scaling out from this range were diluted so as to contain them within it. Anti-HBs, hepatitis B e antigen (HBeAg) and IgM anti-hepatitis B core (HBc) were determined by enzyme-linked immunosorbent assays (Abbott JAPAN Co., Ltd., Tokyo, Japan). Levels of HBV DNA were determined using the COBAS TaqMan HBV v.2.0 kit (Roche Diagnostics, Basel, Switzerland) that has a dynamic range over 2.1–9.0 log copies/mL.

HBV Genotyping

The HBV genotype was determined by a genotype-specific probe assay (Smitest HBV genotyping Kit, Genome Science, Fukushima, Japan) as previously reported [12].

Molecular Evolutionary Analyses

HBV genotype A started to prevail in Japan merely several years ago, suggesting that it was imported to Japan only recently [3, 13]. Therefore, genomic sequences of HBV/A, recovered from sera of patients with acute HBV infection, would be closely related one another and with those reported from abroad. To evaluate this possibility, 20 HBV/A samples were selected randomly, and sequenced by the method reported previously [14].

The number of nucleotide substitutions per site was estimated by the 6-parameter method [15], and a phylogenetic tree was constructed by the neighbor-joining method [16] based on the numbers of substitutions. To confirm the credibility of phylogenetic analyses, bootstrap resampling tests were carried out 1,000 times [17].

Statistical Analyses

Categorical variables were compared by chi-squared test or Fisher's exact test, and continuous variables by the Mann–Whitney U-test. A P value < .05 was considered statistically significant. Receiver operating characteristic (ROC) analysis was performed to compute the area under ROC curves for viral markers to determine cut-off points for predicting the outcome.

RESULTS

Distribution of HBV Genotypes in Patients with Acute Hepatitis B

HBV genotypes were determined in 215 of the 232 (93%) patients with acute hepatitis B. Of the 215 patients, genotype A was detected in 113 (52%), B in 26 (12%), C in 73 (33%), D in 1 (1%), E in 1 (1%), and F in 1 (1%). The distribution of genotypes was compared among four periods during 1994 through 2010 (Table 1). The proportion of patients with genotype A gradually increased to 65.9% in 2007–2010; it was higher than those in the earlier periods (34.4% in 1994–1998 [$P = .002$], 36.8% in 1999–2002 [$P = .002$], and 51.9% in 2003–2006 [$P = .093$]).

Phylogenetic Relationship among HBV Strains of Genotype A

We randomly selected 11 HBV/A strains sampled in 2007–2010 and 9 of those in 2001–2006, and constructed a molecular evolutionary tree (Fig. 1). All the 20 samples had similar nucleotide sequences with a concordance of 99%. They were close to previously reported genotype A2 sequences from Western countries. The results support a possibility that HBV/A was imported to Japan only recently, and has been spreading throughout the country.

Clinical Features among Patients Infected with HBV of Different Genotypes

Clinical features of patients with acute hepatitis B of different genotypes are compared in Table 2. The mean age was no different among patients infected with HBV of different genotypes. The proportion of men was higher in genotype A or B than C infection (93.8% or 80.7% vs. 39.7% [A vs. C, $P < .001$; B vs. C, $P < .001$]).

The maximum alanine aminotransferase (ALT) level was lower in patients with genotype A than C (2,126 ± 938 vs. 2,857 ± 1,668 IU/L, $P = .002$). The maximum bilirubin level was higher in patients with genotype A (7.1 ± 6.4 mg/dL) or C (9.0 ± 7.5 mg/dL) than B (4.8 ± 3.3 mg/dL) (A vs. B, $P = .003$; B vs. C, $P < .001$). Regarding viral markers, the peak HBV DNA level was higher in patients with genotype A than C (6.3 ±

1.7 vs. 4.9 ± 1.5 log copies/mL, $P < .001$). HBeAg was detected in 95 of the 121 (77.3%) patients with genotype A, 24 of the 28 (88.5%) with genotype B, and 37 of the 58 (65.5%) with genotype C (A vs. C, $P = .036$). MSM (men who have sex with men) were more frequent in patients with genotype A than B or C (31.4% vs. 4.8% or 11.3% [A vs. B, $P = .017$; A vs. C, $P = .002$]). Anti-HIV was examined in 72 of the 113 (63.7%) patients with genotype A, seven of the 26 (26.9%) with genotype B, and 58 of the 73 (79.5%) with genotype C, and 1 with genotype E. Anti-HIV was detected in seven of the 72 (9.7%) patients with genotype A, and the other 96 patients tested for anti-HIV showed negative results. All of the seven patients with anti-HIV cleared HBsAg from the serum within 6 months without antiviral treatment.

Among the 215 patients whose HBV genotypes were determined, 159 could be followed until the confirmation of clinical outcomes. The distribution of HBsAg-positive period is compared among patients with different genotypes. Group I (HBsAg persisting for < 3 months) comprised 84 patients; Group II (3–6 months) comprised 54 patients; Group III (> 6–12 months) comprised 15 patients; and Group IV (> 12 months) comprised 6 patients. HBsAg stayed > 6 months in 21 of the 215 (9.8%) patients, including 14 of the 113 (12.4%) with genotype A, 1 of the 26 (3.8%) with genotype B, and 6 of the 73 (8.2%) with genotype C. Among the 21 patients, 15 (71.4%) cleared HBsAg within 12 months from the onset, and were classified into Group III. The remaining six (5 with genotype A and 1 with genotype B), who failed to clear HBsAg within 12 months, were classified into Group IV. All of the six were negative for anti-HIV. The proportion of Group IV was 6.0% in the patients with genotype A, 4.0% in those with genotype B, and 0% in those with genotype C.

The mean duration of HBsAg was 13.9 ± 8.7 weeks in patients with genotype A, 7.1 ± 5.3 weeks in those with genotype B, and 9.6 ± 7.6 weeks in those with genotype C, presuming the duration of HBsAg in Group IV at 12 months. The duration was longer in patients with genotype A than B or C (A vs. B, $P < .001$; A vs. C, $P = .04$).

Prediction of the Outcome by the Duration of HBsAg

Table 2 shows that the duration of HBsAg among patients with genotype A varied to a higher extent than that among those with other genotypes. Therefore, we determined HBsAg and HBV DNA levels serially, and evaluated them for the ability to predict the outcome of acute hepatitis B in patients with genotype A.

Serial changes in HBsAg levels are shown in supplementary figure 1A. HBsAg levels declined more slowly in Group II than I, as well as in Group III than II. In Group IV, HBsAg re-elevated at 12 weeks after the onset. Figure 2 compares HBsAg levels among Groups I–IV at different intervals from the onset. HBsAg at 8 weeks from the onset was useful for distinguishing Group III or IV from Group I or II. Likewise, HBsAg at 12 weeks from the onset was helpful for discriminating among Groups II, III, and IV.

Prediction of the Outcome by HBV DNA

We also studied serial changes of HBV DNA in patients with genotype A, and examined if they also were useful for predicting the clinical outcome of acute hepatitis B. Supplementary figure 1B shows serial changes in HBV DNA levels in patients in four groups. Although the re-elevation of HBV DNA was not observed, the decline of HBV DNA was quite slow in Group IV. Figure 3 compares HBV DNA levels among Groups I–IV at different intervals from the onset. HBV DNA at 4 weeks from the onset was useful for distinguishing Group III or IV from Group I or II. Likewise, HBV DNA levels at 8 weeks from the onset were useful for discriminating between Group IV and Group III, as well as for distinguishing Group III or IV from Group I or II.

Levels of HBsAg and HBV DNA for Predicting Persistent Infection

As the levels of HBsAg at 12 weeks and HBV DNA at 8 weeks from the onset were useful for distinguishing Group IV from the other groups, we evaluated the appropriate levels for predicting persistent infection in patients with genotype A. When we set the

cut-off value of HBsAg at 1000 IU/mL based on the ROC analysis, both the positive
predictive value and the negative predictive value were 100% with high sensitivity
(100%) and specificity (98.1%). Likewise, when we set the cut-off value of HBV DNA
at 10^6 log IU/mL based on the ROC analysis, both the positive predictive value and the
negative predictive value were 100% with high sensitivity (100%) and specificity
(96.4%). Therefore HBsAg at 12 weeks over 1000 IU/mL or HBV DNA at 8 weeks over
10^6 log copies/mL is useful for predicting persistent infection.

DISCUSSION

In Japan, as shown in Table 1, the dominant HBV in acute hepatitis in Japan has been
shifting from genotype C to A [3, 5, 14, 18]. The fact that nucleotide sequences of
HBV/A isolates from patients with acute hepatitis B in this study were very close to one
another suggests that most HBV/A strains were imported recently and have spread
rapidly, which may be attributed to the features of HBV/A in transmission routes and
viral kinetics. We have reported that patients with genotype A tend to have multiple
sexual partners [5]. Consequently, chances of secondary transmission of HBV/A would
be higher than those of other genotypes, which may increase the number of patients who
contract HBV/A infections. On the other hand, HBsAg persisted longer in patients with
genotype A than B or C, which is consistent with the in vivo experiment using
chimera-mice carrying human hepatocytes showing that proliferation of HBV starts
later and lasts longer in genotype A than B or C infection [19].

Our results have shown that 6% of the patients with genotype A develop persistent
infection. As liver cirrhosis or hepatocellular carcinoma can develop in a substantial
population of HBV carriers [20, 21], it is important to distinguish the patients in whom
HBV infection becomes chronic, and follow them carefully. Although polymorphisms in
host genes may be useful for identifying patients who are prone to develop chronic
HBV infection [22], simple surrogate markers for the outcome have not been reported.

Our data indicate that it would be difficult to predict the clinical outcome based on serum levels of viral markers at the first visit alone. This is understandable, because the dose of infecting virus, as well as the interval between infection and the first visit, can vary widely. Hence, we set out to analyze changes in serum levels of viral markers.

As seen in Figure 2, HBsAg levels at 12 weeks from the onset were most useful for discriminating among Groups II, III and IV in the genotype A infection. Therefore, the outcome of acute hepatitis B may be predictable at this time point. Of note is the re-elevation of HBsAg observed in Group IV (Supplementary Fig. 1A). Re-elevation of viral markers suggests prolonged viral proliferation in the liver, and may be useful to identify the patients who may develop chronic infection.

As shown in Figure 3, HBV DNA levels at 4 weeks from the onset can discriminate Groups I/II from III/IV. Furthermore, HBV DNA levels at 8 weeks from the onset can distinguish Group IV from Group I, II or III. Therefore, the combination of HBV DNA levels at weeks 4 and 8 would be useful for predicting the outcome. For the prediction of a chronic outcome, HBV DNA level at 8 weeks from the onset, is a useful surrogate marker of the outcome as well as HBsAg level at 12 weeks. There were differences in viral kinetics among Groups I, II, III and IV.

Our present study showed that 15 out of the 215 patients (7.0%) cleared HBsAg from > 6 to 12 months after the onset. Sixty percent of the 15 patients had HBV/A. Although these patients met the criteria of chronic infection, they finally cleared HBsAg from the sera. Therefore, we would like to propose that transition to chronic infection in acute hepatitis B be judged at 12 months from onset in patients with genotype A; further studies in larger cohorts are necessary. One reason for our proposal is the indication of antiviral treatment. Antiviral treatment to the patients with acute hepatitis B is not indicated because previous studies failed to show the efficacy of antiviral treatments in the patients with acute hepatitis B [23, 24]. However, if patients who really develop chronic infection can be identified and treated by antiviral treatment, the number of those who develop secondary infection may be markedly reduced. Evaluation of the

efficacy of antiviral treatments by prospective studies, based on surrogate markers for the outcome, should be conducted as the next step. HBeAg, which was reported to be useful as a surrogate marker for chronicity, should also be assessed as a surrogate marker [25, 26].

Our study has some limitations. First, the lack of data in early stages made it difficult to study viral kinetics precisely. Secondly, viral kinetics in the infection with each HBV genotype were obtained from a restricted number of patients, not large enough to establish the usefulness of changes in viral markers in earlier stages of HBV infection. Thirdly, anti-HIV was not checked in all the patients for the lack of informed consent. Fourthly, HBsAg and HBV DNA were not determined 24 weeks after onset when discrimination between groups III and IV may be possible more easily. Fifthly, the maximum levels of ALT and bilirubin may be affected by the time of blood test. Validation studies in larger cohorts are necessary to evaluate the feasibility of our hypotheses.

In conclusion, we have shown that viral kinetics and the clinical outcome are different among patients with acute hepatitis B who are infected with HBV of distinct genotypes. HBsAg levels at 12 weeks and HBV DNA at 8 weeks after the onset would be useful to predict the clinical outcome of patients with acute hepatitis B.

Notes

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