Table 1 Baseline characteristics and treatment of 24 ATL patients with resolved HBV infection who underwent HBV DNA monitoring following systemic chemotherapy

	HBV reactivation (+) $n = 3$	HBV reactivation (−) $n = 21$	p value
Median age (range)	59 (58–65)	64 (41–77)	0.822
Sex			0.217
Male	3	9	
Female	0	12	
ATL type of disease			0.090
Acute	1	17	
Lymphoma	2	1	
Chronic	0	2	
Smoldering	0	1	
ECOG performance status			0.530
0 or 1	3	14	
2 or more	0	7	
Baseline HBV status			1.00
Anti-HBc positive and anti-HBs positive	3	18	
Anti-HBc positive and anti-HBs negative	0	3	
Anti-HBc negative and anti-HBs positive	0	0	
Baseline anti-HBs titers (mIU/mL)			0.728
<10	0	3	
≥10, <100	2	8	
≥100	1	10	
Initial chemotherapy regimen[a]			0.396
CHOP	0	7	
VCAP-AMP-VECP	3	10	
Others	0	4	
Mogamulizumab administration[b]			0.576
(+)	2	9	
(−)	1	12	
Allogeneic HSCT[c]			1.00
(+)	1	5	
(−)	2	16	
Year enrolled for HBV DNA monitoring			–
2005–2006	0	0	
2006–2008	0	4	
2008–2009	0	3	
2009–2013	3	14	
Median HBV DNA follow-up time (range)[d]	640 (637–1030)	227 (57–1420)	–

HBV hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *ECOG* Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisolone, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone), *HSCT* hematopoietic stem cell transplantation

[a] Initial chemotherapy regimen for adult T-cell leukemia–lymphoma was given during HBV DNA monitoring

[b] In 2 of 3 HBV-reactivated cases, mogamulizumab was given prior to HBV reactivation

[c] One patient received allogeneic hematopoietic stem transplantation after HBV reactivation

[d] HBV DNA follow-up time indicates the time from the date of baseline HBV DNA measurement until the date of the last HBV DNA measurement

was effective in preventing hepatitis due to HBV reactivation in all three patients. Most of HBV reactivation has been reported to occur in B-cell lymphoma, especially in those who received rituximab-containing chemotherapy [2–4, 6]. This is the first report regarding the risk of HBV reactivation focused on ATL patients with resolved HBV infection, which suggesting that the risk of HBV reactivation in ATL patients may be similar to that in B-cell lymphoma patients [15, 16].

ATL is a mature T-cell lymphoma and human T-cell leukemia virus type-1 plays a role in its pathogenesis. Aggressive ATL has been reported to have a poor prognosis with a median overall survival of approximately 1 year, regardless of intensive chemotherapy [17]. The anti-CCR4 monoclonal antibody, mogamulizumab has been shown recently to be effective and safe for aggressive ATL patients in the setting of monotherapy or combined with conventional chemotherapy [9, 11, 18]. It is expected that mogamulizumab will enable long-term disease control, so more HBV reactivation events may be predicted because CCR4 is a chemokine receptor expressed on T-helper type 2 and regulatory T cells [7, 19], and is thought to have an important

Table 2 Characteristics of 3 patients with HBV reactivation

	Case 1	Case 2	Case 3
Age	65	59	58
Sex	Male	Male	Male
Type of ATL	Lymphoma	Lymphoma	Acute
ECOG performance status	1	1	0
Baseline HBV status			
HBsAg	(−)	(−)	(−)
Anti-HBc titers	98.1 %	3.6 C.O.I	1.5 C.O.I
Anti-HBs titers	20.0 mIU/mL	24.0 mIU/mL	>1000.0 mIU/mL
HBV DNA levels	Not detectable	Not detectable	Not detectable
Chemotherapy regimens before HBV reactivation			
	VCAP-AMP-VECP plus mogamulizumab	VCAP-AMP-VECP	VCAP-AMP-VECP Mogamulizumab CHOP DeVIC etc.
Number of regimens	1	1	7
Allogeneic HSCT[a]	No	Yes	No
After HBV reactivation			
Time to reactivation (day)[b]	90	71	541
HBV DNA levels at reactivation (log copies/mL)	<2.1	<2.1	<2.1
Peak HBV DNA levels (log copies/mL)	2.3	<2.1	<2.1
Anti-HBs titers	17.6 mIU/mL	22.0 mIU/mL	566.5 mIU/mL
HBV genotype	C	C	C
HBV mutation of precore region or basal core promoter	Wild	Wild	NA
Antiviral drugs	Entecavir, lamivudine	Entecavir	Entecavir
Hepatitis due to HBV reactivation	No	No	No
HBV DNA follow-up time (day)[c]	1030	640	637
Outcome	Alive (CR1)	Alive (CR1)	Death due to ATL progression

HBV hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *ECOG* Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone), *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisolone, *DeVIC* dexamethasone, etoposide, ifosfamide, carboplatin, *CR1* first complete response

[a] One patient (case 2) received allogeneic hematopoietic stem transplantation after HBV reactivation

[b] Time to reactivation indicates the time from the date of baseline HBV DNA measurement until the date of the confirmation of HBV reactivation

[c] HBV DNA follow-up time indicates the time from the date of baseline HBV DNA measurement until the date of the last HBV DNA measurement

role in maintaining the balance of the human immune system. The mechanism whereby mogamulizumab causes HBV reactivation is not fully understood; a reduction of numbers of CCR4-expressing cells following this antibody treatment might be associated with an imbalance of antiviral immunity, resulting in the development of HBV reactivation [9, 13]. Although HBV reactivation was confirmed in 2 of 11 patients who received mogamulizumab, this study did not prove that HBV reactivation is associated with mogamulizumab therapy, partly because of the small sample size.

This study has the following limitations: a retrospective study in a single institution with a small sample size, and the diagnosis of HBV reactivation at early stage when only when HBV DNA became detectable (below 2.1 log copies/mL) by PCR. Because antiviral treatments after the onset of hepatitis are often insufficient to control HBV reactivation, preemptive antiviral therapy guided by regular HBV DNA monitoring, whereby the antiviral drug is given immediately when HBV DNA becomes detectable, is recommended by some guidelines to prevent hepatitis due to HBV reactivation [20, 21]. However, the definition of HBV reactivation and cut-off values of HBV DNA levels, along with the timing of initiation of antiviral treatment in patients with resolved HBV infection, have not been fully investigated yet.

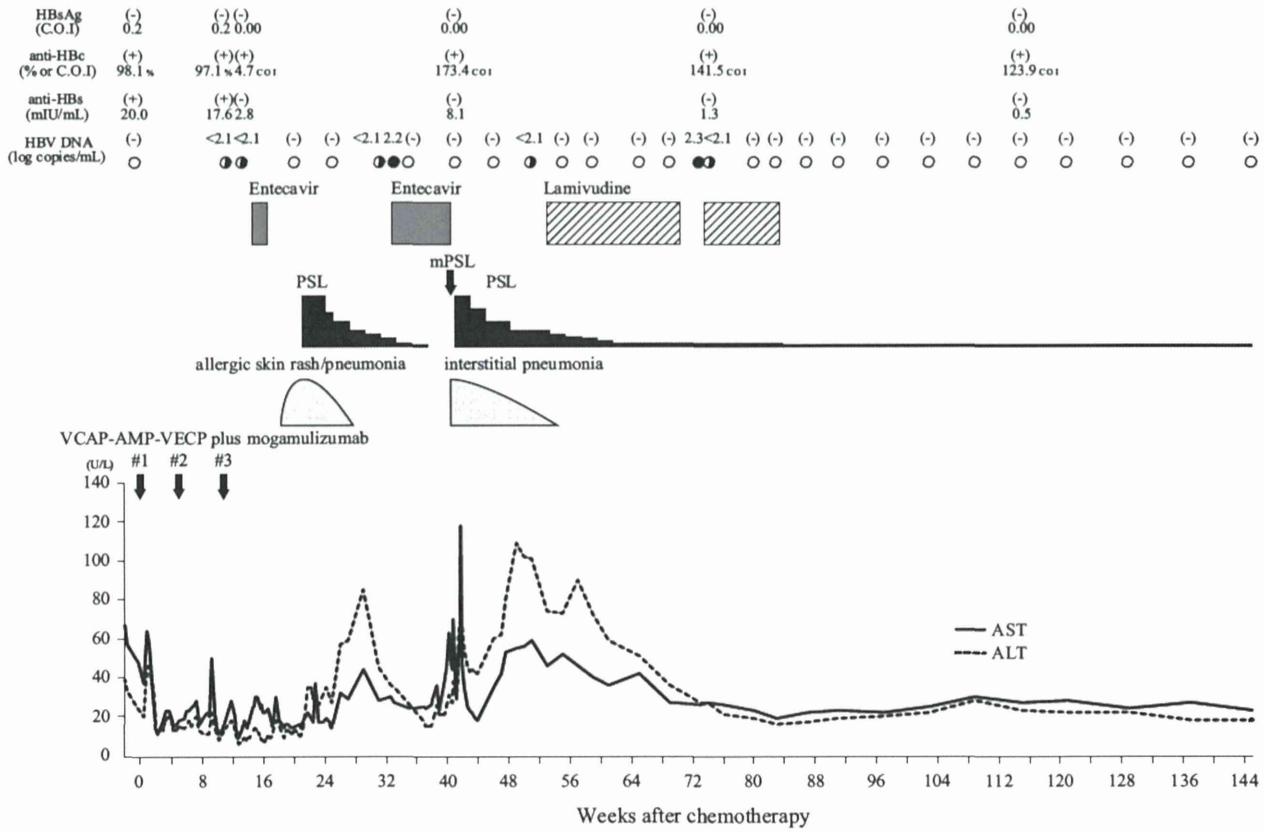

Fig. 2 Clinical course of case 1. A 65-year-old male was diagnosed as having adult T-cell leukemia–lymphoma of lymphoma type and received VCAP-AMP-VECP plus mogamulizumab combined chemotherapy. At 3 months after systemic chemotherapy, HBV reactivation was confirmed with HBV DNA levels below 2.1 log copies/mL and antiviral therapy (entecavir, 0.5 mg/day) was given immediately with no HBV-related hepatitis. He presented with elevation of transaminase levels after detection of HBV DNA, it considered not viral hepatitis but drug-induced liver damage because of transient and slight increase of HBV DNA levels. Because he suffered from an allergic rash and interstitial pneumonia (IP), entecavir could not be continued. Consequently, reemergence of HBV (HBV DNA levels of 2.2 log copies/mL) was observed at 3 months after the first detection of HBV reactivation. However, he discontinued entecavir because of the occurrence of IP, and HBV reactivation was again observed. Lamivudine was given for HBV reactivation, but was discontinued due to mild renal dysfunction, which resulted again in replication of HBV (HBV DNA levels of 2.3 log copies/mL) at 18 months after initiating mogamulizumab-containing chemotherapy. The *open circles* show undetectable PCR signals during HBV DNA monitoring; the *half-filled circles* show PCR signals indicating HBV DNA levels below 2.1 log copies/mL; and the *filled circles* show detectable PCR signals indicating HBV DNA levels of 2.1 log copies/mL or more. *HBV* hepatitis B virus, *PSL* prednisolone, *mPSL* methylprednisolone, *AST* aspartate transaminase, *ALT* alanine aminotransferase, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone)

In conclusion, the incidence of HBV reactivation was 12.5 % in ATL patients with resolved HBV infection following systemic chemotherapy. In mogamulizumab era, further well-designed prospective studies are warranted to estimate the incidence of HBV reactivation and to establish regular HBV DNA monitoring-guided preemptive antiviral therapy for these patients.

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RESEARCH ARTICLE

Validation of Cross-Genotype Neutralization by Hepatitis B Virus-Specific Monoclonal Antibodies by *In Vitro* and *In Vivo* Infection

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Abstract

Vaccines based on hepatitis B virus (HBV) genotype A have been used worldwide for immunoprophylaxis and are thought to prevent infections by non-A HBV strains effectively, whereas, vaccines generated from genotype C have been used in several Asian countries, including Japan and Korea, where HBV genotype C is prevalent. However, acute hepatitis B caused by HBV genotype A infection has been increasing in Japan and little is known about the efficacy of immunization with genotype C-based vaccines against non-C infection. We have isolated human monoclonal antibodies (mAbs) from individuals who were immunized with the genotype C-based vaccine. In this study, the efficacies of these two mAbs, HB0116 and HB0478, were analyzed using in vivo and in vitro models of HBV infection. Intravenous inoculation of HBV genotype C into chimeric mice with human hepatocytes resulted in the establishment of HBV infection after five weeks, whereas preincubation of the inocula with HB0116 or HB0478 protected chimeric mice from genotype C infection completely. Interestingly, both HB0116 and HB0478 were found to block completely genotype A infection. Moreover, infection by a genotype C strain with an immune escape substitution of amino acid 145 in the hepatitis B surface protein was also completely inhibited by incubation with HB0478. Finally, in vitro analysis of dose dependency revealed that the amounts of HB0478 required for complete protection against genotype C and genotype A infection were 5.5 mIU and 55 mIU, respectively. These results suggested that genotype C-based vaccines have ability to induce cross-genotype immunity against HBV infection.

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Introduction

Hepatitis B virus (HBV) is a blood-borne, hepatotropic virus that infects an estimated 350 million people worldwide. Besides the manifestations associated with acute hepatitis, chronic HBV infection constitutes a significantly high risk for the development of liver cirrhosis and hepatocellular carcinoma. HBV strains are classified into eight genotypes based on genetic diversity [1,2] and the prevalence of these genotypes varies geographically [3]. Hepatitis B surface antigen (HBsAg) is the key molecule for HBV entry into the hepatocyte [4] and HBV vaccination establishes host immunity by activating B lymphocytes that produce HBsAg-specific antibodies (anti-HBs) with neutralizing activities. The highly immunogenic region of HBsAg, known as the "a" determinant, comprises two peptide loops in which several amino acids vary among the HBV genotypes [5].

Vaccination of high risk individuals and universal infant/childhood vaccination programs have effectively decreased the incidence of acute HBV infection and consequent chronic hepatitis B [6]. Recombinant vaccines containing HBsAg generated from HBV genotype A2 (gt-A2) have been used worldwide. Although these A2-type vaccines are effective in preventing non-A2 HBV infections [7], investigation of cross-genotype protection is limited in the clinical setting. On the other hand, genotype B (gt-B) and genotype C (gt-C) strains are the most prevalent in east Asian countries [1] and some of these countries, including Japan and Korea, have used recombinant vaccines generated from gt-C for immunoprophylaxis against HBV endemic in these communities [8,9]. In the last decade, however, the spread of gt-A strains imported from foreign countries and the subsequent increase of hepatitis caused by HBV gt-A is a growing concern in Japan [10]. Until now, little is known about whether the gt-C HBV vaccine can induce effective immunity against non-C HBV infection.

Previously, we isolated human monoclonal antibodies (mAbs) against HBV from healthy volunteers who had been immunized with a gt-C type recombinant HBV vaccine (Biimugen), using a cell-microarray system [11–13]. A subsequent report revealed that among these mAbs, HB0116 and HB0478, recognize the first N-terminal peptide loop within the "a" determinant and have HBV-neutralizing activities [14]. In this report, whether these mAbs generated by the gt-C type vaccine can protect gt-A strain infections was investigated using in vitro and in vivo HBV infection models, including primary human hepatocytes (PHHs) and severe combined immunodeficient mice transgenic for urokinase-type plasminogen activator, whose livers were repopulated with human hepatocytes (hereafter referred to as chimeric mice) [15–17]. The neutralizing activities of these mAbs against the frequently isolated immune escape mutant, which has an amino acid substitution of arginine for glycine at residue 145 within the second, C-terminal loop of HBsAg (G145R) [18–20], were also investigated.

Materials and Methods

Ethics statement

This study conformed to the ethics guidelines of the 1975 Declaration of Helsinki as reflected by approval by the Ethics Committee of University of Toyama with written informed consent (Permit Number: 14–123). All animal experiments were carried out in strict accordance with the recommendations in the Guide for the Care Use of Laboratory Animals of the National Institute of Health. The animal protocol was approved by the Ethics Committees of PhoenixBio Co., Ltd (Permit Number: 0253). Chimeric mice were housed in specific pathogen—free facilities at the laboratory of PhoenixBio Co., Ltd. Food and water were delivered ad libitum. Chimeric mice were weighed and anesthetized using isofluorane prior to blood collection from the

orbital vein. The chimeric mice were anesthetized using isofluorane and sacrificed by exsanguination from the heart at the end of the experiment.

HBV-specific mAbs and recombinant peptides

Recombinant HB0116 and HB0478 in IgG form were generated as described previously [14]. Synthetic peptides for the first loop of HBsAg gt-C and gt-A (123–137 gt-C: TCTI-PAQGTSMFPSC; 123–137 gt-A: TCTTPAQGNSMFPSC) were generated also as described previously [14].

The binding activity of each mAb for recombinant peptides was examined by ELISA with streptavidin-coated plates (Nunc, Roskilde, Denmark). Plates were coated with the peptides at 10 μg/mL and nonspecific binding was blocked with PBS containing 3% bovine serum albumin (BSA). Each mAb was added to the wells for 2 hours, followed by washing and reaction with alkaline phosphatase-conjugated anti-human IgG (Sigma, Saint Louis, MO). The O.D. value at 405 nm was evaluated after addition of phosphate substrate (Sigma). Control human monoclonal IgG1 (cIgG, Athens Research & Technology, Athens, GA) was added at the same concentration as the control.

Immunoprecipitation assay

1×10^4 copies of HBV of gt-C, gt-A and G145R (gt-C with an amino acid substitution of arginine for glycine at position 145 of HBsAg) were incubated with 1 μg of mAbs diluted in 2% BSA/PBS or cIgG on a rotating wheel overnight at 4°C and then protein A-Sepharose beads (GE Healthcare) were added to the mixture and incubated for a further 4 hours. The beads were centrifuged briefly to remove the supernatants, washed four times with 1 mL 2% BSA/PBS and resuspended in 30 μL sample loading buffer (Tris/HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0·001% bromophenol blue). After boiling for 5 minutes, 15 μL aliquots were applied to 15% SDS-PAGE and the proteins were separated and transferred to a nitrocellulose membrane. HBsAg was detected using 1 μg/mL of a HB0116/HB0478 mixture, followed by anti-human IgG conjugates of horseradish peroxidase (1:5000, Sigma) as the secondary antibody. The bands were visualized with enhanced chemiluminescence (Amersham Biosciences, Buckinghamshire, UK).

HBV-neutralizing assay using HepaRG cells

The HBV-neutralizing capacities of HB0116 and HB0478 were investigated using the HepaRG cell line (supplied by Biopredic International, Rennes, France). The HepaRG cells were cultured and differentiated as described previously [21,22]. 1×10^4 copies of HBV and 1 μg of each mAb were preincubated for 1 hour at room temperature and then added to HepaRG cells in medium containing 4% polyethylene glycol (PEG) 8000 (Sigma-Aldrich, St. Louis, MO, USA). After overnight incubation, the HepaRG cells were washed gently three times with medium and then cultured with fresh medium. On day 7 after infection, cellular DNA was extracted and HBV DNA was quantified as described previously [14].

In vivo HBV-neutralizing assay using chimeric mice

The chimeric mice were purchased from PhoenixBio Co, Ltd (Hiroshima, Japan). The HBV inocula used in this experiment were prepared as follows: culture supernatants from cells transfected with plasmids expressing HBV gt-C, gt-A, and G145R contained immature HBV virions [16] and chimeric mice were inoculated with these culture supernatants to obtain the

monoclonal and intact infectious virions. After establishing viremia in these mice, the sera were collected and used as inocula after titration in another experimental chimeric mouse.

Firstly, $1x10^4$ copies of the sera of chimeric mice infected with gt-A, gt-C, G145R were incubated at 37°C for 2 hours in the presence of HB0116 and/or HB0478 and injected intravenously into chimeric mice. Five weeks after injection, serum HBV DNA was measured by quantitative polymerase chain reaction (PCR) as reported previously [23].

In vitro HBV-neutralizing assay using PHHs isolated from chimeric mice

Freshly isolated PHHs were purchased from PhoenixBio Co., Ltd (Higashihiroshima, Japan). Briefly, human hepatocytes were collected from the livers of chimeric mice by collagenase perfusion and plated on collagen-coated 96-well multiplates at a density of 6.7×10^4 cells per well. The cells were then grown in dHCGM medium (Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 1 μg/mL of penicillin, 1 μg/mL of streptomycin, 20 mM HEPES, 15 μg/mL of L-proline, 0.25 μg/mL of human recombinant insulin, 50 nM dexamethazone, 5 ng/mL of human recombinant epidermal growth factor, 0.1 mM ascorbic acid, and 2% DMSO).

To investigate HBV kinetics, PHHs were inoculated with serum from HBV gt-C chimeric mice at 5 genomes per cell for 24 hours in the presence of 4% PEG 8000. The sera from chimeric mice contained excess subviral particles including HBs proteins. The cells were then washed three times with the medium to remove the inoculum, and the culture supernatants were collected and replenished with fresh medium on 2, 3, 5, 7, and 12 days post infection (dpi).

To optimize the infectious condition for the analysis of antibody neutralization, HBV gt-C at 10, 3, 1, and 0.3 genomes per cell was preincubated with or without 100 mIU of hepatitis B immune globulin (HBIG) for 2 hours and PHHs were inoculated with the HBV-HBIG mixture for 24 hours with PEG or for 48 hours without PEG. The cells were washed and the supernatants were collected as described above.

Antibody neutralization experiments were performed as follows. HBV gt-C or gt-A inocula at 10 genomes per cells (6.7×10^5 genomes/well) were preincubated with 670, 67, 6.7, or 0.67 ng of HB0478 (corresponding to 550, 55, 5.5, or 0.55 mIU) and exposed to PHHs for 48 hours without PEG. The cells were then washed and the supernatants were collected as described above.

Southern blot analysis of HBV DNA

Southern blot analysis was performed with full-length probes for HBV as described previously [24].

Quantification of HBV DNA, pregenomic RNA and HBsAg

Total RNA and total DNA were extracted from PHHs using ISOGEN (Nippon Gene Co. Ltd., Tokyo, Japan) and SMITEST EX R&D Kit (Genome Science Laboratories, Tokyo, Japan), respectively. Purified total RNA was then reverse-transcribed using a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Extracellular HBV DNA, intracellular HBV DNA and pregenomic RNA were quantified by real-time quantitative PCR using StepOne Plus and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The samples were denatured by incubating for 10 minutes at 95°C and amplified for 45 cycles (95°C 15 seconds, 60°C 60 seconds) with specific primers and TaqMan fluorescent probes. HBV DNA was amplified using primers HBV-F (5′-CACATCAGGATTCCTAGGACC-3′), HBV-R (5′-AGGTTGGTGAGTGATTGGAG-3′), and TaqMan probe HBV-FT (5′-FAM-CAGAGTCTAGACTCGTGGTGGACTTC-TAMRA-3′).

Primers HBV-PC-F (5′-GGTCTGCGCACCAGCACC-3′), HBV-DN-R (5′-GGAAAGAAGT-CAGAAGGCAA-3′) and TaqMan probe HBV-FM (5′-FAM-TCCAAGCTGTGCCTT-MGB-3′) specifically amplify cDNA from precore RNA. Primers HBV-PG-F (5′-CACCTCTGCC-TAATCATC-3′), HBV-DN-R and TaqMan probe HBV-FM amplifies cDNA from both precore RNA and pregenomic RNA. The amount of pregenomic RNA was calculated by subtracting the copy number of precore RNA amplification from that of precore/pregenomic RNA amplification [25]. Extracellular HBsAg was quantified by automated ELISA (Fujirebio Inc., Tokyo, Japan). The detection limits are 2 × 103 copies for HBV DNA, 2 × 102 copies for pregenomic RNA and 0.005 IU/mL for HBsAg.

Results

Influence of genotype and amino acid substitutions on recognition by HBV-specific mAbs

The mAbs HB0116 and HB0478 bind to the first loop (amino acids 123–137) of the "a" determinant and strongly inhibit HBV gt-C infection [14]. Therefore, whether the binding capacity of each mAb is affected by amino acid variation within the first loop was examined using recombinant peptides; there is amino acid variation between genotypes C and A at positions 126 (gt-C: I, gt-A: T) and 131 (gt-C: T, gt-A: N). Both HB0116 and HB0478 bound peptides not only corresponding to the first loop with the gt-C sequence but also corresponding to those with the gt-A sequence, indicating their cross-genotype recognition on binding in vitro (Fig. 1A). The binding capacities to the native HBs proteins of gt-A, gt-C, together with gt-C with the substitution G145R located within the second loop of HBsAg extracellular domain, were also examined. Interestingly, immunoprecipitation assays revealed that HB0116 bound to HBsAg of HBV gt-C and gt-A, but not to G145R, whereas HB0478 could bind to all three proteins (Fig. 1B).

Next, the HBV-neutralizing activity of these mAbs was evaluated using HepaRG cells, which support HBV infection, by inoculating them with a high dose of HBV. Fig. 2 shows that HB0116 suppressed the increase of HBV DNA after inoculation of both HBV gt-C and gt-A, but could not inhibit infection by G145R. However, HB0478 could prevent infection by HBV gt-C, gt-A, and also G145R. These results are consistent with the immunoprecipitation results shown in Fig. 1B and indicate that HB0478 can bind to the first loop, regardless of genotype, and also bind to the G145R substituted protein, which is seen as an antibody escape variant in clinical practice.

HB0116 and HB0478 protect against HBV gt-C and gt-A infections but only HB0478 protects against G145R mutant infection in vivo

The in vivo neutralizing activity of the mAbs was investigated using chimeric mice with human hepatocytes. After 1×10^4 copies of HBV gt-C or gt-A were incubated with HB0116 and/or HB0478, the mixtures were injected intravenously into naïve chimeric mice and serum HBV DNA concentrations were measured for the evaluation of HBV infection at five weeks after injection. Although HBV gt-C infection was confirmed in the control experiment (Group 1, 9.8×10^3 and 1.1×10^4 copies/ml) (Table 1), preincubation of the inoculum with either 1 μg or 10 μg of HB0116 or HB0478 completely blocked HBV infection with both gt-C and gt-A (Groups 2–5 for gt-C, Groups 6–9 for gt-A). Meanwhile, inoculation of the HBV G145R strain into naïve chimeric mice resulted in the establishment of infection (Group 10, 1.0×10^4 and 1.4×10^4 copies/ml) and incubation with 10 μg of HB0116 had no impact on infection by G145R (Group 11, 1.1×10^4–4.4×10^4 copies/ml), whereas as little as 1 μg of HB0478 completely blocked G145R infection (Groups 12 and 15). Apparently, a combination of HB0116 and

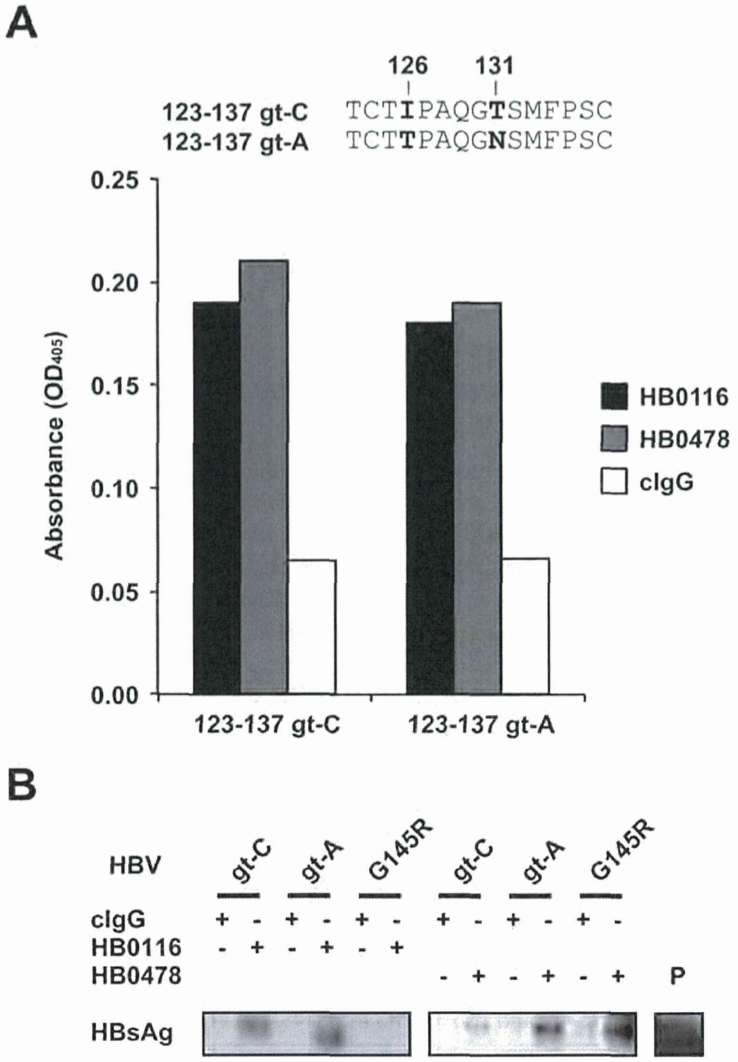

Fig 1. Binding capacity of mAbs HB0116 and HB0478 against with gt-C and gt-A HBsAg and the G145R variant. (A) Binding of mAbs HB0116 and HB0478 to synthetic peptides covering the first external loop of small-HBsAg was demonstrated by ELISA. The sequences of the recombinant peptides used in the analysis are shown above: amino acids which vary between genotype C (gt-C) and genotype A (gt-A) are indicated in bold. The absorbance at 405 nm is shown on the Y axis. Average data of three independent experiments are shown. **(B)** The gt-C, gt-A, and G145R virions were immunoprecipitated with HB0116 or HB0478 and HBsAg in the precipitates was detected by Western blotting. Recombinant HBsAg protein was used as the positive control (P lane). Representative data of three independent experiments are shown.

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HB0478, either at 1 μg or 10 μg protected the chimeric mice from HBV infection (Groups 13 and 14).

Evaluation of PHHs isolated from chimeric mice with human hepatocytes as an in vitro HBV infection model

PHHs isolated from the chimeric mice with human hepatocytes were used to characterize further the neutralizing activity of mAb HB0478. In vitro HBV infection of the PHHs was