HBs G6 mAb and other anti-HBs mAbs were analyzed by double antibody sandwich ELISA in microwell plates coated with purified anti-HBs Mt or anti-HBs A11 mAb. The reactions of both r-wHBsAg and rG145R HBsAg with Mt mAb were blocked by anti-HBs G6 mAb (Table 3), and the former had a much lower effective blocking concentration (for $>50\%$ neutralization rate). The reaction of A11 with r-wHBsAg could only be blocked by a high concentration of anti-HBs G6 mAb, and there was no reaction from A11 with rG145R HBsAg. Under the same reaction conditions, anti-HBs A11 mAb and anti-HBs Mt mAb were tested for their ability to block HBsAg neutralization. When the plates were fully coated with wild-type HBsAg and the neutralization concentration was 100.00 mg/L, the average rate of neutralization was only 6.83% and 1.45% for anti-HBs A11 mAb and anti-HBs Mt mAb, respectively.

3.5 Clinical examination

The G6-ELISA and two commercial ELISA kits (B and C) were compared for their abilities to detect HBsAg in a group of specific clinical sera. The sensitivities of the G6-ELISA and two commercial ELISA kits (B and C) were 0.125, 0.25 and 0.125 ng/mL, respectively. The positive detection rate of the G6 ELISA was 19.21% (34/177 cases), and 6.21% and 14.89% for the two commercial ELISA kits (B and C), respectively ($P<0.01$ and 0.05, respectively). There was also a significant difference in the positive detection rate between two commercial ELISA kits (B and C, $P<0.01$).

The results indicated that anti-HBs G6 mAb, A11 mAb and Mt mAb bound to different antigen determinants of HBsAg. The distance between the binding sites (including framework and space distances) of anti-HBs G6 mAb and anti-HBs A11-mAb on the corresponding antigen determinants of r-wHBsAg was far, but there was still some correlation between the two antigen determinants. Anti-HBs G6 mAb could effectively block the binding of anti-HBs MT-mAb with either wild-type or G145R HBsAg. However, the different blocking efficiencies suggest that the distance between the corresponding antigen determinants of anti-HBs G6 mAb and anti-HBs MT-mAb was shorter than that of anti-HBs G6 mAb and anti-HBs

Table 2 Comparison of the detection abilities of different commercial ELISA kits and the G6m-ELISA kit for partial recombination immune escape mutant HbsAg

reagent name	reaction intensity rank			average coloration and comparison		
	high	middle	low	average coloration rate/%	S.D./%	P^{**}
G6m-ELISA	9	0	3	75.86	42.5814	
commercial ELISA A	4	2	6	49.5358	46.1619	<0.05
commercial ELISA B	4	1	7	49.7133	53.0950	<0.05
commercial ELISA C	4	6	2	58.1408	34.5212	>0.05
commercial ELISA D	6	2	4	62.7992	43.5974	>0.05
commercial ELISA P#	6	6	0	73.9516	21.0668	>0.05

*: reaction intensity rank: high: ⩾80%; middle: 30%–80%; low: ⩽30%. **: compared with G6m-ELISA; #: coated with multi-clone antibody.

Table 3 Initial analysis of the anti-HBs G6 mAb antigen binding sites

coating antibody	interrupted antigen	the concentration of interrupting antibody (anti-HBs G6 mAb, mg/L)				
		uninterrupted	0.01	0.10	1.00	10.00
MT mAb	r-wHBsAg	2.812/0.0%*	2.799/0.005%	2.414/14.2%	0.331/88.2%	0.151/94.6%
	rG145RHBsAg	1.156/0.0%	1.307/−13.1%	0.250/78.4%	0.051/96.59%	0.031/97.3%
A11 mAb	r-wHBsAg	2.379/0.0%	2.456/−0.56%	1.119/54.4%	0.992/59.6%	0.431/82.5%
	rG145RHBsAg	0.033/N**	0.015/N**	0.020/N**	0.014/ N**	0.059/N**

*: Data were presented as interrupted hole *A* value/interruption rate; **: anti HBs A11 mAb could not react with rG145R HBsAg at this coating concentration and the interruption test was not effective.

Table 4 Comparison of the G6-ELISA with two commercial ELISA kits (A and B) for the detection of HBsAg in clinical sera

G6-ELISA	commercial ELISA B		commercial ELISA C		total
	+	−	+	−	
+	26	8	11	23	34
−	0	143	0	143	143
total	26	151	11	166	177

A 11-mAb. It is presumed that the differences in the ability of anti-HBs G6 mAb to bind with the two antigens result from a spatial change in the protein framework of the G145R mutant.

4 Discussion

As with wild-type HBV, it is important for the clinical diagnosis and epidemiologic investigation of HBV IEMS infection to detect genes, antigens and antibodies. The methods, positive rate and positive coverage of the detections are various, and the clinical significances are also different [19–22]. Since the early 1990s, many researchers have studied the difficulties in the detection of IEM HBsAg. They attempted to increase the clinical detection rate of HBsAg using kits coated with polyclonal antibodies, high-affinity antibodies against the non "a" determinant of HBsAg or a group of mAb against the "a" determinants of different IEM HBsAgs to prevent common health problems caused by false-negative diagnoses of the HBV infection [20–22].

Since 2000, many domestic ELISA kits have been able to detect IEM HBsAg to some degrees. However, detection products that have similar detection rates as Abbott chemiluminescence are not available. We attempted to prepare an mAb for the reliable clinical diagnosis of HBV infection and obtained one hybridoma cell line that secreted an anti-HBsAg antibody named "G6". Systematic assessments demonstrated that this cell line could secrete an antibody with high efficiency and the secreted antibody was equal to or better in specificity and affinity than the corresponding antibody in the currently available commercial kits. The sensitivity of G6 ELISA was no less than 0.125 ng/mL based on its ability to detect the wild-type HBsAg.

Further analysis suggested that the binding ability of this mAb with IEM rHBsAg had some of the following characteristics: (1) This mAb could detect more (12/15) IEM rHBsAgs than the mAbs previously reported and its reaction degree showed an evident character that was either high or low. In addition, its capability of detecting IEM rHBsAg was better than that of commercial kits [23,24]. (2) Its ability to bind with IEM HBsAg with mutations at the amino acid sequence sites 139, 145, 144, 143 and 142 located in the "a" determinant of the antigenic loop II and the plasmid production levels was equal to that of the wild-type HBsAg. It was markedly weaker at binding with the site replacement mutants that had mutations located at either the beginning of or nearby the "a" determinant loop I, including amino acid sequence sites 120, 122, 123, and 124. (3) Its binding ability with site 126 mutant (in the "a" determinant loop I) was equal to the wild-type HBsAg, and there existed differences in the ability of the mAb to bind with site 129 mutants with different amino acid replacements. (4) There were no specific rules regarding the ability of the mAb to bind with the multi-site mutants. However, HBsAgs with mutations in the middle or the tail of the "a" determinant loop II could, to some degrees, improve the poor reaction caused by mutations in the beginning of loop I. The mAb was able to bind to HBsAbs with mutations outside the "a" determinant as well as it could do to the wild-type.

The relationship between the structure of IEM HBsAg and the degree of the antigenic shift has attracted much attention. He *et al* [25,26] found that the main reason for the decline in the affinity of the IEM mutant to the primary antibody (which was for wild-type HBsAg) was the difference in the water affinity between the original amino acid and the substitute. Hou *et al* demonstrated that in two patients infected by an IEM HBV with mutations in amino acids 121 to 124, the IEM HBsAg was not positively detected, even when using the anti-HBs polyclonal antibody [27]. The results were verified by our study. On the other hand, both sites 122 and 160 were the key amino acid sites for determining the HBV serotype [28], suggesting that there was some special association between the antigenic shift in special sites (e.g., the antigen binding site of anti-HBs G6 mAb) and the group determinant of HBV, and strong antigenic shift was highly associated with the mutation site.

The blocking tests demonstrated that the antigen binding sites of anti-HBs A11 and anti-HBs Mt mAb were independent of each other. Anti-HBs G6 mAb could block the reactions of anti-HBs A11 and anti-HBs Mt with r-wHBsAg, but the degree of blocking was limited, and its ability to block anti-HBs A11 was lower than the latter, suggesting that the corresponding antigen determinant of anti-HBs G6 mAb was independent of anti-HBs A11 and anti-HBs Mt mAb in the primary structure. In addition, it suggested that there was a significantly larger spatial distance between the corresponding antigen determinants of anti-HBs G6 mAb and anti-HBs A11 mAb than that of anti-HBs G6 mAb and anti-HBs MT mAb. Because the specificity of the anti-HBs A11 mAb to the "a" determinant (or the nearby sites) of HBsAg is most definite, we conclude that there exists some distance in the amino acid sequence between the corresponding antigen determinant of anti-HBs G6 mAb and the HBsAg "a" determinant.

In this study, serum samples were examined using a G6 ELISA and two commercial ELISA kits (B and C). The results demonstrated that G6 ELISA and commercial

ELISA kits B and C had similar sensitivities (0.125, 0.25 and 0.125 ng/mL, respectively), and the positive rate was 19.21% (34/177 cases), 6.21% and 14.89%, respectively. The negative serum was correctly identified by the G6 ELISA and the two commercial ELISA kits. Together, these data indicate that the G6 ELISA has a stronger capability to detect HBsAg than the commercial ELISA kits. The G6 mAb had a strong sensitivity for wild-type HBsAg (0.125 ng/mL), same as the commercial ELISA kits; its blocking efficiency against G145R HBsAg was greater than that against wild-type HBsAg when coated with the anti-HBs Mt mAb (Table 3); it detected more IEM HBsAgs and its ELISA signal intensity was stronger than the tested commercial ELISA kits and/or anti-HBs mAbs (Tables 2 and 3), and we presume that the sera were detected as HBsAg positive only by using the G6 ELISA containing IEM HBV. The recent studies by Ijaz and Louisirirotchanakul *et al* have also provided some clinical evidence [29,30].

References

1. Carman W F. The clinical significance of surface antigen variants of hepatitis B virus. J Viral Hepat, 1997, 4(Suppl 1): 11–20
2. Kao J H, Chen D S. Global control of hepatitis B virus. Lancet Infect Dis, 2002, 2(7): 395–403
3. Günther S, Sommer G, Plikat U, Iwanska S, Wain-Hobson S, Will H, Meyerhans A. Naturally occurring hepatitis B virus genomes bearing the hallmarks of retroviral G → A hypermutation. Virology, 1997, 235(1): 104–112
4. Hunt C M, Mcgill J M, Allen M I, Condeady L D. Clinical relevance of hepatitis B viral mutations. Hepatology, 2000, 31(5): 1037–1044
5. Kfoury Baz E M, Zheng J, Mazuruk K, Van Le A, Peterson D L. Characterization of a novel hepatitis B virus mutant: demonstration of mutation-induced hepatitis B virus surface antigen group specific "a" determinant conformation change and its application in diagnositic assays. Transfus Med, 2001, 11(5): 355–362
6. Weber B. Genetic variability of the S gene of hepatitis B virus: clinical and diagnostic impact. J Clin Virol, 2005, 32(2): 102–112
7. Ogura Y, kurosaki M, Asshina Y, Asahina Y, Enomoto N, Marumo F, Sato C. Prevalence and significance of naturally occurring mutations in the surface and polymerase genes of hepatitits B virus. J Infect Dis, 1999, 180(5): 1444–1451
8. Poovorawan Y, Theamboomlers A, Chongsrisawat V, Sanpavat S. Molecular analysis of the a determinant of HBsAg in children of HBeAg –positive mother upon failure of postexposure prophylaxis. Int J Infect Dis, 1998, 2(4): 216–220
9. Ngui S L, O'Connell S, Eglin R P, Heptonstall J, Teo C G. Low detecion rate and maternal provernance of hepatitis B virus S gene mutants in cases of failed postnatal immunoprophylaxix in England and Wales. J Infect Dis, 1997, 176(5): 1360–1365
10. Ly T D, Servant-Delmas A, Bagot S, Gonzalo S, Férey M P, Ebel A, Dussaix E, Laperche S, Roque-Afonso A M. Sensitivities of four new commercial hepatitis B virus surface antigen (HBsAg) assays in detection of HBsAg mutant forms. J Clin Microbiol, 2006, 44(7): 2321–2326
11. Wuhan Institute of Biological Products in China. Manufactory protocol of seral HBV vaccine. Inner data in the research shop of HBV vaccine manufacture, Wuhan Institute of Biological Products in China. 1987, 76–89
12. Li F H, Yang D L, Yu Z Q, Wang J C, Wang Y K, Hao L J. Establishment of a cell line with anti-monoclonal antibody with two-fold immunoreactivities against both HBsAg and HBcAg. Tong Ji Yi Ke Da Xue Xue Bao,1994, 23(4): 288–291 (in Chinese)
13. Wuhan Institute of Biological Products in China. Research on the one step quickly hepatitis B virus detection ELISA kits. In research on HBs antigen and HBe antigen detection ELISA Kits with monoclonal antibody (Inner data in Wuhan Institute of Biological Products in China). 1993, 33–47
14. TianY J, Qin L, Zhang Z M, Li L, Lie Y C, Yu Z Q, Zhao X P, Yang D L. Study on a transgenic cell line stable expression Cly145Arg mutation HBsAg. Zhong Guo Bing Du Xue, 2005, 20(6): 574–577 (in Chinese)
15. Zhang B, Li F H, Gong J S, Tian Y J, Zhang C Y, Li S J, Chen Y, Huang Y G, Yang D L. Development and Application of Affinity Chromatography for Purification of Recombinant G145R HBsAg. Zhong Guo Sheng Wu Zhi Ping Xue Za Zhi, 2007, 20(10): 767–770 (in Chinese)
16. Tian Y J, Zhang Z H, Li L, Liu S P, Xu Y, Lu M J, Yang D L. Screening and application of a monoclonal antibody against variant hepatitis B surface antigen. Mian Yi Xue Za Zhi, 2008, 24(1): 56–60 (in Chinese)
17. Qian C, Jiang P, Lu C, Yao K. Preparation and characterization of anti-epitopes of SARS coronavirus M protein monoclonal antibody. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi, 2006, 22(2): 213–217 (in Chinese)
18. Zhang C Y, Gong J S, Zhang X Y, Chen Y, Liu J H, Zhang B, Peng J, Huang Y G, Li F H. detection of the serum G145R mutant relative HBsAg and its clinical significant. Zhong Guo Mian Yi Xue Za Zhi, 2008, 24(12): 63–66 (in Chinese)
19. Zhang D H, Lu Z M. Diagnology of Infectious Disease(in Chinese). Shanghai: Publishing House of Shanghai Science and Technology, 2007, 33–41
20. Weber B. Diagnostic impact of the genetic variability of the hepatitis B virus surface antigen gene. J Med Virol, 2006, 78(Suppl 1): S59–S65
21. Liu J H, Huang Y G, Zhang C Y, Peng J, Zhang X Y, Gong J S, Chen Y, Li F H. Development of a method to detect immune escape mutant HBsAg of widely and its initial sue in clinic. Zhong Guo Shi Yang Zheng Duan Xue, 2008, 12(10): 1199–1203 (in Chinese)
22. Zhang J L, Chen J, Li M N, Zhang L, Zhang J, Guo J J, Xie J. Antivariant hepatitis B surface antigen monoclonal antibody and another eight hepatitis B detectors used for quarantine and their work-efficiency comparison. Zhong Guo Yao Wu Yu Lin Chuang, 2005, 5(11): 814–815 (in Chinese).
23. Cooreman M P, van Roosmalen M H, te Morsche R, Sünnen C M, de Ven E M, JansenJ B, Tytgat G N, de Wit P L, Paulij W P. Characterization of the reactivity pattern of murine monoclonal antibodies against wild type hepatitis B surface antigen to G145R and other naturally occurring a loop escape mutations. Hepatology,

1999, 30(5): 1287–1291

24. Levicnik-Stezinar S. Hepatitis B surface antigen escape mutant in a first time blood donor potentially missed by a routine screening assay. Clin Lab, 2004, 50(1,2): 49–51
25. He J W, Wu L,Yiao X, Li H M, Wen Y M. Antigen expression and DNA immunization of 129Leu and 145Arg variants of hepatitis B virus S gene. Zhong Hua Wei Sheng Wu Xue He Mian Yi Xue Za Zhi, 1999, 19(3): 201–205 (in Chinese)
26. Liu F, Zhang Y P, Ma Z M, Wu L, Wen Y M, He L F. Studies on the mechanism and clinical significance of low immunogenicity of hepatitis B virus“a”determinant 129 leu variant. Zhong Hua Wei Sheng Wu Xue He Mian Yi Xue Za Zhi, 2002, 22(3): 279–283 (in Chinese)
27. Hou J, Karayiannis P, Waters J, Luo K, Liang C, Thomas H C. A unique insertion in the S gene of surface antigen-negative hepatitis B virus Chinese carriers. Hepatology, 1995, 21(2): 273–278
28. Schaefer S. Hepatitis B virus: Significance of genotypes. J Viral Hepat, 2005, 12(2): 111–124
29. Ijaz S, Torre F, Tedder R S, Williams R, Naoumov N V. Novel immunoassay for the detection of hepatitis B surface ‘escape’ mutants and its application in liver transplant recipients. J Med Virol, 2001, 63(3): 210–216
30. Louisirirotchanakul S, Kanoksinsombat C, Theamboonlert A, Puthavatana P, Wasi C, Poovorawan Y. Mutation of the “a” determinant of HBsAg with discordant HBsAg diagnostic kits. Viral Immunol, 2004, 17(3): 440–444