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Prevalence of the 'a' Determinant Mutant in Japanese Adult HBV Carriers Seropositive for Both HBsAg and Anti-HBs

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ABSTRACT

To analyze whether the lack of hepatitis B virus (HBV) clearance in the presence of antibody to hepatitis B surface antigen (anti-HBs) is associated with the frequent infection of mutant HBV that have several mutations within 'a' region, we determined the entire nucleotide sequence of 'a' region of S gene in 15 adult Japanese HBV carriers seropositive for both hepatitis B surface antigen (HBsAg) and anti-HBs, whose serum HBV-DNA level vary from very low to very high. Unique mutant strains with various amino acid changes such as Thr-141 and Ser-126 were detected in only three patients (20%) of the HBV carriers examined, while only the wild-'a' type virus was detected in the rest of the patients. These results suggest that the seropositivity for both HBsAg and anti-HBs does not always indicate the presence of 'a'-mutant virus. However, unique nucleotide deletion mutants in pre-S1/pre-S2 region and amino acid mutation at the start codon of the pre-S2 region were detected in 7 patients of 12 HBV carriers without 'a' mutant (58.3%). The viral persistence in these patients may be linked to the selection of these escape mutants in the presence of anti-HBs. (Jikeikai Med J 2002 ; 49 : 31-41)

Key words : 'a' determinant mutant, pre-S deletion mutant, anti-HBs positive carrier

Introduction

After the establishment of an effective vaccination for preventing hepatitis B virus (HBV) infection, the HBV carrier rate in Japan has been dramatically reduced. However, once HBV infects a host chronically, there is, as yet, no clinically available therapy for its eradication. The immune reaction against HBV has been thought to be an important factor inducing HBV-related morbidity. Among the HBV-related clinical markers, HBV surface antigen (HBsAg) is the serological hallmark of HBV infection. Persistence of HBsAg for more than 6 months implies chronic infection with HBV and the appearance of antibody to hepatitis B surface antigen (anti-HBs) marks the recovery from hepatitis B infection. However, some patients have shown a coexistence of HBsAg and anti-HBs.

The envelope proteins of HBV are coded in the preS/S gene, and they are important targets for immune-mediated viral elimination[1]. In chronic infection of HBV, clearance of HBsAg or development of anti-HBs is usually associated with the elimination of HBV from the infected patient. Seroconversion from HBsAg to anti-HBs usually indicates the clearance of HBV, and most patients with chronic hepatitis B show a significant improvement in their clinical status along with the seroconversion.

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However, several studies have reported the persistence of HBV-DNA or detection of HBsAg in some patients with anti-HBs[2,3], although it is surprising that HBV-DNA or HBsAg are detectable in the presence of anti-HBs. Although the co-occurrence of HBsAg and anti-HBs has been thought to be rare among HBV carriers, recent studies indicate that their coexistence is not a rare serological event[2,3]. Indeed, it has been reported that such patients represent about 21-32% of all HBV-infected patients[2,3]. This phenomenon occurs when the anti-HBs are directed to one of the subtype determinants and not the common 'a' determinant, and when they are unable to neutralize the HBV so that these patients appear to be HBV carriers.

HBV shares a common immunodominant and immunoprotective determinant on the surface antigen, called 'a' determinant of HBsAg[4-6]. This determinant is coded within codon 124-147 in the S gene, and it consists of two conformational loops in which a disulphide bridge between Cys-124 and Cys-137 forms the first loop and one between Cys-139 and Cys-147 forms the second loop[7]. It is believed that the neutralizing antibodies against HBV envelope proteins are mainly targeted toward this conformational epitope[1,7,8]. Recently, 'a' mutant HBV having several mutations within the 'a' region, such as Arg-145 or Aln-126, was frequently detected in vaccinated babies who became HBV carriers after the vaccination or were treated with immunoglobulin against HBV[7,9-12]. These variants showed much lower binding capacity to anti-'a' monoclonal antibodies[6,10]. Since these mutant viruses may have become dominant during immunological selection, thereby escaping the host immune system, they were named immune escape mutants[7,9-13]. However, it is unclear whether such 'a' mutant viruses are detectable in Japanese adult HBV carriers who are seropositive for both HBsAg and anti-HBs, as is the case with the vaccinated babies. These are only two published studies of S gene analysis in Japanese adult HBV carriers who possessed HBsAg and anti-HBs[3,13]. These studies reported that 'a' mutant viruses are frequently detected and that all, or at least some, of the HBV clones from those carriers had in-phase deletions in the 5′ terminus of the pre-S2 region. Most of them were restricted to patients whose serum HBV-DNA titer remained very high, however, those of HBV carriers with low-titter serum HBV-DNA have not been adequately documented to date. To address these issues, the present study sought to determine the entire nucleotide sequence of the 'a' region of HBV in 15 Japanese HBV carriers with anti-HBs, whose serum HBV-DNA levels varied from very low to very high. We also discuss the clinical significance of the detected mutations. Furthermore, we determined the nucleotide sequence of the pre-S1/pre-S2 region of HBV in these HBV carriers. As a control, we also determined the nucleotide sequence of the 'a' region and the pre-S1/pre-S2 region of HBV in eight Japanese adult HBV carriers without anti-HBs.

MATERIALS AND METHODS

Patients

Serum samples were taken from 23 Japanese HBV carriers. Of these 23 individuals, 15 were seropositive for both HBsAg and anti-HBs, and 8 were positive for HBsAg but negative for anti-HBs. All patients were followed at the Division of Gastroenterology and Hepatology, Department of Internal Medicine, The Jikei University School of Medicine from 1992 to 1997 (Table 1). The ethics committee in The Jikei University approved this study and informed consent was obtained from all subjects.

In the 15 HBV carriers with anti-HBs, 11 were seropositive for HBV e antigen (HBeAg) [HBeAg+], while the remaining 4 were negative for HBeAg [HBeAg−]. The HBV subtype of all patients was adr. All patients but one (Patient 14) had chronic liver disease (Table 1). They had elevated serum alanine aminotransferase (ALT) levels for at least 2 years. A liver biopsy was performed in 12 patients, and histological diagnosis showed liver cirrhosis (LC) in 1 patient, and chronic active hepatitis (CAH) in 11 patients. The serum level of HBV-DNA ranged from less than 3 pg/ml to 5,300 pg/ml. One patient (Patient 14) appeared to be an asymptomatic carrier (ASC). Her serum ALT levels had been within normal limits in repeated examinations for at least 3

Table 1. Clinical and laboratory data of 23 HBV carriers

Patient no.	Age/ Gender	HBsAg (COI)※	anti-HBs (COI)※	HBeAg/ anti-HBe	HBV-DNA (pg/ml)	ALT (IU/l)	Liver Histology
HBV carriers with anti-HBs							
1	34/M	46.1	8.6	+/−	220	91	CAH*
2	35/M	54.8	6.5	+/−	5,300	501	CAH
3	23/F	124.5	20.8	+/−	300	18	N.D.†
4	42/M	71.1	4.7	+/−	<3	61	N.D.
5	32/F	74.9	19.9	+/+	<3	58	CAH
6	25/F	96.3	37.2	+/±	930	421	CAH
7	24/M	75.1	6.7	+/−	140	140	CAH
8	51/M	46.5	2.0	−/+	180	79	LC‡
9	43/M	98.7	2.9	+/−	500	61	CAH
10	34/M	67.2	5.6	+/−	320	82	CAH
11	37/M	70.5	3.2	−/+	252	45	CAH
12	37/F	53.9	17.7	−/±	<3	16	CAH
13	24/F	42.0	3.2	+/−	760	126	CAH
14	52/F	54.2	11.0	−/+	<3	13	N.D.
15	44/M	92.5	6.2	+/−	460	277	CAH
HBV carriers without anti-HBs							
16	51/M	80.5	0.1	+/−	1,150	147	CAH
17	31/M	127.7	0.0	+/−	3,300	81	CAH
18	24/F	62.1	0.5	+/−	580	145	CAH
19	31/F	101.3	0.0	+/−	2,400	137	CAH
20	35/M	79.7	0.2	−/+	160	176	CAH
21	40/M	98.7	0.0	+/−	1,500	22	N.D.
22	43/M	67.2	0.2	−/+	51	129	LC
23	29/M	56.5	0.3	−/+	<3	18	N.D.

※Cut-off index in RIA
† Not done
* Chronic active hepatitis
‡ Liver cirrhossis

years; her serum level of HBV-DNA was no higher than 3 pg/ml.

As a control, we also studied eight HBV carriers without anti-HBs, five with CAH, one with LC, and two ASC. In these controls, five patients were HBeAg+ and the remaining three were HBeAg−. Serum ALT levels of CAH and LC patients were elevated, whereas those of ASC remained within normal limits in repeated examinations for at least 3 years. Their serum level of HBV-DNA ranged from less than 3 pg/ml to 3,300 pg/ml.

None of the 23 subjects were found to be positive for anti-HCV (Ortho Japan, Tokyo, Japan).

HBV markers

HBsAg, anti-HBs, HBeAg, and antibody to HBV e antigen (anti-HBe) were assayed using a solid-phase radioimmunoassay (Abbott. Laboratories, North Chicago, IL). The levels of serum HBV-DNA were determined using a solution hybridization assay (Genostics, Abbott Laboratories).

Amplification of the 'a' region and the pre-S1/pre-S2 region of HBV DNA

Nucleic acids were extracted with phenol and chloroform from 120 μl of serum samples obtained from each patient, and were treated with 2.5 mg/ml proteinase K, 0.5% (w/v) sodium dodecyl sulfate

(SDS) by a method described previously[14,15]. After the extraction, HBV-DNA was precipitated with ethanol, dried, and dissolved in 20 μl of double-distilled water (DDW). The synthetic primers used for the amplification of the 'a' region of the HBV genome were as follows: the sense primer A1: 5′-ATCCTGCTGCTATGCCTCAT-3′ (nt 410-429), and the antisense primer A2: 5′-ATGGCACTAGTAAACTGAGC-3′ (nt 689-670). With these primers, a 261-bp fragment covering the entire 'a' region was amplified by PCR. The synthetic primers used for the amplification of the pre-S1/pre-S2 region of the HBV genome were A3: 5′-CAAACAATCCAGATTGGGAC-3′ (nt 2960-2979), and A4: 5′-TAGAAAATTGAGAGAAGTCCA-3′ (nt 280-260). With these primers, a 536-bp fragment covering the pre-S1/pre-S2 region was amplified by PCR. Amplification was performed in a 100 μl reaction mixture containing 10 μl of serum DNA sample, 0.5 μM of each primer, 200 μM of dNTP, 50 mM of KCl, 10 mM of Tris-HCl (pH 8.3), 1.5 mM of MgCl, 2,200 μg/ml of gelatin, and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Corp, Norwalk, CT). The PCR reaction was cycled 35 times for 1 minute of denaturation at 95°C, for 1 minute of annealing at 50°C, and for 2 minutes of extension at 72°C, followed by the final extension at 74°C for 5 minutes in a programmable thermal cycler (DNA Thermal Cycler PJ 480, Perkin-Elmer Corp). After the amplification, the PCR products were purified from the excess primers with Wizard PCR Prep purification columns (Promega, Southampton, England). The purified PCR products were then precipitated with ethanol and dissolved in 20 μl of DDW.

Sequence of the 'a' region and pre-S1/pre-S2 region of HBV DNA

The sequences of the entire 'a' region and the pre-S1/pre-S2 region were determined by direct sequencing using the dye-terminator method (Applied Biosystem Japan, Tokyo) with sense and antisense PCR-primers, and Taq DNA polymerase. The sequence reactions were performed according to the manufacture's protocol and were analyzed by an ABI 373A autosequencer (Applied Biosystem Japan). We examined each sequence bi-directionally twice in order to prevent errors in the mutations due to misincorporation of Taq polymerase in the PCR. The analyzed sequences were compared with consensus sequences of HBV subtype adr[16-18]. A computer-assisted homology search was carried out using the DNASIS software packages (Hitachi Software, Tokyo).

Statistical analysis

The statistical analysis for the comparison of clinical features between 'a'-mutant type and 'a'-wild type was done using the unpaired Student's t-test and χ^2 test in the Statview-J 4.02 program, with two-tailed P values <0.05 indicating significance.

RESULTS

The distribution of amino acid changes in the 'a' determinant region of HBV in HBV carriers with both HBsAg and anti-HBs

Table 2 summarizes the nucleotide mutations and the deduced amino acid residue substitutions of the 'a' determinant region amplified from 23 HBV carriers. Unique amino acid changes were found in the 'a' determinant region in 3 of 15 HBV carriers with anti-HBs (20%), whereas only the 'a'-wild type viruses were detected in 8 HBV carriers without anti-HBs. Figs. 1 and 2 show the entire nucleotide sequences and the deduced amino acid residue sequences of the 'a' determinant region in these three patients. In these patients two kinds of rare and unique mutant viruses were identified (Table 2 and Figs. 2 and 3). An amino acid change from Ile (ATT) or Thr (ACT) to Ser (AGT) substitution at the 126th codon was detected in two patients (Patients 2, and 3). Another amino acid change from Lys (AAA) to Thr (ACA) substitution at the 141st codon was detected in Patient 1. In addition, several unique amino acid changes near the 'a' region, such as Thr (ACC) to Ile (ATC) substitution at the 123rd codon and Pro (CCC) to Leu (CTC) substitution at the 151st codon, were also identified in this strain. Fig. 3 is a diagrammatic representation of

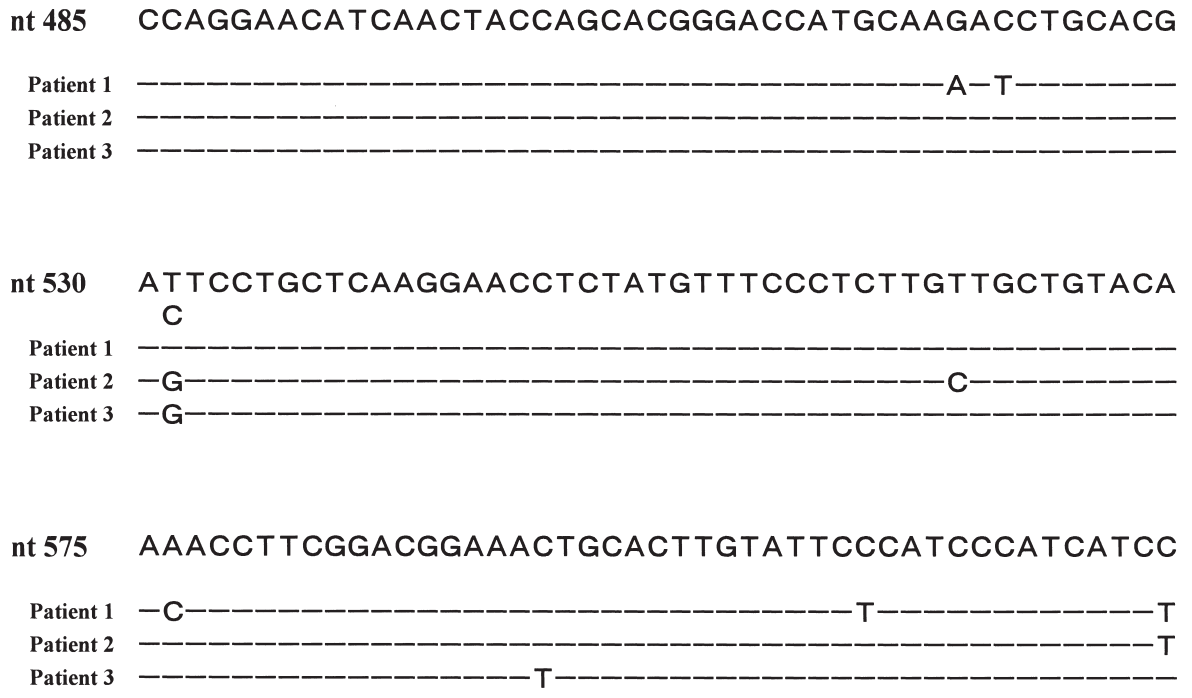

Fig. 1. Nucleotide sequences of the 'a' determinant region and near this region of HBV DNA in HBV carriers who are seropositive for both HBsAg and anti-HBs (Patients 1-3).
Top line indicates the consensus 'a' determinant sequence of the subtype adr HBV[16-18]. Nucleotide sequences below indicate those of 'a' mutant viruses detected in this study. A dash denotes the same nucleotide as the consensus.

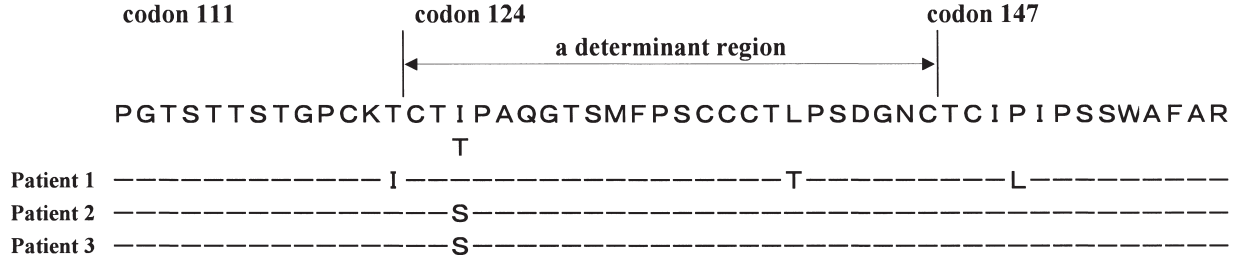

Fig. 2. Deduced amino acid residue sequences of the 'a' determinant region and near this region of HBV DNA in HBV carriers who are seropositive for both HBsAg and anti-HBs (Patients 1-3).
Top line indicates the consensus 'a' determinant sequence of the subtype adr HBV[16-18]. Amino acid sequences below indicate those of mutant viruses detected in this study. A dash denotes the same amino acid as the consensus.

amino acid changes within and near the 'a' determinant region. Six silent mutations were found in four patients (Fig. 1) as follows. A G→A mutation was detected at nt 520 in Patient 1, a T→C mutation was detected at nt 565 in Patient 2, a C→T mutation was detected at nt 592 in Patient 3, an A→C mutation was detected at nt 544 and a C→T mutation was detected at nt 586 in Patient 7 (not shown in Fig. 1), and a C→T mutation was detected at nt 592 in Patient 8 (not shown in Fig. 1). Then we examined the HBV-subtype determining the nucleotide position. The d/y determining codon 122 in all patients was 122-Arg, indicating subtype d. The w/r determining codon 160 in all patients was 160-Arg, indicating subtype r. Thus, the subtype of HBV in 23 patients was determined to be adr genomically.

We compared the clinical features between HBV carriers who had 'a' mutants and those of the remain-

Table 2. Amino acid changes in the 'a' determinant region and nucleotide deletions in the pre-S1/pre-S2 regions of HBV-DNAin 23 HBV carriers

Patient no.	Amino acid changes in 'a' determinant region	Nucleotide deletions in pre-S1/pre-S2 regions
HBV carriers with anti-HBs		
1	S123 Thr→Ile, S141 Lys→Thr, S151 Pro→Leu	none
2	S126 Ile/Thr→Ser	none
3	S126 Ile/Thr→Ser	none
4	none	pre-S1/pre-S2 deletion nt 3013-nt4*
5	none	none
6	none	none
7	none	pre-S1 deletion nt 3020-nt 3202*/ pre-S2 deletion nt 47-nt 55
8	none	pre-S2 1st codon Met→Ile†
9	none	none
10	none	pre-S1 deletion nt 3020-nt 3202*
11	none	none
12	none	pre-S2 deletion nt 8-nt 58
13	none	none
14	none	pre-S2 deletion nt 8-nt 58
15	none	pre-S1/pre-S2 deletion nt 3127-nt 58
HBV carriers without anti-HBs		
16	none	none
17	none	none
18	none	none
19	none	none
20	none	none
21	none	none
22	none	none
23	none	none

* Bone pre-S deletion mutant and wild-type viruses were detected
† Amino acid change from Met (ATG) to Ile (ATA) at the start codon of pre-S2 region was detected

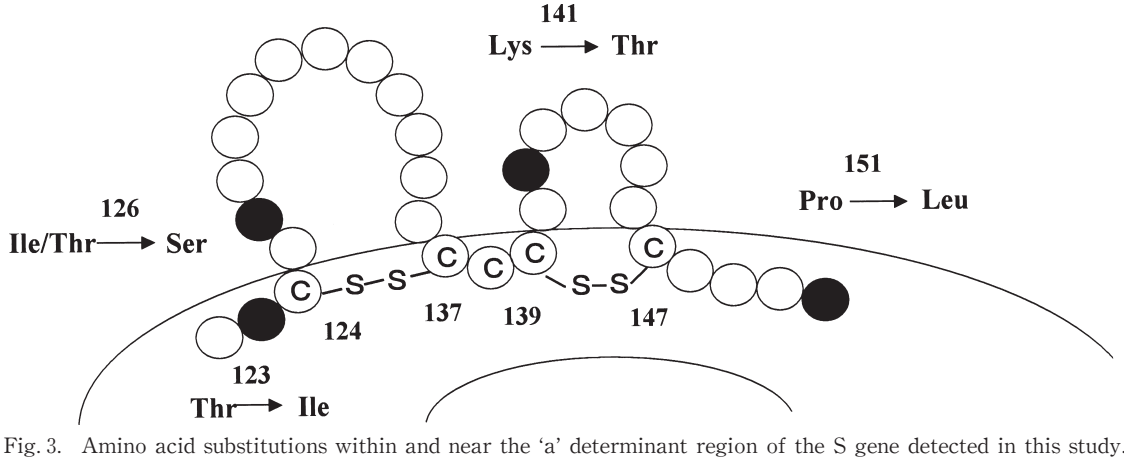

Fig. 3. Amino acid substitutions within and near the 'a' determinant region of the S gene detected in this study. The conformational structure of the 'a' determinant region was proposed by Carman et al.[7].

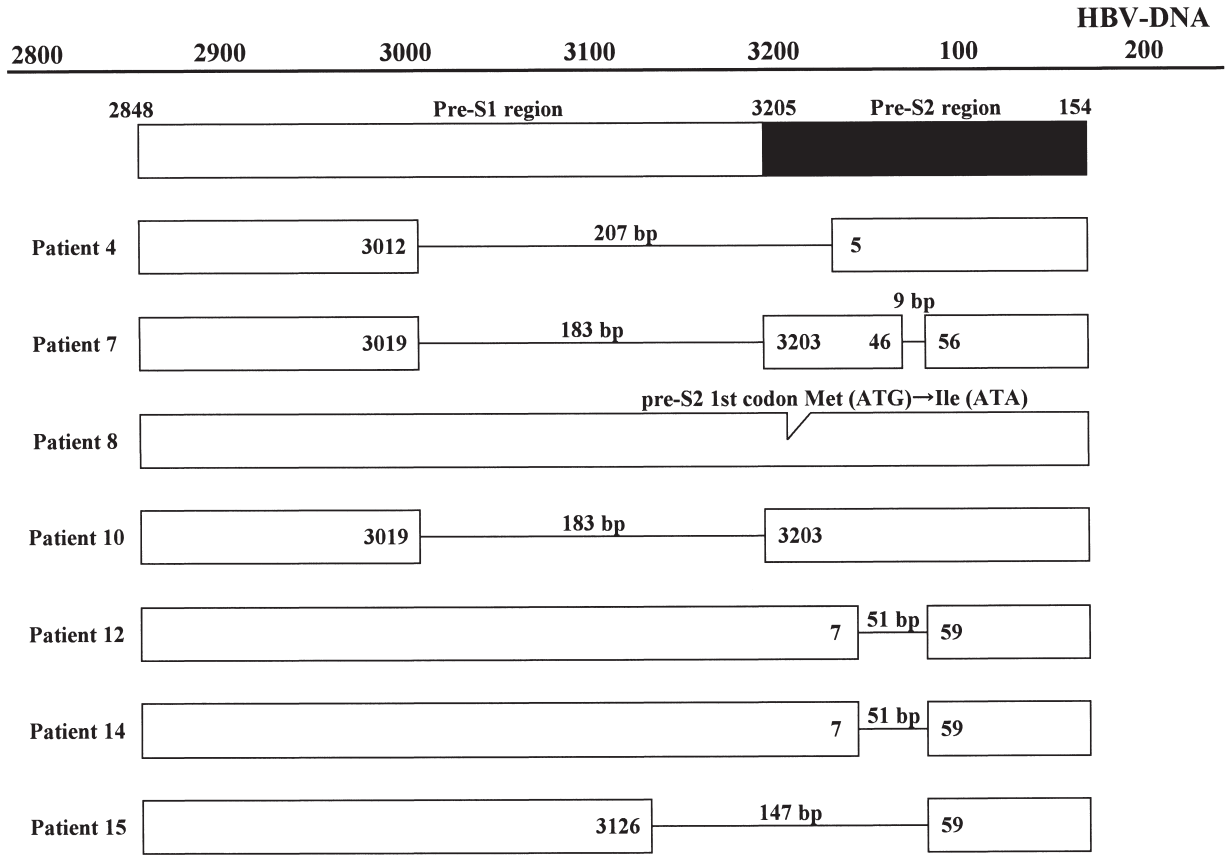

Fig. 4. Deletions in the pre-S1/pre-S2 regions of HBV DNA in HBV carriers who are seropositive for both HBsAg and anti-HBs.
The ranges of the pre-S1/pre-S2 regions with nucleotide numbers are shown above. Solid lines indicate deletions with their spans in base pairs, and the numbers of nucleotides flanking them are shown. Unique deletion mutants were detected in six patients (Patients 4, 7, 10, 12, 14, and 15). An amino acid substitution from Met to Ile at the start codon of the pre-S2 region was detected in one patient (Patient 8).

ing patients with the 'a'-wild type, and found no statistically significant difference between these two groups in the titer of HBsAg and anti-HBs, and in the levels of HBV-DNA and ALT. Neither were there any significant differences in the histological findings (Table 1).

Nucleotide deletions in the pre-S1/ pre-S2 region of HBV in HBV carriers with both HBsAg and anti-HBs

Table 2 and Fig. 4 show deletions of nucleotides in the pre-S1 region and/or the pre-S2 region. Unique deletion mutants were detected in 6 (Patients 4, 7, 10, 12, 14, and 15) of 15 HBV carriers with anti-HBs (40%), whereas no deletion mutant was found in the remaining 8 HBV carriers without anti-HBs. All the six individuals with pre-S deletion mutants had no amino acid change within the 'a' determinant region. The 3′ terminus of the pre-S1 region and the 5′ terminus of the pre-S2 region were favored sites of deletion. All deletions of nucleotides in the pre-S1/pre-S2 region were a multiple of three. Wild pre-S1 strains were also detected in the four patients with pre-S1 deletion mutants (Patients 4, 7, 10, and 15). We detected an amino acid substitution from Met (ATG) to Ile (ATA) at the start codon of the pre-S2 region in another patient (Patient 8). In contrast, no significant mutation or deletion was found in the control HBV carriers without anti-HBs (Patients 16-23).

DISCUSSION

It remains unclear whether the lack of HBV clearance in the presence of anti-HBs is associated with frequent infection with the 'a' mutant virus in adult HBV carriers. Several studies speculated that 'a' mutant viruses are not neutralized by anti-HBs, therefore HBV with 'a' mutants are able to exit escaping from anti-HBs[2-13]. In the present study we investigated the nucleotide sequence of the 'a' determinant region and the pre-S1/pre-S2 region in serum samples collected from 15 Japanese HBV carriers who were seropositive for both HBsAg and anti-HBs. We found a low prevalence of 'a' mutant viruses in HBV carriers with both HBsAg and anti-HBs in sera. We initially assumed that the persistent infection of HBV despite the presence of anti-HBs may be linked to frequent infection of 'a' mutants not neutralized by anti-HBs, because 'a' escape mutants such as Arg-145 or Aln-126 have frequently been detected in vaccinated babies or HBsAg-negative HBV carriers[7,9-12]. However, 'a' mutant strains with various amino acid changes, such as Thr-141 or Ser-126, were detected only in 20% of the HBV carriers examined, although we might have missed the minor population strains because of using the direct sequencing method. In particular, we failed to find any of the 'a' mutant in four HBV carriers (Patients 4, 5, 12, and 14) with low-titered serum HBV-DNA (<3 pg/ml). These results suggest that seropositivity for both HBsAg and anti-HBs does not necessarily indicate the presence of the 'a' mutant virus in serum.

Our findings are somewhat different from those of previous studies[3,13] with respect to the frequency of the 'a' mutants. Yamamoto et al[13] studied six cases of HBV carriers with anti-HBs, and found 'a' mutants in five cases (83.3%). They found Ser-126 in one patient, Asn-126 in one patient, Asn-126 and Arg-145 in one patient, and Arg-145 in the remaining two patients. Kohno et al[3] also examined five cases of HBV carriers with HBsAg and anti-HBs, and they identified 'a' mutants in four cases (80%). They identified Asn-130 and Gly-145 in one case, Asn-130 in two cases, and Ser-126 in one case. Such a high prevalence of 'a' mutants is not consistent with our findings. This discrepancy may come from the difference in the HBV strains. Both of the previous studies included patients with relatively high serum HBV-DNA titers, while our subjects were HBV carriers with HBV-DNA titers ranging from very low to very high. These data may indicate a difference in the viral replication competence or the characters of the examined viruses. It may be that the 'a' mutant strains in the previous studies were able to efficiency evade the circulating anti-HBs immunity due to a lower antigenicity of their HBsAg[6,7,10].

We identified a rare and unique mutant in one patient (Patient 1). Several amino acid changes within and near the 'a' loop region, such as Thr-141, Ile-123, and Leu-151, were identified in this strain. This mutant HBV is a unique mutant that has not been identified in previous studies, to our knowledge. Another unique mutation, Ser-126 was detected in two patients, although it had been identified in previous studies[3,6,13], and its binding capacity of to several anti-'a' monoclonal antibodies was shown to be remarkably reduced in comparison with that of the wild type[6]. Therefore, it may be that these amino acid changes may alter the conformational structure of the 'a' determinant region, and the viral persistence in the presence of anti-HBs may be linked to the selection of these 'a' mutants. While each of these three patients showed high levels of serum HBsAg (Table 1), the previous studies[3,10,11] have reported that even the well-known escape 'a' mutants, such as Arg-145 or Asn-130, could show high levels of HBsAg in sera when their serum HBV-DNA levels were high. To discuss the significance of detected 'a' mutations on the conformational antigenic nature of the 'a' determinant, future studies should focus on further functional analysis such as evaluating the expression of several antigenic determinants on HBsAg with mapped monoclonal anti-HBs antibodies, or clarifying the quasispecies nature of the 'a' determinant region by subcloning.

To further investigate the mechanism of viral persistent infection in the presence of anti-HBs, we studied the nucleotide sequences of the pre-S1 and pre-S2 regions in our patients. We found that unique pre-S1/pre-S2 deletion mutants, or the pre-S2 start

codon mutant, were frequently detected in patients with anti-HBs (46.7%). Since this region codes the promoter region of the surface antigens[19], deletions or mutations in this region may affect the production of HBsAg or modify the character of the HBsAg[3,13,20]. Therefore, these pre-S gene mutants may contribute to viral persistence under the immune pressure of anti-HBs. In particular, the pre-S1 defective mutant with a 183-base pairs deletion (nt. 3020-3202) was detected in two patients (Patients 7 and 10). Gerken et al[21] also identified this pre-S1 defective mutant with the deletion exactly at the same position in a patient with hepatocellular carcinoma who was negative for HBsAg and positive for anti-HBs. Since the deleted sequence overlapped the pre-S2 promoter region and the B and T cell recognition sites of the pre-S1 region[22], selection of this defective mutant may be associated with the unusual serological state wherein HBsAg and anti-HBs exit simultaneously. Nucleotide deletions in the pre-S1 region were also observed in other patients (Patients 4 and 15). The former half segment of the pre-S1 region was well conserved in all four patients. Since the pre-S1 residues 21-47 are required for binding hepatocytes plasma membrane[23], virological factors might have acted to conserve the N-terminal portion. Since wild pre-S1 strains were also detected in these patients, it may be that defective viruses could replicate with the helper co-infecting virus, which has a complete viral genome[24,25]. Pre-S2 deletion mutants or the pre-S2 start codon mutant, resulting in the disability of synthesis of intact the pre-S2 protein, were observed in seven patients (Patients 4, 7, 8, 10, 12, 14, and 15). The deletion mutations in the pre-S2 region may be permissible for the replication of mutants, since the pre-S2 protein is not essential for HBV replication or infectivity[3,26].

In the remaining five patients (Patients 5, 6, 9, 11 and 13), we found neither a significant mutation in the 'a' determinant region nor a deletion in the pre-S1/pre-S2 region. This suggests that there may be different immunological features among the patients we studied. The most reasonable explanation for the mechanism of the coexistence of HBsAg and anti-HBs in these patients may involve the difference between the subtype of the infecting HBV and the subtype-specificity of the anti-HBs, as shown in previous studies[2,3,27-29]. Kohno et al[3] examined the specificity of the anti-HBs of each serum sample and found that serum without the 'a' mutant or pre-S deletion mutant contained anti-HBs specific for the w determinant, while the subtype of the mainly infecting HBV was adr. They hypothesized that adr-HBV could maintain the infection because they could escape the neutralizing effect of the anti-w antibody. This same mechanism may have been in our five remaining patients without the 'a' mutants. To clarify further the mechanism of viral persistence despite coexisting anti-HBs, future studies should focus on determining the subtype specificity of anti-HBs in each serum sample by testing the hemagglutination ability of the detector cells coated with small HBsAg particles of adr, adw, ayr, or ayw.

In conclusion, our results indicated that there is a low prevalence of 'a' mutants and frequent detection of pre-S deletion mutants in Japanese adult HBV carriers seropositive for both HBsAg and anti-HBs. Unique 'a' determinant mutants such as Thr-141 and Ser-126, or several pre-S1/ pre-S2 deletion mutants, or the pre-S2 start codon mutant, were detected in 10 of 15 (66.7%) HBV carriers with anti-HBs, whereas such pre-S/S mutants were not detected in 8 HBV carriers without anti-HBs. These results suggest that the selection of these 'a' mutants or pre-S1/pre-S2 deletion mutants may contribute to viral persistence under the immune pressure of anti-HBs.

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