Acta Medica Okayama

Volume 38, *Issue* 2 1984 *Article* 12

APRIL 1984

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Takao Tsuji[*] Kenji Takahashi[†]

Kunihiko Naito[‡] Hideo Nagashima[**]

[*]Okayama University,

[†]Okayama University,

[‡]Kumayama Municipal Hospital,

[**]Okayama University,

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Takao Tsuji, Kenji Takahashi, Kunihiko Naito, and Hideo Nagashima

Abstract

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*PMID: 6731028 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **38,** (2), 175-180 (1984)

DETECTION OF SERUM BLOCKING FACTORS AND ANTIBODIES TO THE ALBUMIN RECEPTOR ON HBsAG PARTICLES IN HEALTHY PERSONS AND PATIENTS WITH LIVER DISEASES

Takao TSUJI, Kenji TAKAHASHI, Kunihiko NAITO* and Hideo NAGASHIMA**

*Health Research Center, Okayama University, Okayama 700, Japan, * Department of Internal Medicine, Kumayama Municipal Hospital, Okayama 709-08, Japan and ** First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan*

Received November 16, 1983

Abstract. An enzyme-linked immunosorbent assay (ELISA) for the detection of serum blocking factors (BF), or antibodies to the albumin receptor on HBsAg particles, was developed, and its clinical usefulness was examined in healthy persons and patients with liver diseases. Thirteen of 80 anti-HBs-positive female (16.3 %) had BF, but all 25 male anti-HBs-positive, 41 female and 32 male anti-HBs-negative subjects were negative for BF. The activity of BF in BF-positive cases was not associated with the positive reciprocal hemagglutination titer of anti-HBs. For a neutralization test of BF, the BFs from 5 cases were absorbed with IgG-immunobeads. It was determined that these IgG-BFs were antibodies to the albumin receptors on HBsAg particles. No significance between positive-BF and abnormal S-GPT levels was recognized. These results suggest that the present test for the detection of BF, or anti-albumin receptor antibody, different from anti-HBs, might be useful for diagnosis of hepatitis B and as a marker for HB virus.

Key words : HBV, blocking factor to albumin receptor, antibody to albumin receptor, albumin receptor, ELISA.

The importance of polymerized human serum albumin (pHSA) in the adherence of hepatitis B virus (HBV) to human and chimpanzee hepatocytes has been confirmed (1). The binding-site of HBV with pHSA seems to be in an albumin receptor (1, 2). Recently, it was reported that the albumin receptor might be associated with another polypeptide determinant different from the antigenic determinant of HBsAg (3-5). However, a host immune response to the albumin receptor is unknown. In the present study, an enzyme-linked immunosorbent assay (ELISA) for detecting the binding activity of pHSA (pHSA-BA) (6) to albumin receptor was used as a test to detect the serum blocking factors (BF) or the antibodies to the HBV albumin receptor.

MATERIALS AND METHODS

Serum samples were obtained from 105 anti-HBs-positive persons (93 healthy and 12 with abnormal S-GPT levels) and 73 anti-HBs-negative persons (51 healthy and 22 with ab-

normal S-GPT levels). For the purpose of detecting anti-HBs and HBsAg, passive hemagglutination (PHA) and reversed passive hemagglutination (RPHA) (Fujizoki kits, Japan) were routinely performed, respectively. For detecting the binding of BF to albumin receptor on HBsAg particles, ELISA was used for the detection of pHSA-BA, as previously reported (6). Anti-HBs-coated microplates were prepared according to the method of Wolters *et al.* (7).

For the polymerization of human serum albumin (pHSA) and preparation of horseradish peroxidase (HPRO)-labelled pHSA, the methods of Lenkei *et al.* (8) and Nakane *et al.* (9) were used. The concentration of protein of the pooled conjugate was 500 μg per ml. The conjugate was diluted 100 times with 1 % BSA, 0.05 % Triton X-100 and 20 mM Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl (BSA-Triton-TBS), and divided into 3.6 ml lots. Then, 0.1 ml of HBeAg-positive standard serum (RIA cut-off ratio of HBeAg 7.0) was diluted 20-times with 0.05 % Triton X-100, and 20 mM Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl (Triton-TBS), was added to an anti-HBs-coated well. The mixture was incubated at 37 ℃ for 1 h in a moist chamber. The wells were aspirated and washed 3 times with Triton-TBS. After washing, 0.05 ml of the test serum and 0.05 ml of Triton-TBS were added to each well, incudated at 37 ℃ for 1 h in a moist chamber, removed by aspiration, and washed 3 times with Triton-TBS. Then, 0.1 ml of the HRPO-labelled pHSA was added to each well, to incubated at 37 ℃ for 1 h in a moist chamber, and removed by aspiration. The wells were afterwards washed 4 times with Triton-TBS. A freshly prepared solution (0.1 ml) of o-phenylene diamine (0.4 mg/ml) and urea peroxide (0.2 mg/ml) in 0.4 M phosphate-citrate buffer (pH 5.0) was added to each well and incubated in the dark at room temperature for 50 min. The enzyme reaction was stopped by adding 0.05 ml of 4 N sulfuric acid. The absorbance of the brown product of the enzyme reaction was measured at 492 nm with a Photo-Elisa II type colorimeter (Organon, Oss, Netherlands). The mean optical density (OD) of 10 HBV marker negative-healthy persons was 3.37 $\pm$ 0.13 (m $\pm$ SD). The blocking percentage of BF was calculated by the inhibition percent of ELISA for pHSA-BA as follows ;

$$\text{Positive blocking } \% = \frac{[\text{Mean optical density (OD) of sera from 10 healthy persons}] - (\text{OD of test serum})}{(\text{Mean OD of sera from 10 healthy persons})} \times 100$$

$\geqslant$ 8.0 (2 $\times$ SE) %

For examining the immunoglobulin class of BF, immunobeads [IgG (Immnobead RAH-3), IgA (Immunobead RAH-13) and IgM (Immunobead RAH-21) : Bio-Rad Laboratories, U.S.A.] were used. The immunobeads were washed 2 times with Triton-TBS and adjusted to the original concentration. Then, 0.07 ml of the test serum and 0.07 ml of the washed-immunobead were mixed and incubated at 37 ℃ for 1 h. After incubation, the mixture was centrifuged at 12,000 r.p.m. for 5 min, and 0.1 ml of the supernatant was added to each HBsAg-reacted well. After incubation, the above mentioned procedure was repeated. The neutralization percentage was calculated by :

$$\text{Neutralization } \% = \frac{(\text{OD of test serum} + \text{immunobead}) - (\text{OD of test serum})}{(\text{Mean OD of the sera of 10 healthy persons}) - (\text{OD of test serum})} \times 100$$

S-GPT level and other liver function tests were carried out in fresh sera by the routine methods. For examining the host immune response excluding anti-HBs, antibody to insoluble liver cell membrane antigen (anti-LM) was detected by our previously reported indirect immunofluorescence technique using acetone-fixed rat liver section (10).

RESULTS

The frequency of the detection of BF in sera from healthy persons and patients with abnormal S-GPT levels is shown in Table 1. The cases with positive BF were restricted to anti-HBs-positive females. Thirteen of 80 anti-HBs-positive

TABLE 1. THE FREQUENCY OF THE DETECTION OF SERUM BLOCKING FACTOR (BF) TO THE ALBUMIN RECEPTOR ON HBsAG PARTICLES IN SERA FROM HEALTHY PERSONS AND PATIENTS WITH ABNORMAL S-GPT LEVELS

Reciprocal PHA titer of anti-HBs	Female			Male			Total		
	No. tested	BF to albumin receptor		No. tested	BF to albumin receptor		No. tested	BF to albumin receptor	
		No. positive	%		No. positive	%		No. positive	%
≥ 5120	2(0)	1(0)		0	0		2(0)	1(0)	
2560	5(0)	4(0)		2(0)	0		7(0)	4(0)	
1280	7(1)	4(1)		0	0		7(1)	4(1)	
640	6(0)	1(0)		2(1)	0		8(1)	1(0)	
320	25(3)	2(0)	8.0	6(1)	0		31(4)	2(0)	6.5
160	13(2)	0	0	5(0)	0		18(2)	0	
80	15(2)	0	0	7(2)	0		22(4)	0	
40	7(0)	1(0)		3(0)	0		10(0)	1(0)	
Total of anti-HBs positive	80(8)	13(1)	16.3	25(4)	0	0	105(12)	13(1)	12.4
< 20	41(11)	0	0	32(1)	0	0	73(22)	0	0

( )=No. of patients with abnormal S-GPT levels

TABLE 2. CLINICAL FEATURES OF 13 FEMALE SUBJECTS WITH POSITIVE SERUM BLOCKING FACTORS TO ALUBIMIN RECEPTOR ON HBsAG PARTICLES

Case No.	Age	Histroies of		Reciprocal PHA titer of anti-HBs	Grade of anti-LM
		blood transfusion	hepatitis		
181	74	−	−	320	+
235	46	+	−	2560	−
236	57	−	−	1280	−
305	76	−	+	2560	±
394	48	+	−	640	±
444	56	−	−	40	+
470	46	−	−	1280	+++
493	58	−	−	1280	++
665	66	−	−	320	+
821	36	−	−	2560	−
824	61	−	+	1280	+
885	56	−	−	≥ 5120	±
1035	36	−	−	320	+

Only case 824 had abnormal liver function test results (S-GPT=76 IU, ZTT=20.5 u).

Table 3. Thirteen females with positive serum blocking factors (BF) to albumin receptor on HBsAG particles, and the activity of BF

Case No.[a]	Blocking % (Activity of BF)	Neutralization % by use of various immunobeads		
		IgG	IgA	IgM
181	21.8	44.3	< 4.0	< 4.0
235	89.4	< 4.0	< 4.0	< 4.0
236	18.0	21.6	36.5	26.6
305	70.5	< 4.0	< 4.0	< 4.0
394	43.5	14.0	< 4.0	< 4.0
444	8.7	57.1	31.2	25.9
470	24.4	26.1	< 4.0	< 4.0
493	16.2	46.8	< 4.0	< 4.0
665	16.0	38.2	< 4.0	< 4.0
821	61.5	< 4.0	< 4.0	< 4.0
824	54.8	< 4.0	< 4.0	< 4.0
885	61.3	< 4.0	< 4.0	< 4.0
1035	54.4	< 4.0	< 4.0	< 4.0

a The same subjects as in Table 2.

females (16.3 %) showed positive BF, but all 25 male anti-HBs-positive, 41 female anti-HBs-negative and 32 male anti-HBs-negative subjects showed negative BF. In an abnormal S-GPT group, 1 of 8 anti-HBs-positive females (12.5 %) showed positive BF. No significance between the detection of BF in healthy persons and in patients with abnormal S-GPT levels was observed. The clinical features of 13 BF-positive subjects are shown in Table 2, and the immunoglobulin class of BF is shown in Table 3. Sera from Case No. 181, 394, 470, 493 and 665 contained the IgG class of BF. In sera from Case No. 236 and 444, all 3 types of immunobeads inhibited the activity of BF. The sera of the other 6 cases were not inhibited by any of the types of immunobeads. Sera with BF-IgG also contained anti-LM, and all of the serum donors were healthy.

DISCUSSION

Infection with HBV has been associated with three distinct viral antigens (HBsAg, HBcAg and HBeAg), separate antibodies, a virus-specific DNA polymerase, and a circular, double strand molecule of DNA (11-13). Each of these HBV-associated antigens can induce an antibody response in humans. Sensitive methods for detecting an antibody to HBsAg (Anti-HBs) (14, 15), an antibody to HBcAg (anti-HBc) (16) and an antibody to HBeAg (anti-HBe) (17) have been developed, and these antibody responses have been well characterized. However, the antibody response to the binding-site of HBV with pHSA (anti-albumin receptor antibody) is not yet clear.

In the present study, the authors developed an ELISA method for detecting

the serum blocking factor (BF) or anti-albumin receptor antibody, and examined the clinical usefulness of its detection in healthy persons and patients with abnormal S-GPT levels. As a result, BF was detected in sera from 13 (including 1 patient with abnormal S-GPT level) of 80 anti-HBs-positive females (including 8 patients with abnormal S-GPT levels) (16.3 %), but not in 25 male anti-HBs-positive and 73 anti-HBs-negative subjects (41 females and 32 males). The immunoglobulin-class of BF, of 5 cases was IgG, and 2 cases with low blocking % of BF showed 3 immunoglobulin classes (IgG, IgA and IgM) of BF. These BFs showing immunoglobulins might be the antibodies to the albumin receptor on HBsAg particles. However, the components of other BFs not absorbed with immunobeads are not clear. In the antibody response to other target antigens, excluding HBV-associated antigens, anti-LM (10, 18) different from antibody to liver specific lipoprotein (anti-LSP) was detected in 4 of 5 IgG BF-positive females.

These results suggest that the present ELISA test for detecting anti-albumin receptor antibodies or the blocking factors (BF) different from anti-HBs, might be useful for diagnosis of hepatitis B and as a marker for prophyraxis of HBV.

Acknowledgements. The authors wish to express their profound thanks to Professor Takahiro Yamabuki and Miss Suzue Mimura of the Health Research Center, Okayama University.

This work was supported by a Grant-in-Aid (No. 58570321) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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