Table 2. Oligonucleotides Used as PCR Primers

Sense Primers			Antisense Primers		
Name	Nucleotide Sequence	Position	Name	Nucleotide Sequence	Position
HBX					
HB-1	5′ CTCTCTCGGAAATACACCTC	220–1239	B-2	5′ GTAACTCCACAGAAGCTCCA	1818–1799
HB-3	5′ TGCCAACTGGATCCTGCGCGGGACGTCCTT	264–1293	B-4	5′ GGCTTGAACAGTAGGACATG	1742–1723
HBS					
HB-5	5′ AAGACCTGCACGATTCCT	391–408	B-6	5′ TAGAGGTAAAAAAGGGACTC	672–654
HB-7	5′ TTCGCAAGATTCCTATGGG	99–517	B-8	5′ GCCCCCAATACCACATCA	634–617
HBC					
HB-9	5′ AACTTTTTCACCTCTGCCT	690–1708	B-10	5′ GCTTGCCTGAGTGCTGT	1945–1929
HB-11	5′ ACTGTTCAAGCCTCCAAGC	1731–1749	B-12	5′ AAGGAAAGAAGTCAGAAGGC	1848–1829
cccHBV					
P23	5′ CTGAATCCCGCGGACGACCC	1443–1462	P24	5′ ACCCAAGGCACAGCTTGGAGG	1891–1871
P25	5′ GTCTGTGCCTTCTCATCTGCC	1553–1573	P26	5′ AGATGATTAGGCAGAGGTGAAAAA	1846–1823
Cassette-ligation					
X-5	5′ ACTCTACCGTCCCCTTCTTCATGTGCCGTT	1351–1380	C-1	5′ GTACATATTGTCGTTAGAACGCGTAATACGACTCA	
X-6	5′ CTCTTTACGCGGTCTTTTTGTCTGTGCCTTC	1404–1434	C-2	5′ CGTTAGAACGCGTAATACGACTCACTATAGGGAGA	

HBV DNA Integrated in Human Genome

Our results provide evidence that HBV DNA was integrated into human genome in four of the seven patients with HCV infection in whom HCC developed after complete responses to IFN therapy (cases 2, 5, 6, and 7). HBV DNA was integrated into chromosome 11q23 in case 2, chromosome 22q11.23-12 in case 5, chromosome 11q12 in case 6, and chromosomes 11q13 and 14q32 in case 7. In case 6, HBV DNA was integrated into protein-coding sequences, hypothetical LOC387771 protein, the function of which remains unclear. HCC developed more than 3 yr after clearance of HCV in the patients with HBV DNA integration (Table 4). The interval from HCV eradication to the diagnosis of cancer was significantly longer in HCC with HBV DNA integration than in HCC without it. In HCC without HBV DNA integration, noncancerous liver tissue showed cirrhosis. In HCC with HBV DNA integration, the fibrosis stage of liver tissue was 1 or 2.

Clinical Courses of the Seven Patients

In four patients, in whom more than 3 yr had elapsed since the clearance of HCV RNA, HBV DNA was integrated into the human genome of HCC. In two of the three patients without HBV DNA integration, the surrounding liver showed cirrhosis. In contrast, the surrounding liver of HCC with HBV DNA integration did not progress to cirrhosis. Four patients are alive as of this writing. Tumor recurrence has not been detected in two of these patients (Fig. 2). The other three patients have died: two died of tumor progression and one of a myocardial infarction at operation. There was no correlation between clinical outcome after surgical treatment and HBV DNA integration.

DISCUSSION

In the present study, HCC developed in 4 (2.8%) of 144 patients who were regularly followed up after complete eradication of HCV by IFN monotherapy. Previous studies showed that HCC developed in 8 (2.2) of 363 patients, 6 (4.2%) of 142 patients, and 27 (2.3%) of 1,197 patients with sustained virus responses to IFN (5–7). Our findings are consistent with these results. The interval from the end of IFN therapy to the detection of HCC varied in these reports. In patients with a short interval, HCC most likely existed before the eradication of HCV by IFN. Makiyama *et al.* described the relation between tumor doubling time and the interval to the detection of HCC (7). They estimated that more than 6 yr were required for a single tumor cell to proliferate into a tumor measuring 1 cm in diameter. To our knowledge, 11 cases (including 2 in the present study) in which more than 60 months elapsed from the end of IFN therapy to the detection of HCC have been documented (5–7, 17–19). The longest interval between the end of treatment and diagnosis was 103 months, recorded in a patient in our study. HCC with long intervals between therapy and detection developed in noncirrhotic liver, usually not present in HCC with continuous HCV

Table 3. Histological Findings and the Existence Status of HBV DNA in Patients with HCC

Case	Tumor Size (mm)	Tumor Histology	Resected Liver (Stage)	Resected Liver (Grade)	HBV DNA In Serum	HBV DNA In Liver	cccHBV	HBV DNA Integration
1	19 × 18	Poorly	2	1	−	−	−	−
2	60 × 45	Poorly	2	1	−	+	+	chr. 11q22-23
3	50 × 45	Moderate	4	2	−	−	−	−
4	21 × 19	Poorly	4	2	−	−	−	−
5	30 × 22	Moderate	1	2	−	+	+	chr. 22q11
6	16 × 18	Moderate	2	2	−	+	−	chr. 11q12
7	40 × 30	Poorly	1	1	−	+	−	chr. 11q13 and 14q32

infection, suggesting that other etiologies are responsible for HCC developing a considerable time after the elimination of HCV. Available evidence thus indicates that factors other than chronic HCV infection play a role in the development of HCC detected after the eradication of HCV. This explanation seems more plausible rather than assuming that a tumor present before the start of IFN therapy grew for more than 5 yr without being detected by imaging studies, performed at regular intervals.

Few studies have examined the status of HCC in patients without HBsAg and antibodies to HCV antigen (anti-HCV), so called non-B, non-C patients. Patients with exposure to aflatoxin B1, alcohol addiction, diabetes mellitus, primary biliary cirrhosis, and steatohepatitis are considered at high risk for HCC (20–24). However, none of the patients in our study had these conditions. A recent review proposes that occult HBV is a carcinogenic factor, particularly in the absence of other risk factors for HCC (25). Pollicino *et al.* suggested that occult HBV infection is an independent factor for carcinogenesis in patients with chronic hepatitis C (13). In the present study, HBV DNA was found in four of seven cases of HCC, and cccHBV, virus-growing form, was also detected in two cases. HBV DNA was not detected in the serum of any patient. Small amounts of HBV, detected only in liver, are unlikely to induce hepatic injury or cause inflammation leading to carcinogenesis. In contrast, hepatocytes with HBV DNA integrated into the genome may be transformed, independently of the amount of HBV present. In our patients with occult HBV infection, HBV DNA was integrated into the human genome of HCC. There was a protein coding sequence near the HBV DNA integration site in one of the four HCC with HBV DNA integration. In this case, HBV DNA appeared to directly affect protein expression near the integration site. Integrated HBV DNA most likely caused instability of the human genome, without directly activating or disrupting protein function. A recent European study has reported that HCC was not detected for 5 yr in patients who had sustained virological responses to IFN monotherapy (26). The relation between HBV and HCC may be affected by patient demographics. Henry *et al.* reported that in an area with a high prevalence of HBV infection, occult HBV infection is common among patients with cryptogenic liver cirrhosis (27). To our knowledge, the annual incidence of HCC is higher in Japanese patients with HCV than that in countries in which

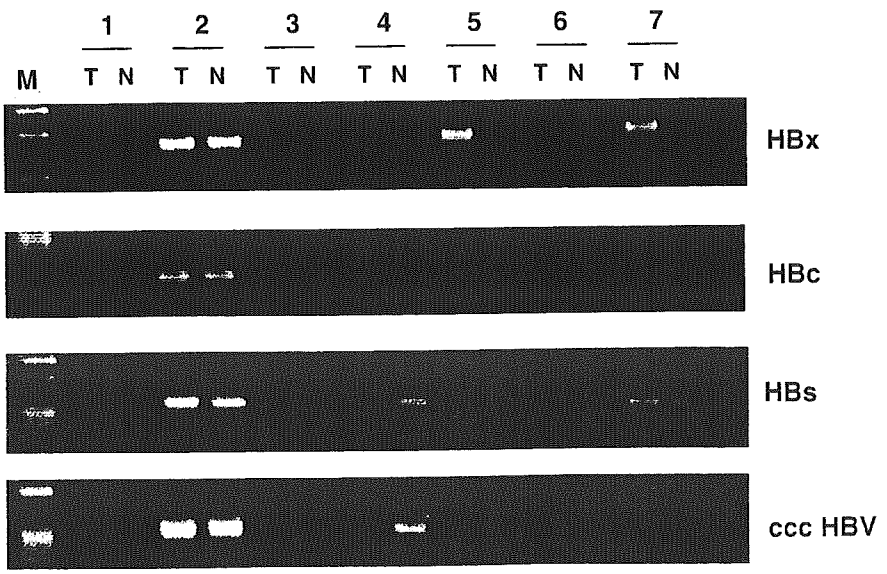

Figure 1. Amplification of HBV DNA and covalently cccHBV in resected liver. M, Marker (*pBR322/AluI*); cccHBV, covalently closed circular HBV.

Table 4. Relations Between Clinical Findings and HBV DNA Integration in HCC

	HCC with HBV DNA Integration (n = 4)		HCC Without HBV DNA Integration (n = 3)		*p*
Age	61 ± 3		64 ± 2		0.295
Interval (month)	67 ± 30		17 ± 4		0.0364*
Tumor size (mm)	32 ± 15		29 ± 16		0.7688
Tumor histology	Moderately D. HCC	2	Moderately D. HCC	1	
	Poorly D. HCC	2	Poorly D. HCC	2	
Fibrosis of resected liver	Stage 1	2	Stage 1	0	
	Stage 2	2	Stage 2	1	
	Stage 3	0	Stage 3	0	
	Stage 4	0	Stage 4	2	

Results are shown as means ± SD.
*Statistically significant.

HBV infection is rare. Occult HBV coinfection or past HBV infection in patients with HCV may partially account for the different incidences of HCC. We have previously reported that HBV DNA integration induces hepatocarcinogenesis in Japanese patients with HCV (28). HBV DNA integration was detected in HCC obtained from the patients with HCV, irrespective of the response to IFN therapy. A history of HCV infection thus seems to increase the risk of HCC in patients with HBV DNA integration.

In conclusion, our findings provide compelling evidence that occult HBV, especially integrated HBV, plays an important role in the development of HCC in patients with HCV eliminated by IFN therapy. Our results confirm the hypothesis that the elimination of HCV by IFN is not the endpoint of therapy for liver disease.

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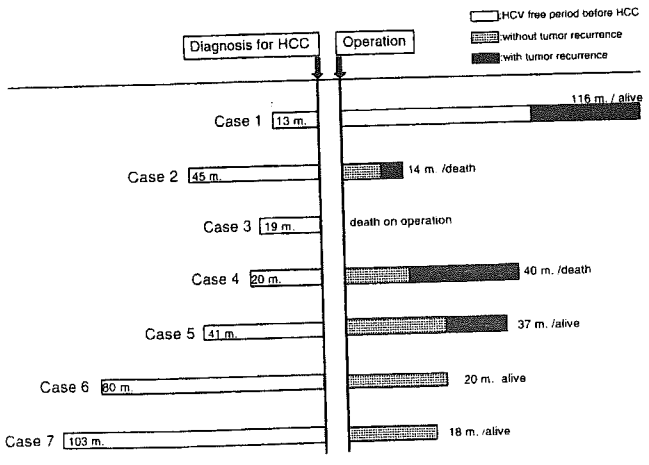

Figure 2. Clinical courses of seven patients with HCC who remained HCV free after IFN treatment. White bar indicates the period from the end of IFN treatment to the diagnosis of HCC. HCV RNA was not detected in any patient after treatment. Small dotted bar shows tumor-free period after liver resection. Meshed bar shows period with tumor recurrence. Two patients died of their tumors, and one died of a myocardial infarction at operation.

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Distinct Geographic Distributions of Hepatitis B Virus Genotypes in Patients With Acute Infection in Japan

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Genotypes of hepatitis B virus (HBV) were determined in 145 patients with acute hepatitis B from various districts in Japan to establish their geographic distribution and evaluating the influence on the clinical illness and outcome. Genotypes were A in 27 (19%) patients, B in 8 (5%), C in 109 (75%) and mixed with B and C in the remaining one (1%). Genotype A was more frequent in metropolitan than the other areas (21/69 (30%) vs. 6/76 (8%), $P < 0.001$). On phylogenetic analysis, seven of the nine (78%) HBV/A isolates selected at random clustered with those from Europe and the United States, while the remaining two with those of subgroup A′ prevalent in Asia and Africa. Maximum ALT levels were lower (2069 ± 1075 vs. 2889 ± 1867 IU/L, $P = 0.03$) and baseline HBV DNA titers were higher (5.90 ± 1.45 vs. 5.13 ± 1.36 log genome equivalents (LGE)/ml, $P = 0.002$) in patients infected with genotype A than C. Hepatitis B surface antigen persisted longer in patients infected with genotype A than C (1.95 ± 1.09 vs. 1.28 ± 1.42 months, $P = 0.02$). HBV infection became chronic in one (4%) patient with genotype A and one (1%) with genotype C infection. Fulminant hepatic failure developed in none of the patients with genotype A, one (13%) with genotype B and five (5%) with genotype C. The point mutation in the precore region (A1896) or the double mutations in the basic core promoter (BCP) region (T1762/A1764) were detected in none of the patients with genotype A, two (25%) with genotype B and 27 (26%) with genotype C. In conclusion, genotype A is frequent in patients with acute hepatitis B in metropolitan areas of Japan, probably reflecting particular transmission routes, and associated with longer and milder clinical course than genotype C. ***J. Med. Virol. 77:39–46, 2005.*** © 2005 Wiley-Liss, Inc.

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INTRODUCTION

The clinical outcome in patients with acute hepatitis B varies widely. Although hepatitis is self-limited in most patients, the clinical features range from asymptomatic to fulminant hepatic failure, while some patients become carriers of hepatitis B virus (HBV) [Chan HL and Lok, 1999; Chan HLY, 1999]. Factors that determine the clinical outcome remain unknown.

Viral nucleotide (nt) mutations have been shown to influence the clinical outcome of acute hepatitis B. Mutations in the precore region (A1896) and the basic core promoter (BCP) region (T1762/A1764) are common in patients with fulminant hepatic failure [Carman et al., 1991; Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Hawkins et al., 1994; Sato et al., 1995; Baumert et al., 1996; Chu et al., 1996]. Viral factors other than these mutations may influence the clinical outcome of acute hepatitis B.

Eight genotypes of HBV have been identified by sequence divergence greater than 8% in the entire genome, and they are designated by capital alphabet letters from A to H [Okamoto et al., 1988; Norder et al., 1994; Stuyver et al., 2000; Arauz-Ruiz et al., 2002]. Furthermore, recombinant HBV strains consisting of two different genotypes have been reported [Bollyky et al., 1996; Morozov et al., 2000]. Genotype distribution is different in different countries and even in distinct areas of the same country [Orito et al., 2001a; Kao, 2002; Kato et al., 2002; Miyakawa and Mizokami, 2003]. Therefore, surveys on genotype distribution may be helpful in identifying transmission routes and evaluating clinical relevance.

It has been shown that the clinical outcome of chronic hepatitis B is influenced by HBV genotypes. In Asian patients with chronic hepatitis B, genotype C is associated with later seroconversion of hepatitis B e antigen (HBeAg) and more severe liver damage than genotype B [Kao et al., 2000; Orito et al., 2001b; Chu et al., 2002; Ding et al., 2002; Sugauchi et al., 2002a]. Likewise, a study from India has shown that genotype D is associated with more severe liver disease than genotype A [Thakur et al., 2002]. Genotype A is peculiar in that A1896 in the precore region occurs infrequently, because it causes instability of the stem-loop structures of the pregenome encapsidation signal [Li et al., 1993; Lok et al., 1994]. These reports suggest that HBV genotypes also influence the clinical characteristics of acute hepatitis. Recent studies on small numbers of patients with acute hepatitis B suggest that the clinical course may differ among infections with distinct HBV genotypes [Mayerat et al., 1999; Kobayashi et al., 2002; Ogawa et al., 2002]. However, the association between viral genotype and severity of liver disease remains uncertain in acute HBV infection.

To evaluate the effect of HBV genotypes on the clinical characteristics of acute hepatitis B, a multi-center study on 145 patients was conducted in Japan.

MATERIALS AND METHODS

Patients

During 1992 through 2001, serum samples were collected from 147 patients diagnosed with acute hepatitis B in our institutions. Only patients from whom sera at the onset of hepatitis were stored were included in this study. Sixty-nine (47%) patients lived in metropolitan areas (Kawasaki, Tokyo and Tokorozawa), while the others in Kurume, Ube, Osaka, Gifu, Nagoya and Sapporo. Criteria for the diagnosis of acute hepatitis B were: (1) Acute onset of liver injury without a history of liver dysfunction and detection of hepatitis B surface antigen (HBsAg) in serum; and (2) IgM antibody to HBV core (anti-HBc) in high titer. Co-infection with hepatitis A virus or hepatitis C virus was excluded by serological tests.

Among the 147 patients, acute hepatitis B in six (4%) was complicated by hepatic encephalopathy and prolonged prothrombin time for the diagnosis of fulminant hepatic failure. Other two (1%) patients remained positive for HBsAg for longer than 6 months, and they were considered to have acquired chronic infection.

Sera from the 147 patients with acute hepatitis B were examined virologically, and the results were correlated with clinical and demographic characteristics. Informed consent was obtained from each patient for the purpose of this study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Ethics Committees of our institutions.

Determination of HBV DNA

Levels of HBV DNA were determined using transcription-mediated amplification (TMA) and hybridization-protection assay (Chugai Diagnostics Science Co., Ltd., Tokyo, Japan) after the protocol as reported [Kamisango et al., 1999]. The range of detection by TMA was from 3.7 log genome equivalents (LGE)/ml ($10^{3.7}$ copies/ml corresponding to 5,000 copies/ml) to 8.7 LGE/ml ($10^{8.7}$ copies/ml). In 16 of 86 studied sera, levels of HBV DNA were under 3.7 LGE/ml and categorized in 3.7 LGE/ml.

Genotyping HBV

HBV genotypes in most samples were determined with commercial enzyme immunoassay kits (HBV Genotype EIA, Institute of Immunology Co. Ltd., Tokyo, Japan) involving monoclonal antibodies to genotype-specific epitopes in the preS2-region, as reported previously [Usuda et al., 1999, 2000; Kato et al., 2001]. Genotypes in 18 (12%) samples were determined by genotype-specific probe assay (Smitest HBV Genotyping Kit, Genome Science, Fukushima, Japan). In brief, DNA extracted from serum was amplified by the polymerase chain reaction (PCR) with three sense primers (s1: 5′-ACC AAC CCT CTG GGA TTC TTT CC-3′, s2: 5′-ACC AAT CCT CTG GGA TTC TTC CC-3′ and s3: 5′-AGC AAT CCT CTA GGA TTC CTT CC-3′ [nt 2902–2924]) and an antisense primer (as1: 5′-GAG CCT GAG GGC TCC ACC C-3′ [nt 3091–3073]) biotinated at the 5′-end;

they were deduced from conserved sequences in the preS1 region of HBV. The biotin-labeled and amplified HBV DNA was denatured in an alkaline solution, and tested for hybridization to probes specific for one or other of the seven genotypes (A–G) immobilized on wells of a 96-well microplate. Thereafter, hybiridization was detected by staining with the streptavidine-horseradish peroxidase (HRP) conjugate [Kato et al., 2003].

Subtypes of genotype B, in terms of Ba with the recombination with genotype C and Bj without it were determined by direct sequencing of precore and core regions by the method reported previously [Sugauchi et al., 2002b].

Amplifying and Sequencing HBV DNA of Genotype A Isolates

A subgroup of genotype A is reported with the designation of A' from South Africa, Philippines, Malawi, and Belgium [Bowyer et al., 1997; Kramvis et al., 2002; Sugauchi et al., 2004]. Randomly selected HBV/A samples were classified into genotype A and subtype A' by sequencing the S region. For amplification and sequencing, the entire S region was divided into two fragments, spanning nt 3130–478 and nt 378–878, respectively, and they were amplified by two-stage PCR. The outer primers for amplification of the 1st fragment were: 5'-ACC AAT CGG CAG TCA GGA AG-3' (sense: nt 3121–3140) and 5'-CTG GAA TTA GAG GAC AAA CG-3' (antisense: nt 488–469) and the inner primers were: 5'-CAG TCA GGA AGG CAG CCT ACT-3' (sense: nt 3130–3150) and 5-AGG ACA AAC GGG CAA CAT AC-3' (antisense: nt 478–459). The outer primers for amplification of the 2nd fragment were: 5'-TGT CCT GGT TAT CGC TGG AT-3' (sense: nt 359–378) and 5'-CAA CGT ACC CCA ACT TCC AA-3' (antisense: nt 909–890) and the inner primers were: 5'-TGT GTC TGC GGC GTT TTA TC-3' (sense: nt 378–397) and 5'-ATG AAG TTT AGG GAA TAA CC-3' (antisense: nt 878–859).

The first stage of amplification was carried out in a thermal cycler for 40 cycles (94°C, 1 min; 55°C, 1 min; 72°C, 1 min) in 100 μl of the reaction mixture containing 200 μM dNTPs, 1.0 μM each of primers and 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$ and 0.001% (wt/vol) gelatin) and 2 U of Ampli-Taq polymerase (Perkin Elmer Cetus Corp., Connecticut). PCR products (2 μl) were subjected to the second stage of amplification under the same conditions as the first stage. Standard precautions to avoid contamination were exercised during PCR, with a negative control serum included in each run.

Amplification products were purified on Wizard PCR preps DNA purification resin (Promega, Wisconsin), and sequenced bidirectionally with the Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, California) using the PCR primers. Sequencing was performed in an automated DNA sequencer (ABI 377: PE Applied Biosystems).

The nucleotide sequences of HBV/A isolates from patients were compared with those of 25 reference HBV/A strains including subtype A' retrieved from the DDBJ/EMBL/GenBank database, as well as representatives of the other six major genotypes (B–G). Phylogenetic trees were constructed with the mega program version 2.1 using the Kimura two-parameter matrix and the neighbor-joining method [Sugita et al., 1991]. To confirm the reliability of phylogenetic tree analysis, bootstrap resampling, and reconstruction were carried out 500 times.

Detection of Point Mutations in the Precore and BCP Regions of HBV

Mutation in the precore region for A1896 was detected by enzyme-linked minisequence assay (Smitest HBV Pre-C ELMA, Roche Diagnostics, Tokyo, Japan) and mutations in the BCP region for T1762/A1764 were detected by enzyme-linked specific probe assay (Smitest HBV Core Promoter Mutation Detection Kit; Genome Science Laboratory, Tokyo, Japan) according to the manufacturer's instructions, after the principles described previously [Orito et al., 2001b]. The results were recorded as "the wild-type" and "the mutant-type" expressed dominantly by HBV isolates.

Statistical Analysis

Data were analyzed by chi-square test or Fisher's exact test for categorical data and Student's *t*-test or Mann–Whitney *U*-test for continuous variables. *P*-values less than 0.05 were regarded as statistically significant. Logistic regression (backward logistic regression) was used in the multivariate analysis to evaluate the factors associated with differences between genotypes A and C.

RESULTS

Distribution of HBV Genotypes

HBV genotypes were determined in 145 of the 147 (99%) patients with acute hepatitis B; they were untypeable in the remaining two patients (Table I). Genotype A was detected in 27 (19%) patients, B in 8 (5%), C in 109 (75%), and mixed genotypes with B and C in the remaining one (1%). In the 69 patients with acute hepatitis B from metropolitan areas (Tokyo, Kawasaki, and Tokorozawa), genotype A was found in 21 (30%), B in 5 (7%), and C in 43 (63%). In the 76 patients from the other areas in the mainland, by contrast, genotype A occurred in 6 (8%), B in 3 (4%), C in 66 (87%), and mixed genotypes with B and C in one (1%). Thus, genotype A was significantly more frequent in patients with acute hepatitis B from the metropolitan than the other areas (30% vs. 8%, $P < 0.001$).

Demographic and Clinical Differences Among Patients Infected With HBV of Distinct Genotypes

Clinical and demographic backgrounds in patients with acute hepatitis B who were infected with HBV of

TABLE I. Demographic and Clinical Differences Among Patients With Acute Hepatitis Who Were Infected With HBV of Distinct Genotypes

Features	Genotypes of HBV				Differences (A vs. C)	
	A (n = 27)	B (n = 8)	C (n = 109)	B/C (n = 1)	Univariate (*P*-value)	Multivariate logistic regression (*P*-value)
Areas					<0.001	0.03
Metropolitan (n = 69)	21 (30%)	5 (7%)	43 (63%)	0		
Others (n = 76)	6 (8%)	3 (4%)	66 (87%)	1 (1%)		
Age (years)	29.3 ± 8.0	35.7 ± 10.1	36.6 ± 13.6	51	0.016	0.152
Male	25 (93%)	7 (88%)	69 (57%)	1 (100%)	0.003	0.018
Transmission routes						
Heterosexual	15 (56%)	3 (37%)	52 (48%)	0	0.197	
Homosexual	5 (19%)	1 (13%)	2 (2%)	0	<0.001	0.133
IV drugs	0	0	8 (7%)	0	0.280	
Unknown	7 (25%)	4 (50%)	47 (43%)	1 (100%)	0.102	
Fulminant hepatic failure	0	1 (13%)	5 (5%)	0	0.582	
ALT (IU/L)[a]	2069 ± 1075	2952 ± 1106	2889 ± 1867	646	0.030	0.084
Bilirubin (mg/dl)[a]	10.7 ± 14.1	10.3 ± 4.9	7.8 ± 6.7	4.8	0.533	
ALP (IU/L)[a]	476 ± 161	501 ± 94	432 ± 116	No data	0.542	
HBeAg	24/26 (92%)	4/8 (50%)	57/93 (61%)	1/1 (100%)	0.357	
Precore and BCP mutations						
Precore (1896A)	0/27	1/8 (13%)	20/102 (20%)	No data	0.250	
BCP (1762T/1764A)	0/27	1/6 (17%)	14/75 (19%)	No data	0.357	
Precore or BCP	0/27	2/8 (25%)	27/102 (26%)	No data	0.096	

[a]Maximum data are shown for alanine aminotransferase (ALT), bilirubin and alkaline phosphatase (ALP).

different genotypes are compared in Table I. Patients with genotype A were younger than those with genotype C (29.3 ± 8.0 vs. 36.6 ± 13.6 years, $P = 0.016$). The proportion of male patients was higher in genotype A than C infection (93% vs. 57%, $P = 0.003$). The main route of transmission identified in the patients with acute hepatitis B was extramarital heterosexual contacts. Homosexual activity was more frequent in patients with genotype A than C (5/27 (19%) vs. 2/109 (1.8%), $P < 0.001$).

The maximum ALT levels were lower in patients with genotype A than B or C infection (2069 ± 1075, 2952 ± 1106 and 2889 ± 1867 IU/L, respectively: A vs. B, $P = 0.02$; A vs. C, $P = 0.03$). The maximum bilirubin and alkaline phosphatase levels were no different among patients infected with HBV of different genotypes. Fulminant hepatic failure developed in one (13%) patient with genotype B and five (5%) with genotype C; no patients with genotype A came down with it. Evolution into chronic infection occurred in two patients (one with genotype A and one with genotype C). The remaining 137 (96%) patients ran a non-fulminant and self-limited disease.

HBeAg was found in 24 of the 26 (92%) patients with genotype A, 4 of the 8 (50%) with genotype B and 57 of the 93 (61%) with genotype C; it was no different between genotype A than genotype C infection ($P = 0.357$). Of the six patients with fulminant hepatic failure, only one (17%) had HBeAg.

With logistic multivariate regression analysis, the variables for differences between genotypes A and C were sex (odds ratio (OR), 6.45; 95% confidence interval (CI), 1.378–30.213; $P = 0.0018$) and area (OR, 0.25; 95% CI, 0.076–0.830; $P = 0.0024$).

Routes of transmission were compared between genotypes A and C in patients with acute hepatitis B from metropolitan areas. Although the mean age was no different, frequently the proportion of male patients was higher in genotype A than C infection (20/21 (95%) vs. 28/43 (65%), $P = 0.012$). Homosexual patients had more frequently genotype A than C infection (5/21 (24%) vs. 1/44 (2%), $P = 0.012$). Additionally heterosexuals with multiple unspecific partners had in genotype A more frequently than C infection (7/12 (58%) vs. 6/26 (23%), $P = 0.035$, respectively). However, with logistic multivariate regression analysis, none of these variables differed between genotype A and C infections.

Figure 1 compares serum HBV DNA levels on admission among patients infected with different genotypes. HBV DNA levels were higher in patients with genotype A than C (5.90 ± 1.45 vs. 5.13 ± 1.36 LGE/ml, $P = 0.002$).

Among the 145 patients whose HBV genotypes could be determined, 54 (A: 15, B: 4, and C: 35) were followed for HBsAg in serum every 2–4 weeks until it disappeared. The time between the first and last detection of HBsAg was defined as the duration of HBsAg, and compared between patients infected with HBV of genotypes A and C (Fig. 2a). The duration of HBsAg was longer in patients with genotype A than C infection (1.95 ± 1.09 (n = 15) vs. 1.28 ± 1.42 months (n = 35), $P = 0.02$). When patients with fulminant hepatic failure were excluded, the mean duration of HBsAg in patients with genotype C became longer, but it was still shorter

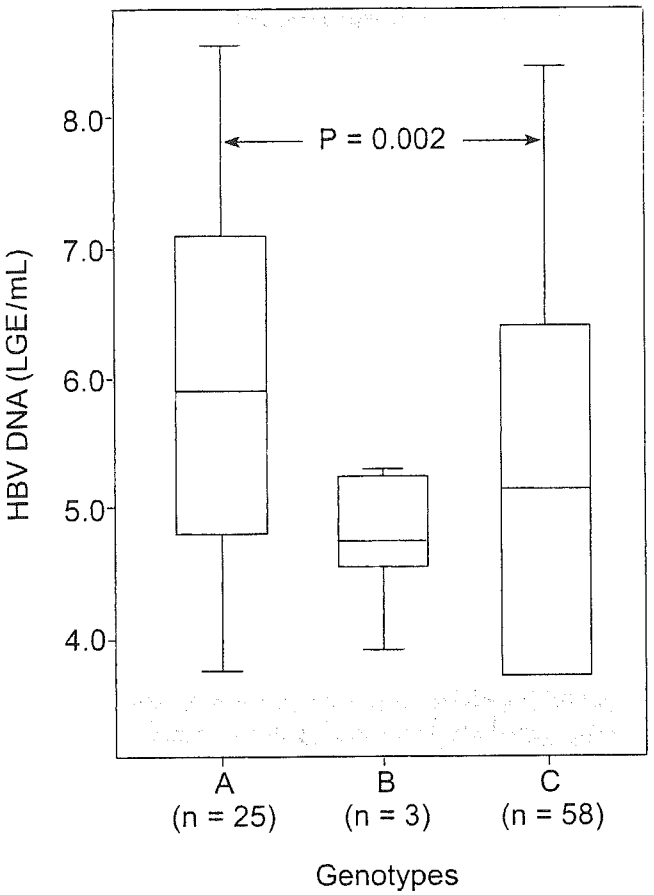

Fig. 1. HBV-DNA levels in patients with acute hepatitis B with genotypes A, B, or C at the presentation. Box plots are given with horizontal lines for the medians, upper and lower edges indicating the 25th and 75th centiles, respectively, and bars represent the extremes without including outliers. Shaded areas are outside the range of detection by the TMA method.

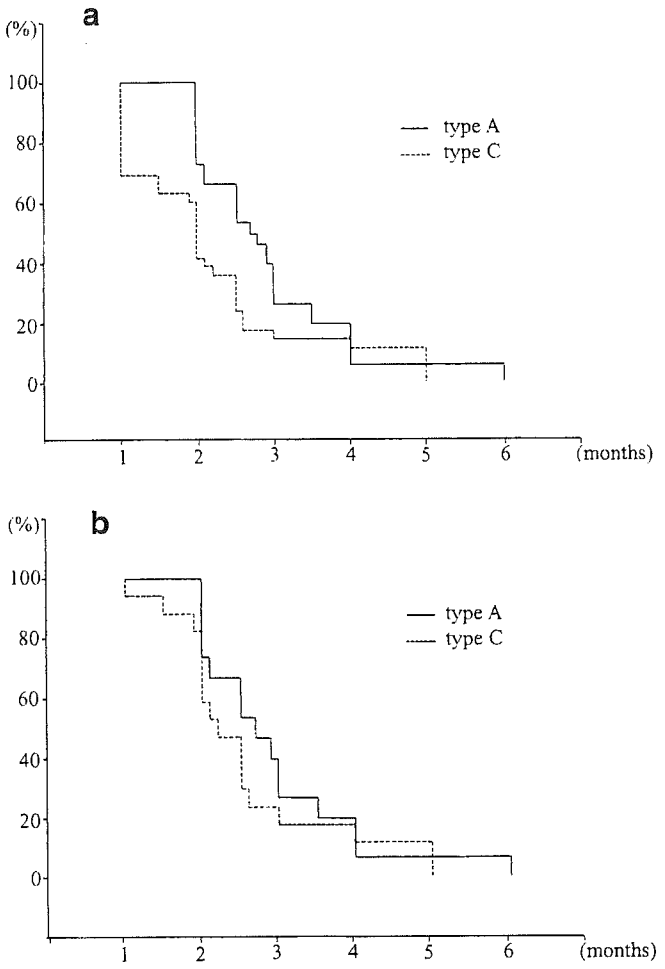

Fig. 2. The duration of HBsAg in patients with acute hepatitis B with genotypes A or C. The results are shown for (**a**) all patients, and (**b**) patients with the wild-type sequences both in precore and BCP regions of HBV.

than that in those with genotype A (1.95 ± 1.09 (n = 15) vs. 1.41 ± 1.42 (n = 31) months, $P = 0.03$).

Subtypes of Genotypes A and B

Among the 27 HBV/A isolates, 9 were selected at random and the entire S region was amplified and sequenced for them. Seven of them were classified into genotype A and the remaining 2 into subgroup A′. The sequence divergence within the seven genotype A isolates ranged from 0.12% to 2.01% in pair-wise comparison, while that between two subgroup A′ and seven genotype A isolates spanned from 5.70% to 6.53%.

A phylogenetic tree was constructed on the entire S-gene sequences from these nine sequences along with those from 31 HBV isolates retrieved from the database (Fig. 3). The seven (78%) HBV isolates classified into genotype A clustered with reported HBV/A isolates, while the remaining two isolates classified into subgroup A′ (cases 3 and 4) joined the branch of subgroup A′.

Six of the eight HBV/B isolates were available for analysis of subtypes. Two (both from the metropolitan area) were classified as Ba and the remaining four, including two from Tokyo and two from the other areas, as Bj. One of the four patients infected with subtype Bj developed fulminant hepatic failure, while the remaining three with subtype Bj as well as the two with subtype Ba ran a non-fulminant course.

Point Mutations in the Precore and Basic Core Promoter Regions of HBV

All the 27 HBV isolates of genotype A in which mutations were sought had the wild-type sequences both in the precore and BCP regions. In contrast, of the 102 genotype C isolates whose precore and BCP sequences were examined, 27 (26%) had mutations in the precore or BCP regions ($P = 0.096$). Furthermore, of the four genotype C isolates from patients with fulminant hepatic failure whose genetic mutations could be determined, three had mutations in the BCP region (T1762/A1764) and two had a mutation in the precore region (A1896). Only one isolate had the wild-type sequences both in the precore and BCP regions. Of

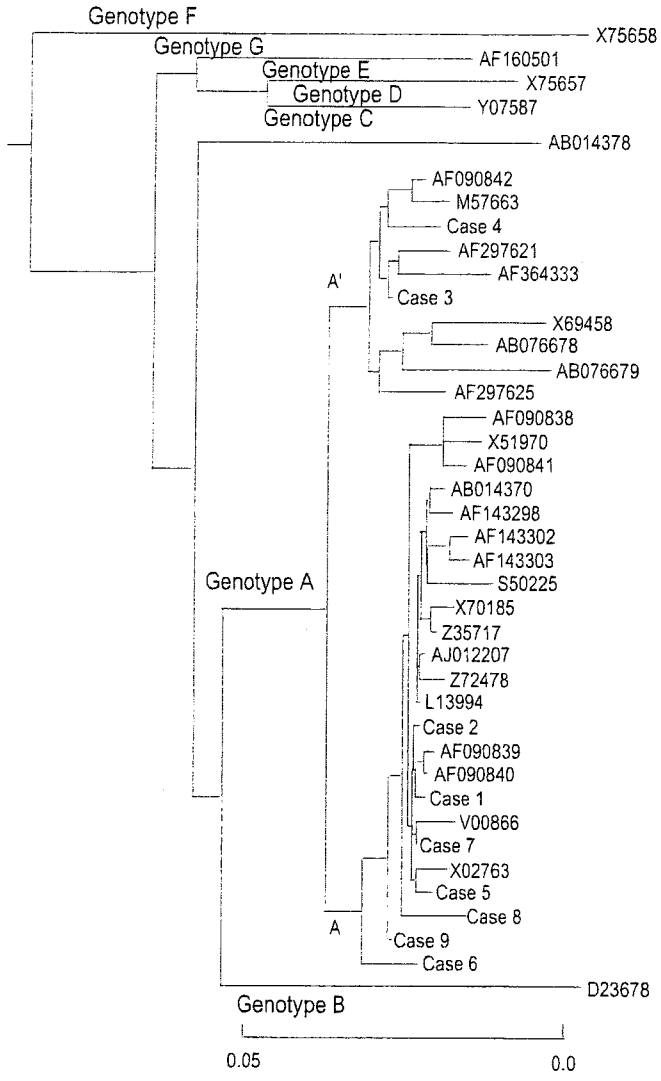

Fig. 3. A phylogenetic tree constructed on HBV DNA sequences spanning the major *S*-gene of all known HBV genomes, including the nine of genotype A. The horizontal bar indicates the number of nucleotide substitutions per site. Accession numbers are shown for the isolates, which have been deposited in the DDBJ/EMBL/GenBank databases. HBV sequences in cases 1–9 were determined in the present study. The HBV/A sequences from cases 1, 2, and 5–9 clustered with the European-American genotype A, while those from cases 3 and 4 clustered with genotype A' that is the African subgroup of genotype A.

the eight genotype B isolates, two (25%) had mutations in the precore or BCP region (Table I).

To examine further differences between genotype A and C infections, patients infected with HBV strains with the wild-type sequences both in precore and BCP regions were compared. The maximum ALT levels were still lower in patients with genotype A than C infection (2069 ± 1075 and 2594 ± 1015 IU/L, respectively, $P = 0.02$), but the maximum bilirubin and alkaline phosphatase levels were no different amongst patients infected with HBV of distinct genotypes. There were no differences in the duration of serum HBsAg between patients with genotype A and C infections (1.95 ± 1.09 vs. 1.58 ± 1.24 months, $P = 0.35$) (Fig. 2b).

DISCUSSION

The salient finding in this study is that infection with HBV genotype A is frequent in patients with acute hepatitis in Japan, lending support to previous studies [Kobayashi et al., 2002; Ogawa et al., 2002]. Substantial portion of patients with acute hepatitis were infected with genotype A, which is detected rarely among patients with chronic hepatitis in Japan [Orito et al., 2001a; Kobayashi et al., 2002]. Genotype A prevails in North-Western Europe, United States, Central Africa, and India [Kao, 2002; Miyakawa and Mizokami, 2003]. This genotype may be prevalent in countries elsewhere, since the distribution of HBV genotypes has not been examined in many districts of the world. Phylogenetic analysis has shown that seven (78%) HBV/A strains of the nine patients examined with acute hepatitis B were of the European-American type. Although the HBV/A sequences from four, (cases 1, 2, 5, and 7) clustered with those reported previously, those from three (cases 6, 8, and 9) were separated genetically (Fig. 3), which suggests their distinct geographic origin.

Notably, the genotype distribution differed between patients with acute hepatitis B from metropolitan areas and the others including many large cities. As genotype A is seen rarely in patients with chronic hepatitis [Orito et al., 2001a; Kobayashi et al., 2002], it is suspected that genotype A in metropolitan areas has a distinct geographic origin. Many patients with genotype A infection in these areas had a history of extramarital sexual contacts with plural unspecified partners. Such sexual behavior may increase the risk of infection with genotype A. In support of this view, most homosexual people in Tokyo who have human immunodeficiency virus type I are coinfected with HBV genotype A [Koibuchi et al., 2001]. Taken together, homosexual activity would increase the risk of genotype A infection in metropolitan areas. Further molecular analysis on HBV isolates from transmitters and recipients will verify this hypothesis. With respect to genotype B, both Ba, and Bj subtypes [Sugauchi et al., 2002b] were detected. Although the number of studied patients was small, patients with subtype Ba were found in the Tokyo metropolitan area exclusively. Whether subtype Ba intrinsic to the metropolitan area has a peculiar geographic origin is currently unknown and awaits further analyses.

Another point made in this study is that HBV genotypes influence clinical features and the outcome of acute hepatitis B. It has been shown that the proportion of patients who develop chronic HBV infection is close to 10% in European and American countries [Sherlock S, 1997] but rare in Japan [Kobayashi et al., 2002]. Recent studies suggest that chances for evolution into chronicity may differ among patients acutely infected with HBV of distinct genotypes [Mayerat et al., 1999; Ogawa et al., 2002]. Our study has shown that patients with genotype A had higher HBV DNA and lower ALT levels, as well as a longer duration of HBsAg in serum. Development of chronic hepatitis was seen in one of the 27 (4%) patients with genotype A as against one of the 109 (1%)

with genotype C infection. Although the number of patients studied was not large enough for statistical evaluation, the transition to chronic infection may be more frequent in infection with genotype A than the other genotypes, insofar as higher viral loads can predict chronic infection [Fong et al., 1994]. Further studies on more patients are required to evaluate whether or not viral persistence occurs more often after HBV infection with genotype A than the other genotypes.

Patients with fulminant hepatic failure in the present study were infected with either genotypes B or C; no patient with genotype A developed hepatic failure. As mutations at nt 1896 in the precore and nt 1762/1764 in the BCP regions, which are found frequently in patients with fulminant hepatic failure [Carman et al., 1991; Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Hawkins et al., 1994; Sato et al., 1995; Baumert et al., 1996; Chu et al., 1996], were not detected in patients with genotype A, low frequency of fulminant hepatic failure associated with genotype A infection may be attributed to the lack of these mutations. The high frequency of HBeAg in genotype A infection may also be related to low frequency of fulminant hepatic failure. However, interpretation on this data should be made carefully, because the number of patients studied was small. Further research is necessary to determine if the genotype itself affects the clinical course of acute hepatitis B.

In summary, (1) infection with HBV genotype A is common in patients with acute hepatitis in Japan; (2) patients with genotype A are more frequent in metropolitan areas and may be associated with particular sexual behavior; (3) patients with genotype A have a milder but longer course of infection, which may lead to increased risk of progression to chronic disease.

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Differences of Hepatocellular Carcinoma Patients with Hepatitis B Virus Genotypes of Ba, Bj or C in Japan

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Key Words
Hepatocellular carcinoma, epidemiology · Subtypes Ba/Bj, hepatitis B · Hepatitis B virus, genotypes B/C

Abstract
Hepatitis B virus (HBV) genotypes B (HBV/B) and C (HBV/C) are prevalent in Asia. Recently HBV/B has been classified into two subtypes, HBV/Ba which is ubiquitously found in Asia, and HBV/Bj which is specific in Japan. In addition, the frequency of positive HBeAg has been reported to be higher in patients with HBV/Ba than those with HBV/Bj. However, little is known about the differences between patients with various genotypes who developed hepatocellular carcinoma (HCC). In 296 serum samples of HCC patients collected from all over Japan, HBV genotypes were determined with the restriction fragment length polymorphism. HBV/A was detected in 1.0%, HBV/Ba in 4.4%, HBV/Bj in 7.4%, and HBV/C in 86.5%. In the Tohoku district and Okinawa, HBV/Ba, HBV/Bj and HBV/C were found in 6.7, 40.0 and 48.9%, compared to 4.0, 1.6 and 93.2% in the other districts in Japan. HBV/Bj patients were more frequently found in the group older than 65 years while HBV/Ba patients were found in all age groups. The frequency of positive HBeAg in HBV/Bj patients was significantly low compared to that in the other patients. More than 60% of the patients with HCC had cirrhosis as the underlying liver diseases. However, in HBV/Ba patients aged 50 years or younger, 80% of them had chronic hepatitis, while 87.5% of those aged older than 50 years had cirrhosis. These data suggest that great differences exist among patients with HCC infected with different genotypes.

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Introduction

In Japan, in more than two thirds of the patients with hepatocellular carcinoma (HCC) the disease is associated with hepatitis C virus (HCV). However, hepatitis B virus (HBV) is the major causative agent of HCC in Asian countries. All strains of HBV isolated from various countries can be classified into 8 HBV genotypes, HBV genotype A (HBV/A) to HBV/H, according to their phylogenetic relationships [1–3]. It has been reported that the clinical and virologic manifestations of patients with chronic HBV infection show significant differences among the different HBV genotypes [4–6]. In addition, specific distributions of HBV genotypes have been demonstrated among areas and countries [4, 7]. In south-east Asian countries, such as Japan, Taiwan, or China, HBV/B and HBV/C are prevalent [5, 7, 8].

In Japanese patients with HCC, the patients with HBV/B are rare and their mean age is high [7, 9]. However, in Taiwanese patients with HCC, a high proportion of younger patients have HBV/B. Until now, it is still unclear why younger Taiwanese patients with HBV/B develop HCC while Japanese patients with HBV/B rarely develop HCC, only in older age.

Recently, we demonstrated that HBV/B strains should be divided into two subtypes, HBV/Ba and HBV/Bj, according to their genetic relationship, and that HBV/Ba is found ubiquitously in Asian countries while HBV/Bj is found only in Japan [10, 11]. It was reported that HBeAg was found more frequently in patients with chronic infection with HBV/Ba than in those with chronic infection with HBV/Bj (32 vs. 9%) [12]. However, it is still unknown whether etiological and virologic differences are found between the HCC patients with HBV/Ba and HBV/Bj. Thus, in the patients with HCC, the difference between the subtypes of HBV/Ba and HBV/Bj might explain the etiological or clinical differences between Japan and Asia where HBV/Bj and HBV/Ba are endemic, respectively.

So, the aim of this study was to investigate the differences in the etiological, virologic and clinical characteristics among Japanese HCC patients with different HBV genotypes, such as HBV/Ba, HBV/Bj or HBV/C.

Patients and Methods

Patients with HCC

Two hundred and ninety-six patients with HCC were consecutively collected from 19 hospitals throughout Japan during January 2001 to December 2002. All the patients were chronically positive for HBsAg, and negative for anti-HDV, anti-HCV and anti-HIV. The diagnosis of HCC was reached clinically with ultrasound, computerized tomography, magnetic resonance imaging, angiography, tumor markers and biopsy if possible. The diagnoses of chronic hepatitis (CH) and liver cirrhosis (LC) were principally done by liver biopsy. However, a proportion of patients with ascites, jaundice or severe thrombocytopenia were diagnosed by ultrasound, computerized tomography and liver function tests. The serum samples and clinical data were collected from these patients with written informed consent. This study was conducted according to the ethical guidelines in our hospitals.

Virologic Assays

In all serum samples, HBsAg (CLIA, Fujirebio, Japan, detection limit 0.13 ng/ml), HBcAg (CLIA, Fujirebio, Japan) and anti-HBc (CLIA) were tested. Serum HBV DNA was detected by nested polymerase chain reaction (PCR) with the primers derived from the S gene. The patients were not enrolled in this study if the serum HBV DNA was not detected by PCR. The HBV genotype was determined by restriction fragment length polymorphism as described previously [13]. In brief, the S gene of HBV DNA was amplified by nested PCR. Then the products were sequentially digested by the restriction enzyme, *AlwI*, *EarI*, *HphI*, *NciI* and *NlaIV*, respectively. The HBV genotype was determined by the size of the digested PCR product which was electrophoresed on agarose gel. When the test results were inconclusive, the sequences of the S region were determined directly, then the genotype was decided by phylogenetic analysis [13, 14]. When patients were found to have HBV/B, the subtypes Ba and Bj were determined by restriction fragment length polymorphism [11]. In brief, at nucleotide position 1838 in the pre-core region, only A was found in patients with HBV/Ba while only G was found in those with HBV/Bj. The restriction enzyme detection system was established targeting the discrimination of this difference in nucleotides with the restriction enzyme, *SpeI* and *MseI* after the pre-core region was amplified by PCR.

Statistical Analysis

The data were statistically analyzed by Student's t test, nonparametric Mann-Whitney test, and χ^2 test where appropriate. A p value of <0.05 was regarded as statistically significant.

Results

HBV Genotypes and Clinical Findings

Of the 296 patients, 223 were male and 73 were female. The mean age was 55.1 ± 10.8 (range 26–81) years. The clinical findings are shown in table 1. Thirty-five percent of the patients were positive for HBeAg. Regarding the HBV genotypes, 3 patients (1.0%) were HBV/A, 13 (4.4%) HBV/Ba, 22 (7.4%) HBV/Bj, 256 (86.5%) HBV/C, and 2 (0.7%) of mixed genotype (HBV/B and C). The clinical findings by HBV genotype are shown in table 2. There were no significant differences in the mean levels of total bilirubin, AST and ALT among patients with different HBV genotypes. However, the mean ALP level and γ-

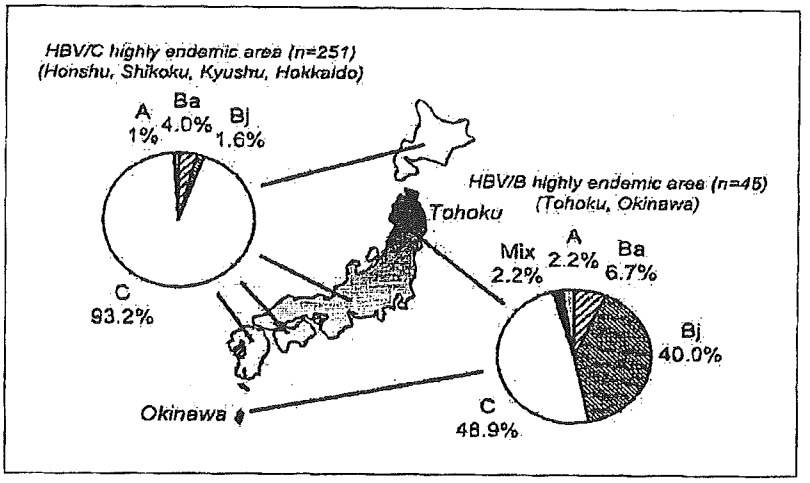

Fig. 1. The geographic distribution of HBV genotypes in Japan. In the Tohoku district, the northern area of mainland Japan, and Okinawa, the most southern islands, 48.9% of HCC patients were HBV/C, 6.7% were HBV/Ba, and 40.0% were HBV/Bj. In contrast, in other parts of Japan, Hokkaido, Honshu, Shikoku and Kyushu, 93.2% were HBV/C, 4.0% were HBV/Ba and 1.6% were HBV/Bj.

Table 1. Characteristics of 296 HBsAg-positive Japanese patients with HCC collected from all over Japan

Male:female	223:73
Age, years	55.1 ± 10.8[a]
Total bilirubin, mg/dl	1.5 ± 1.9
AST, IU/l	78.5 ± 103.9
ALT, IU/l	63.0 ± 69.8
ALP, IU/l	321.1 ± 225.4
γ-GTP, IU/l	108.4 ± 174.4
HBeAg, % positive	35.0
Anti-HBe, % positive	64.8
HBV genotype	
HBV/A	3 (1.0%)
HBV/Ba	13 (4.4%)
HBV/Bj	22 (7.4%)
HBV/C	256 (86.5%)
Mix	2 (0.7%)

[a] Mean ± SD.

Table 2. Clinical findings of the HCC patients with HBV genotypes of Ba, Bj or C

	HBV genotype		
	Ba	Bj	C
Age, years	55.4 ± 12.9	66.6 ± 10.6	54.0 ± 10.7
		p < 0.01	p < 0.01
Total bilirubin, mg/dl	1.0 ± 0.4	1.2 ± 0.7	1.5 ± 2.0
AST, IU/l	173.9 ± 352.6	51.6 ± 42.1	82.6 ± 113.4
ALT, IU/l	102.4 ± 162.9	33.9 ± 16.8	66.5 ± 74.9
ALP, IU/l	147.7 ± 126.6	209.8 ± 95.4	343.9 ± 238.0
		p < 0.05	
γ-GTP, IU/l	78.6 ± 55.9	63.1 ± 45.9	110.5 ± 186.7
			p < 0.05

GTP level of the HBV/C patients was significantly higher than those with HBV/Ba and HBV/Bj, respectively ($p < 0.05$).

Geographic Distribution of HBV Genotypes

The geographic distribution of HBV genotypes was area-specific in Japan (fig. 1). This specific distribution of HCC patients was in accord with that of all the patients including asymptomatic carriers, CH and LC patients, as described previously [7]. Namely, in the Tohoku district, the northern area of the Japanese mainland, and Okinawa, the most southern islands, 22 (48.9%) of HCC patients were HBV/C, 3 (6.7%) were HBV/Ba, and 18 (40.0%) were HBV/Bj. In contrast, in other areas of Japan, Hokkaido, Honshu, Shikoku and Kyushu, 234 (93.2%) were HBV/C, 10 (4.0%) were HBV/Ba, and 4 (1.6%) were HBV/Bj ($p < 0.01$).

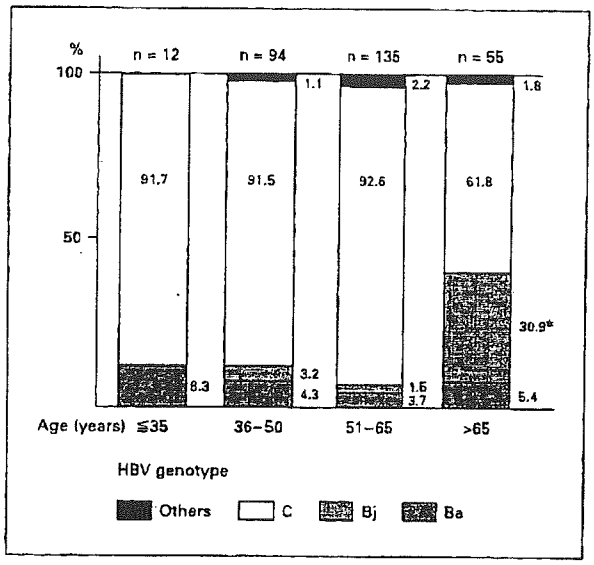

Fig. 2. The distribution of HBV genotypes in each age group. In groups aged 35 years or younger, 36–50 years, and 51–65 years, more than 90% of HCC patients had HBV/C. On the other hand, in the group aged older than 65 years, only 61.8% of patients had HBV/C while 30.9% had HBV/Bj (* $p < 0.01$, group aged older than 65 years vs. other age groups). More patients with HBV/Ba were in the younger aged group, although the number of patients with HBV/Ba was small in all the groups.

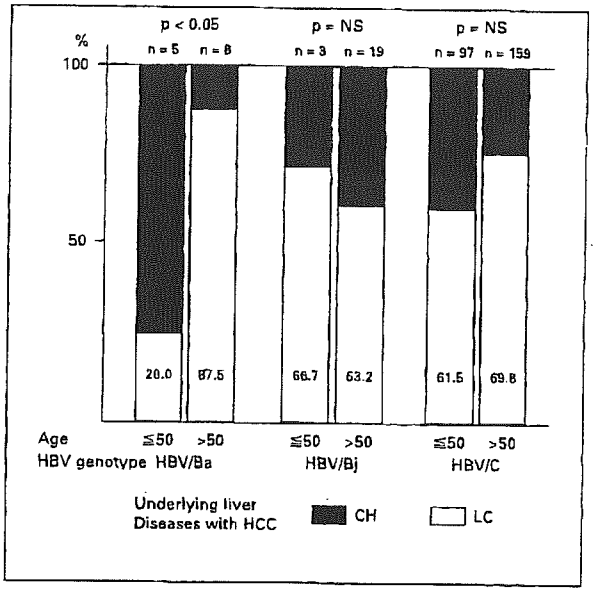

Fig. 4. The underlying liver diseases, chronic hepatitis (CH) or liver cirrhosis (LC), in HCC patients. In patients with HBV/Ba, only 25.0% of the group aged 50 years or younger had LC, while 85.7% of the group aged older than 50 years had LC ($p < 0.01$). However, in patients with HBV/Bj or HBV/C, the ratios of the underlying liver diseases were approximately identical even when compared by age.

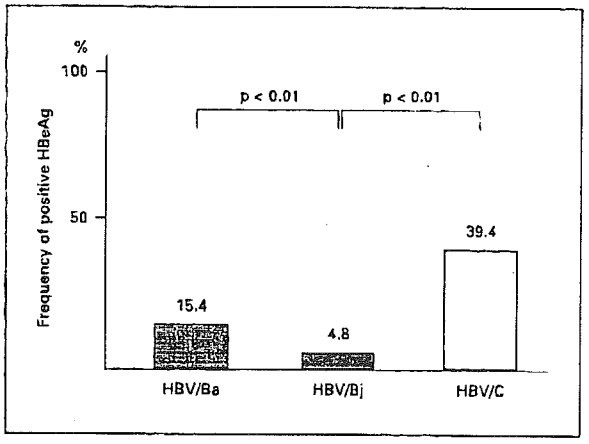

Fig. 3. The frequency of patients with positive HBeAg in each HBV genotype. The frequency of positive HBeAg was 4.8% in patients with HBV/Bj, compared with 39.4% in those with HBV/C (Bj vs. C, $p < 0.01$), and 15.4% in those with HBV/Ba (Bj vs. Ba, $p < 0.01$).

Mean Age and Frequency of Positive HBeAg among Patients with Each Genotype

The mean age of HBV/Bj patients (66.6 ± 10.6 years) was significantly higher than those with HBV/Ba (55.4 ± 12.9 years, $p < 0.01$) and HBV/C (54.0 ± 10.7 years, $p < 0.01$; table 2). The distribution of HBV genotypes in each age group is shown in figure 2. In groups aged 35 years or younger, 36–50 years, and 51–65 years, more than 90% of HCC patients had HBV/C. On the other hand, in the group aged older than 65 years, only 61.8% of the patients had HBV/C while 30.9% had HBV/Bj ($p < 0.01$, group aged older than 65 years vs. other age groups). HBV/Ba tended to be found in the younger age group although the number of patients with HBV/Ba was small in all groups.

The frequency of positive HBeAg was 4.8% in patients with HBV/Bj, compared with 39.4% in those with HBV/C (Bj vs. C, $p < 0.01$), and 15.4% in those with HBV/Ba (Bj vs. Ba, $p < 0.01$; fig. 3).

Underlying Liver Diseases

All HCC patients had underlying chronic liver diseases, such as CH or LC. We compared the underlying liver diseases among those aged 50 years or younger and those aged older than 50 years by HBV genotype (fig. 4). In 13 patients with HBV/Ba, only 1 (20.0%) of the 5 patients aged 50 years or younger had LC, while 7 (87.5%) of the 8 patients aged older than 50 years had LC ($p < 0.05$). However, in patients with HBV/Bj or HBV/C, the ratios of underlying liver diseases were approximately identical even when compared by age.

Discussion

The clinical and virologic features of patients with chronic HBV infection are specific according to their HBV genotypes [4, 15]. However, to date, there has been no report on the relationship between the HBV genotypes of Ba, Bj and C, and the clinical characteristics of HCC patients. We therefore analyzed the relationship between the clinical characteristics of Japanese HCC patients identified throughout Japan, and their HBV genotypes, including the HBV subtypes of Ba and Bj. In this study, we demonstrated that HBV/Ba (4.4%), HBV/Bj (7.4%) and HBV/C (86.5%) were found in Japanese HCC patients, and that there were distinct clinical differences among the three HBV genotypes, in geographic distribution, age distribution, and the frequency of positive HBeAg.

Of the Japanese patients with chronic HBV infection, including asymptomatic carriers, CH, LC and HCC, 1.7% were HBV/A, 12.2% HBV/B, 84.7% HBV/C, 0.4% HBV/D, and the others 1.0%, as reported previously [7]. In this study, we collected 296 serum samples from patients with HCC throughout Japan. In addition, we recently developed a new method for detecting HBV/Ba and HBV/Bj with restriction fragment length polymorphism [11]. Thus, we showed that 1.0% was HBV/A, 4.4% HBV/Ba, 7.4% HBV/Bj, 86.5% HBV/C, and mixed genotype 0.7% in Japanese HCC patients. This prevalence in HCC patients is almost identical to that in all patients with chronic HBV infection [7]. In addition, the geographic distribution of HBV/B and HBV/C in HCC patients is also identical to that in all patients. However, when we analyzed the HBV subtypes of HBV/Ba and HBV/Bj in patients with HBV/B, a high proportion of patients with HBV/Bj is found in the highly endemic HBV/B area, the Tohoku district and Okinawa, while the prevalence of HBV/Ba is approximately identical between the highly endemic HBV/C area, the other areas of Japan, and the highly endemic HBV/B area. Thus, HBV/Bj is specifically distributed in the Tohoku district and Okinawa.

As reported previously, HBV/Ba is ubiquitous in all Asian countries including Japan, although HBV/Bj is specific to Japan and is not found in other countries [11]. In Okinawa, it is reported that a high proportion of patients with chronic HBV infection have HBV/B and a good prognosis compared with patients with HBV/C [16, 17]. In contrast, in Taiwan, close to Japan, a higher proportion of patients aged 50 years or younger with HBV/B have HCC and CH [15]. The underlying liver diseases in those who developed HCC were compared among each HBV genotype group. In the HBV/Ba group, up to 75% of the patients aged 50 years or younger had CH as the underlying liver disease, compared with patients aged over 50 years. On the other hand, in the group with HBV/Bj or HBV/C, more than 60% of the patients had LC regardless of their age. The mean age of the patients with HBV/Ba in Japan is more than 10 years younger than those with HBV/Bj. So, more younger patients with HBV/Ba tend to have CH than the other patients. However, the molecular mechanism is unclear why patients with HBV/Ba develop HCC at a younger age and often have CH.

It is unclear why Japanese patients with HBV/B have a good prognosis while Taiwanese patients with HBV/B often have more advanced liver diseases, such as HCC. The frequency of patients positive for HBeAg in the HBV/Ba and HBV/C groups was higher than in the HBV/Bj group. So, the viral activity of HBV may be higher in patients with HBV/Ba or HBV/C than those with HBV/Bj. Thus, these differences in subtypes of HBV/Ba and Bj could be one of the reasons why the discrepancy in prognosis exists between Japanese and Taiwanese patients with HCC.

The differences in DNA sequences between HBV/Ba and HBV/Bj can be characterized in the core gene [10]. It has been reported that HBV/Ba, not HBV/Bj, recombines with HBV/C in the core gene. The product of the core gene is reported to be a cytotoxic T-cell epitope [18], suggesting that patients with HBV/Ba and HBV/C may be exposed to severe immune responses for destroying hepatocytes compared with those with HBV/Bj. In addition, patients with HBV/Ba more often have core promoter mutations at nucleotide 1762/1764 than those with HBV/Bj [11], which is associated with more advanced liver diseases [6, 19]. Taken together, these facts may indicate a poor prognosis in patients with HBV/Ba compared to those with HBV/Bj.

In the patients with HBV/C, the mean ALP and γ-GTP levels were higher than those with the other genotypes. In this study, there may exist some bias of regarding the tumor size of HCC between patients with HBV/C and the other patients. It is considered that more patients with a rather large size of HCC were found in the patients with HBV/C, resulting in elevation in ALT and γ-GTP levels.

To investigate the hepatocarcinogenesis and risk factors of HCC, it is important to study the differences in host, environmental and viral factors. The various genetic alterations, such as mutations of cancer-associated genes or loss of some chromosomes, are found in the HCC cells [20]. However, the genetic polymorphism varies among populations [21]. The differences in host genomes are still unknown between Japanese and other Asian populations. The association of environmental factors, such as air, water and food contaminated with some chemical agents, and HCC is still unclear, although aflatoxin affects the mutation of p53 in HCC [22]. However, with respect to the viral factors, a survey of the distribution of HBV genotypes or subtypes will be important clues for solving these problems.

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Mode of HCV Infection Examined by Polymorphism of Hypervariable Region-1 in Cases of Acute Hepatitis C After Accidental Exposure to Blood-Borne Pathogens

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Acute hepatitis C is known to respond better to interferon therapy than chronic hepatitis C. The reason for this difference remains unclear. The present study was undertaken to examine HCV quasispecies in blood from patients with acute hepatitis C caused by accidental exposure to blood-borne pathogens and in blood from the source patients. Three patients who developed hepatitis C (recipient patients; R-Pt.) and two patients who served as a source of HCV infection (source patients; S-Pt.) were the subjects of this study. The number of quasispecies and the genetic diversity in hypervariable region-1 (HVR-1) were examined on the basis of fluorescence single-strand conformation polymorphism and sequence analysis (FSSA). On the day of the accident, the number of quasispecies and genetic diversity were 13 and 36 in S-Pt.1 and 6 and 20 in S-Pt.3, respectively. At the time of diagnosis of acute hepatitis, the number of quasispecies and nucleotide diversity were 2 and 2 in R-Pt.1, 2 and 0 in R-Pt.2, and 4 and 0 in R-Pt.3, respectively. Immediately before the start of treatment, the number of quasispecies and genetic diversity were 4 and 4 in R-Pt.1, 2 and 0 in R-Pt.2, and 3 and 0 in R-Pt.3., respectively. In three R-Pts, interferon therapy resulted in eradication of HCV. These findings indicate that in the early stage of HCV infection, only a portion of HCV transmitted from S-Pts to R-Pts can proliferate. The low number of quasispecies of HCV appears to be one of the reasons why acute hepatitis responds well to interferon therapy. ***J. Med. Virol. 75:35–41, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: HCV; acute hepatitis C; hypervariable region-1; needlestick accident

INTRODUCTION

Thanks to advances in diagnosis, including the introduction of nucleic acid amplification testing (NAT), there is now little risk of transfusion-related hepatitis C virus infection in Japan [Japanese Red Cross NAT Screening Research Group, 2000]. However, health care workers always face the risk of accidental exposure to blood-borne pathogens by needlestick puncture while working in hospitals. The risk of HCV infection due to needlestick accidents involving HCV patients is several percent at most [Sodeyama et al., 1993; Liddle, 1996]. To date, however, no valid method has been established for preventing HCV infection due to accidental exposure to blood-borne pathogens in medical facilities. Although attempts at prophylactic interferon therapy have been made to prevent HCV infection, beginning immediately after accidental exposure, some cases of acute hepatitis C have developed despite this treatment [Nakano et al., 1995]. Acute hepatitis C is known to follow a chronic course in many cases [Alter et al., 1992]. It has been reported that response rates to interferon therapy were higher for acute hepatitis C than for chronic hepatitis C [Omata et al., 1991]. It remains unclear why acute hepatitis C responds to interferon therapy better than chronic hepatitis C.

The present study was undertaken to compare polymorphism of the hypervariable region-1 (HVR-1) of HCV in blood sampled from recipient patients who developed acute hepatitis C following accidental exposure to blood-borne pathogens at a known time and

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