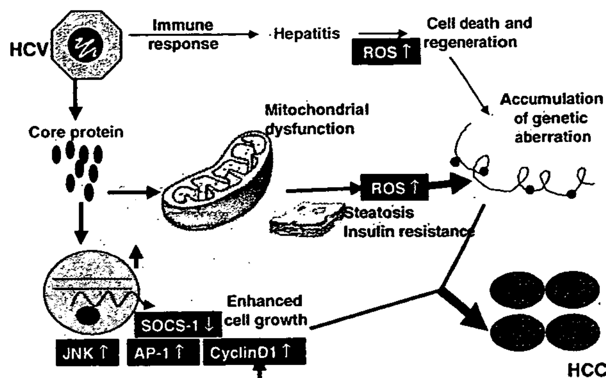

Figure 2 Molecular pathogenesis of liver disease in hepatitis C virus (HCV) infection. Induction of oxidative stress together with hepatic steatosis by the HCV core protein would play a pivotal rôle in the development of hepatocellular carcinoma (HCC). Alterations in cellular gene expressions, such as tumor necrosis factor-α (TNF-α) or suppressor of cytokine signaling-1 (SOCS-1), and those in the intracellular signaling pathways including c-Jun N-terminal kinase (JNK) would be coaccelerators to hepatocarcinogenesis in HCV infection. ROS, reactive oxygen species.

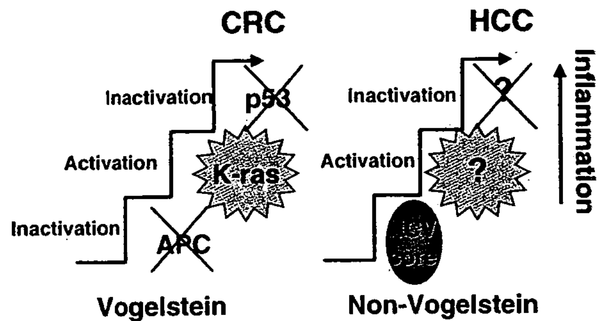

Figure 3 Mechanism of hepatitis C virus (HCV)-associated hepatocarcinogenesis. Multiple steps are required in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that genetic mutations accumulate in hepatocytes. However, in HCV infection, some of these steps may be skipped in the development of hepatocellular carcinoma (HCC) in the presence of the core protein. The overall effects achieved by the expression of the core protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations, required for carcinogenesis. By considering such a 'non-Vogelstein-type' process for the induction of HCC, a plausible explanation may be give for many unusual events occurring in HCV carriers. CRC, colorectal cancer; APC, *adenomatous polyposis coli*.

types of hepatitis, such as autoimmune hepatitis. The basis for the overproduction of oxidative stress may be ascribed to the mitochondrial dysfunction.[13,15] The function of the electron transfer system of the mitochondrion is suggested in association with the presence of the HCV core protein.[16] Hepatic steatosis in hepatitis C may work as fuel for oxidative stress overproduction.[14,17,18]

Other possible pathways would be the alteration of the expression of cellular genes, interacting with cellular proteins, and modulation of intracellular signaling pathways (Fig. 2). For an example, tumor necrosis factor (TNF)-α and interleukin-1β have been found to be transcriptionally activated.[19] The core protein has also been found to interact with some cellular proteins, such as retinoid X receptor (RXR)-α, which play pivotal roles in cell proliferation and metabolism.[20] The mitogen-activated protein kinase (MAPK) cascade is also activated in the liver of the core gene transgenic mouse model. The MAPK pathway, which consists of three routes, c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK), is involved in numerous cellular events including cell proliferation. In the liver of the core gene transgenic mouse model prior to HCC development, only the JNK route is activated. Downstream of the JNK activation, transcription factor activating factor (AP)-1 activation is markedly enhanced.[19,21] Far downstream, both the mRNA and protein levels of cyclin D1 and CDK4 are increased (Fig. 2). The suppression by HCV core protein of the suppressor of cytokine signaling (*SOCS*)-*1*, a tumor suppressor gene, may also contribute to hepatocarcinogenesis. Thus, the HCV core protein modulates the intracellular signaling pathways and gives an advantage to hepatocytes for cell proliferation.

Such an effect of the core protein on the MAPK pathway, combined with that on oxidative stress, may explain the extremely high incidence of HCC development in chronic hepatitis C (Fig. 2).

Hepatocarcinogenesis in HCV infection: A mechanism distinct from those in other cancers

The results of our studies on transgenic mice have indicated a carcinogenic potential of the HCV core protein *in vivo*; thus, HCV may be directly involved in hepatocarcinogenesis. In research studies of carcinogenesis, the theory by Kinzler and Vogelstein has gained popularity.[22] They have proposed that the development of colorectal cancer is induced by the accumulation of a complete set of cellular gene mutations. They have deduced that mutations in the *adenomatous polyposis coli* gene for inactivation, those in *K-ras* for activation and those in the *p53* gene for inactivation accumulate, which cooperate toward the development of colorectal cancer.[22] Their theory has been extended to the carcinogenesis of other cancers as well, called 'Vogelstein-type' carcinogenesis (Fig. 3).

On the basis of the results for the induction of HCC by the HCV core protein, we would like to introduce a mechanism different from that of Kinzler and Vogelstein for hepatocarcinogenesis in HCV infection. We do allow a multistage process in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that many mutations accumulate in hepatocytes. Some of these steps, however, may be skipped in the development of HCC in HCV infection to which the core protein would contribute (Fig. 3). The overall effects achieved by the expression of the viral protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations, required for carcinogenesis.

By considering such a 'non-Vogelstein-type' process for the induction of HCC, a plausible explanation may be given for many unusual events happening in HCV carriers.[23] Now it does not seem so difficult as before to determine why HCC develops in persistent HCV infection at an outstandingly high incidence. Our theory may

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also give an account of the non-metastatic and multicentric de novo occurrence characteristics of HCC, which would be the result of persistent HCV infection.

References

1 Saito I, Miyamura T, Ohbayashi A *et al.* Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. *Proc. Natl Acad. Sci. USA* 1990; **87**: 6547–9.
2 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J. Hepatol.* 1998; **28**: 930–8.
3 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671–5.
4 Yotsuyanagi H, Shintani Y, Moriya K *et al.* Virological analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. *J. Infect. Dis.* 2000; **181**: 1920–8.
5 Moriya K, Yotsuyanagi H, Shintani Y *et al.* Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. *J. Gen. Virol.* 1997; **78**: 1527–31.
6 Moriya K, Fujie H, Shintani Y *et al.* Hepatitis C virus core protein induces hepatocellular carcinoma in transgenic mice. *Nat. Med.* 1998; **4**: 1065–8.
7 Smith GS, Walford RL, Mickey MR. Lifespan and incidence of cancer and other diseases in selected long-lived inbred mice and their F 1 hybrids. *J. Natl Cancer Inst.* 1973; **50**: 1195–213.
8 Koike K, Moriya K, Ishibashi K *et al.* Expression of hepatitis C virus envelope proteins in transgenic mice. *J. Gen. Virol.* 1995; **76**: 3031–8.
9 Koike K, Moriya K, Yotsuyanagi H *et al.* Sialadenitis resembling Sjögren's syndrome in mice transgenic for hepatitis C virus envelope genes. *Proc. Natl Acad. Sci. USA* 1997; **94**: 233–6.
10 Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. *Hepatology* 1992; **15**: 572–7.
11 Lerat H, Honda M, Beard MR *et al.* Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. *Gastroenterology* 2002; **122**: 352–65.
12 Moriya K, Fujie H, Yotsuyanagi H *et al.* Subcellular localization of hepatitis C virus structural proteins expressed in transgenic liver. *Jpn J. Med. Sci. Biol.* 1997; **50**: 169–77.
13 Moriya K, Nakagawa K, Santa T *et al.* Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocellular carcinogenesis. *Cancer Res.* 2001; **61**: 4365–70.
14 Moriya K, Todoroki T, Tsutsumi T *et al.* Increase in the concentration of carbon 18 monounsaturated fatty acids in the liver with hepatitis C: analysis in transgenic mice and humans. *Biochem. Biophys. Res. Commun.* 2001; **281**: 1207–12.
15 Okuda M, Li K, Beard MR *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; **122**: 366–75.
16 Moriya K, Tajima A, Tsutsumi T *et al.* Hepatitis C virus core protein insults mitochondrial function through reducing the ETS complex 1 activity. In: *10th International Meeting on Hepatitis C and Related Viruses, Kyoto, 2003*. Osaka: Japan Linkage, 2003; 73.
17 Shintani Y, Fujie H, Miyoshi H *et al.* Hepatitis C virus and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840–8.
18 Koike K, Moriya K. Metabolic aspects of hepatitis C: steatohepatitis distinct from NASH. *J. Gastroenterol.* 2005; **40**: 329–36.
19 Tsutsumi T, Suzuki T, Moriya K *et al.* Intrahepatic cytokine expression and AP-1 activation in mice transgenic for hepatitis C virus core protein. *Virology* 2002; **304**: 415–24.
20 Tsutsumi T, Suzuki T, Shimoike T *et al.* Interaction of hepatitis C virus core protein with retinoid X receptor-α modulates its transcriptional activity. *Hepatology* 2002; **35**: 937–46.
21 Tsutsumi T, Suzuki T, Moriya K *et al.* Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. *Hepatology* 2003; **38**: 820–8.
22 Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996; **87**: 159–70.
23 Koike K, Tsutsumi T, Fujie H *et al.* Role of hepatitis viruses in hepatocarcinogenesis. *Oncology* 2002; **62**: 29–37.

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Original Article

Amino acid substitutions in the S region of hepatitis B virus in sera from patients with acute hepatitis

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Background: An increase in the number of acute hepatitis patients with hepatitis B virus (HBV) of non-indigenous genotypes may reduce the efficacy of vaccination against HBV.

Methods: We have determined the amino acid (aa) sequences in the major hydrophilic region (MHR) in the S region of HBV in patients with acute hepatitis B and compared those with the ones from HBV strains used for the production of HBV vaccines commercially available in Japan.

Results: Of 48 patients studied, 11 were infected with genotype A, 11 with genotype B and 26 with genotype C HBV. The aa sequences of the nine genotype A isolates were the same as the aa sequence of J02205 which is used for the production of one of the commercially available recombinant vaccines. The aa sequences of the 11 genotype B isolates differed from the aa sequence of J02205 in two or three amino acids. Of the 26 genotype C isolates, 22 had the same aa sequence as X01587 which is used for the production of another recombinant vaccine. The remaining genotype C isolates had aa substitutions at aa131, which have a potential to alter the hydropathy and the three-dimensional structure of the MHR. The differences among the three current HBV vaccines in aa sequences in the MHR theoretically alter the hydropathy and three-dimensional structure.

Conclusion: Our results suggest that the transmission of HBV isolates with different genotypes or with aa substitutions in the MHR might reduce the efficacy of currently available HBV vaccines in the protection of HBV infections.

Key words: genotype, hepatitis B virus, major hydrophilic region, vaccine

INTRODUCTION

ABOUT 300 MILLION people in the world are chronically infected with hepatitis B virus (HBV). Chronic infection may eventually lead to liver cirrhosis or hepatocellular carcinoma.[1–4] To prevent the transmission of this virus, vaccination has been introduced in many countries. Indeed, universal vaccination has not only reduced the number of infected individuals, but also the number of deaths related to HBV.[5,6]

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In Japan, in 1985, a national project was started to vaccinate children born to HBV-infected mothers. The chances of vertical transmission from HBV-carrying mothers have decreased. Recently, the prevalence of HBV in Japan has decreased to approximately 0.6%.[7]

Because the number of individuals infected with HBV has decreased, the number of patients with acute hepatitis B, mainly caused by horizontal transmission from HBV carriers, should also have decreased. However, in Japan, the number of patients with acute hepatitis B has recently increased (Yatsuhashi H. *et al.*, 2004, unpubl. data).

The increase in the number of patients with acute hepatitis B may, in part, be the result of patients carrying novel HBV genotypes imported from abroad. For

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example, in recent years, genotype A HBV has often been detected in patients with acute hepatitis B.[8,9]

Genotype A HBV is transmitted from individuals who live in or have immigrated from other countries to Japan. Its infection is characterized by a high viral load and a long hepatitis B surface antigen (HBsAg) positivity period. The transition of acute hepatitis B with genotype A HBV infection to the chronic state has been reported recently.[8,10] Decreasing the transmission rate of genotype A HBV is therefore important for the control of the disease. Introducing universal vaccination for adolescents or adults is a measure to be considered.

The effectiveness of universal vaccination depends on the reactivity of vaccines against HBV. HBsAg binds antibody to hepatitis B surface antigen (anti-HBs) produced against HBV vaccines mainly via the 'a' determinant region (aa124–aa149). This region contains common antigenic epitopes of all subtypes (adw, adr, ayw, ayr) of HBsAg and lies in the major hydrophilic region (MHR) between aa99 and aa169. Amino acid (aa) substitutions in the MHR, particularly in the 'a' determinant region, can alter B cell epitopes of HBsAg, leading to immunological escape from the host immunity induced by either vaccination or previous infection.[11] Therefore, if HBV prevalent in Japan has aa substitutions in the MHR, the effect of universal vaccination may be reduced.

In Japan, three types of HBV vaccine (Bimmugen, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan; Heptavax, Merck & Co., Whitehouse Station, NJ, USA; and Meinyu, Meiji Dairies, Tokyo, Japan) are now available. Efficacy and immunogenicity of vaccines are not always comparable or identical.[12,13] Whether giving a single vaccine effectively prevents the transmission of all genotypes of HBV is an important but still unsolved problem. Elucidating the aa substitutions in the MHR may give a clue to this problem.

The purpose of the present study is to determine the difference of the aa sequences in the MHR of HBV among isolates from patients with acute hepatitis and also the difference of the aa sequences among viral strains used for the production of anti-HBV vaccines, and to find ways to use currently available vaccines as effective prophylaxes.

METHODS

Patients

FROM 1992 TO 2001, serum samples were collected from 48 patients diagnosed with acute hepatitis B in our institutions. Only patients whose serum samples were stored at the onset of hepatitis were included in this study. All the 48 patients ran a self-limited clinical course. No patients subsequently developed fulminant hepatic failure or chronic sequelae.

The criteria for the diagnosis of acute hepatitis B were the following: (i) an acute onset of liver injury without a history of liver dysfunction and positivity for HBsAg in serum; and (ii) immunoglobulin M (IgM) antibody to HBV core antigen (anti-HBc) at a titer of more than 2.5 of cut-off index. Coinfection with a hepatitis A virus or a hepatitis C virus was excluded by serological tests. None of the patients had previously received any vaccination against HBV.

Serum samples from the 48 patients with acute hepatitis B were examined virologically, and the results were examined for correlations with clinical characteristics. Informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committees of our institutions.

Determination of HBV-DNA

Hepatitis B virus DNA level was determined using transcription-mediated amplification (TMA) and a hybridization protection assay (Chugai Diagnostics Science, Tokyo, Japan) using the protocol of Kamisango *et al.*[14] The range of detection using TMA was from 3.7 log genome equivalents (LGE)/mL (i.e. $10^{3.7}$ copies/mL corresponding to 5000 copies/mL) to 8.7 LGE/mL ($10^{8.7}$ copies/mL). In seven of 34 studied serum samples, the level of HBV-DNA was lower than 3.7 LGE/mL and these were categorized as 3.7 LGE/mL.

Genotyping HBV

Hepatitis B virus genotypes were determined using commercial enzyme immunoassay kits (Smitest HBV Genotyping kit; Genome Science, Fukushima, Japan). In brief, DNA extracted from serum was amplified by polymerase chain reaction (PCR) using three sense primers (i.e. s1: 5'-ACCAACCCTCTGGGATTCTTTCC-3', s2: 5'-ACCAATCCTCTGGGATTCTTCCC-3', and s3: 5'-AGCAATCCTCTAGGATTCCTTCC-3' [nt 2902–2924]) and an antisense primer (i.e. as1: 5'-GAGCCTGA GGGCTCCACCC-3' [nt 3091–3073]) biotinylated at the 5' end; their sequences were deduced from conserved sequences in the pre-S1 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 of the seven HBV genotypes (A-G) immobilized on wells of a 96-well

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microplate. Thereafter, hybridization was detected by staining with the streptavidin-horseradish peroxidase (HRP) conjugate.[15]

Amplifying and sequencing the S region of HBV-DNA

The entire aa sequence of MHR in the S region was amplified by two-stage PCR using genotype-specific primers. The outer primers for the amplification of the first fragment were 5′-TTTCCACCAAGCTCTGCAA-3′ (sense: nt 9–28) and 5′-TTCAGGGAATAACCCCATCT-3′ (antisense: nt 872–853) for genotype A, 5′-CTCCA CCACTTTCCA GACT-3′ (sense: nt 1–22) and 5′-CAACTCCCAATTACATATCCC-3′ (antisense: nt 899–879) for genotype B and 5′-TTACAGGCGGGG TTTTTCTT-3′ (sense: nt 70–89) and 5′-TACAGACTT GGCCCCCAATA-3′ (antisense: nt 771–752) for genotype C. The inner primers were 5′-AGAGTCAGGGGCC TGTATTTT-3′ (sense: nt 35–55) and 5′-AGGGAATAA CCCCATCACTTT-3′ (antisense: nt 869–849) for genotype A, 5′-TTCAAGATCCCAGAGTCAGG-3′ (sense: nt 24–43) and 5′-AGGGAATATCCCCACCTTTTT-3′ (antisense: nt 869–849) for genotype B and 5′-CGGGGTT TTCTTGTTGACA-3′ (sense: nt 77–97) and 5′-CCCAAT ACCACATCATCCATA-3′ (antisense: nt 758–738) for genotype C.

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 reaction mixture containing 200 mM dNTPs, 1.0 mM each of primers and 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 Ampli-Taq polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). PCR products (2 µL) were subjected to the second stage of amplification under the same conditions as those in the first stage. Standard precautions to avoid contamination were taken during PCR, with a negative control serum sample included in each run.

Amplification products were purified on Wizard PCR preps DNA purification resin (Promega, Madison, WI, USA), and sequenced bidirectionally with a Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, USA) using the above-mentioned PCR primers. Sequencing was performed in an automated DNA sequencer (ABI 377; PE Applied Biosystems).

The nucleotide sequences of HBV isolates from the patients were compared with those of three reference HBV strains which are used for vaccine production.[16–18]

Phylogenetic trees were constructed with the Mega Program version 2.1 (Center for Evolutionary Functional Genomics, The Biodesign Institute, Tempe, AZ, USA) using the Kimura two-parameter matrix and the neighbor-joining method.[19] To confirm the reliability of phylogenetic tree analysis, boot-strap resampling, and reconstruction were carried out 500 times.

Hydrophobicity and secondary structure analysis

The hydrophobicity profile of the MHR of the S region was predicted by computer-assisted Kyte-Doolittle analysis (an estimate of hydrophobicity based on the bulk phase partitioning of side chain hydrophobicity alone)[20] with GENETYX-MAC software (version 10.1; Software Development, Tokyo, Japan).

The secondary structures of the amino acids in the same region were predicted by computer-assisted Robson[21] and Chou-Fasman analyses[22] with the GENETYX-MAC software.

Statistical analyses

Data were analyzed by the chi-squared test for categorical data and Student's *t*-test or the Mann–Whitney *U*-test for continuous variables. *P*-values less than 0.05 were regarded as statistically significant.

RESULTS

Distribution and clinical characteristics of HBV genotypes

HEPATITIS B VIRUS genotype was determined in the 48 patients with acute hepatitis B. Genotype A was detected in 11 (23%) patients, genotype B in 11 (23%) and genotype C in 26 (54%).

The clinical and demographic backgrounds of the patients with acute hepatitis B who were infected with HBV of different genotypes are shown in Table 1. The mean ages of all the groups were similar. The proportion of male to female patients was higher in genotype A infection than in genotypes B or C infection (100%, 73% and 64%, respectively: A *vs* B, $P = 0.22$; A *vs* C, $P = 0.01$; B *vs* C, $P = 0.16$). The maximum alanine aminotransferase (ALT) levels were lower in patients with genotype A infection than in patients with genotypes B or C infection (1646 ± 1123, 3085 ± 1119 and 2545 ± 981 IU/L, respectively: A *vs* B, $P = 0.01$; A *vs* C, $P = 0.03$; B *vs* C, $P = 0.89$). The maximum HBV-DNA levels were not significantly different between the

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Table 1 Demographic and clinical differences among patients with acute hepatitis infected with HBV of distinct genotypes

Features	Genotypes of HBV			Differences (*P*-value)		
	A ($n = 11$)	B ($n = 11$)	C ($n = 26$)	A *vs* B	A *vs* C	B *vs* C
Age (years)	30.6 ± 7.5	28.1 ± 5.1	31.1 ± 9.1	0.41	0.87	0.33
Gender (M:F)	11:0	8:3	15:11	0.22	0.01	0.16
ALT (IU/L)	1646 ± 1123	3085 ± 1119	2545 ± 981	0.01	0.03	0.89
HBV-DNA (LGE/mL)	6.8 ± 1.7	6.6 ± 2.1	5.2 ± 1.2	0.60	0.23	0.06

ALT, alanine aminotransferase; HBV, hepatitis B virus.

genotypes (6.8 ± 1.7, 6.6 ± 2.1 and 5.2 ± 1.2 LGE/mL, respectively: A *vs* B, $P = 0.60$; A *vs* C, $P = 0.23$; B *vs* C, $P = 0.06$).

Amino acid sequence of the S region

The aa sequence of the S region between aa27 and aa203 was determined in the 48 sequences. Figure 1 shows a phylogenetic tree constructed using the 48 sequences and 15 published sequences (four for genotype A, three for genotype B, three for genotype C, one for genotypes D, E, F, G and H). Among the 48 sequences we studied, 11 were classified into genotype A, 11 into genotype B and 26 into genotype C.

The aa sequence of the region between aa101 and aa163 including MHR (aa111-aa156) was compared among 48 sequences and three HBV sequences (X01587, J02205 and huGK-14) currently used for anti-HBV vaccine production. As shown in Figure 2, the aa sequences of X01587 (used for Bimmugen) and J02205 (used for Heptavax) differed in eight amino acids (i.e. aa110, aa113, aa114, aa126, aa131, aa143, aa160 and aa161). The aa sequence of huGK-14, which is used for the HBV-vaccine Meinyu, differed from that of X01587 in six amino acids and from that of J02205 in two amino acids.

Nine of the 11 isolates classified into genotype A had the same aa sequence as J02205. The remaining two isolates (AB289727 and AB289728) differed from J02205 at aa161 (Fig. 2).

Ten of the 11 isolates classified into genotype B had the same aa sequence as J02205 except for two amino acids (aa114 and aa131). The remaining isolate had another aa substitution at aa112 (Fig. 2).

As shown in Figure 2, 22 of the 26 isolates classified into genotype C had the same sequence as X01587. The remaining four isolates (from patients 10, 24, 30 and 48) had the same sequence as X01587 except for one aa substitution at aa131; the threonine (aa131) of X01587 was substituted with proline for three isolates (AB289714, AB289720 and AB289736) and with alanine for one isolate (AB289701).

Hydrophobicity and secondary structure analysis

As mentioned above, the aa sequences of the MHR from four isolates differed from that of X01587 only at aa131. Furthermore, the aa sequence of the MHR differed between X01587 and J2205 in eight amino acids. We compared the hydropathy and secondary structure of the MHR among J02205, X01587 and two isolates with genotype C (one isolate with proline at aa131 and one with alanine at aa131). The results of Kyte-Doolittle hydropathy analysis based on the hydropathy index are shown in Figure 3. The substitution with alanine-131 was found to alter the patterns on the hydropathy plot, whereas the substitution with proline-131 was found to have little effect. A substitution with alanine-131 could increase the hydrophobicity of the first loop of the MHR, which may affect the antigenicity of HBV.

The secondary structure of our isolate with alanine-131 by Chou-Fasman analysis predicted an α-helix configuration for the region from aa126 to aa135 instead of the β-configuration predicted for the same region of X01587. The predicted secondary structure of our isolate with proline-131 coincided with that of X01587. In contrast, by Robson prediction, the secondary structure of our isolate with alanine-131 coincided with that of X01587; however, that of our isolate with proline-131 was found to have lost a turn structure between aa131 and aa134, which was predicted for X01587.

DISCUSSION

VACCINATION IS THE key to controlling HBV infection. In countries with a high prevalence of HBV infection, universal vaccination is effective not only for controlling viral infections but also for decreasing the incidence of hepatocellular carcinoma.[5,23] Even in

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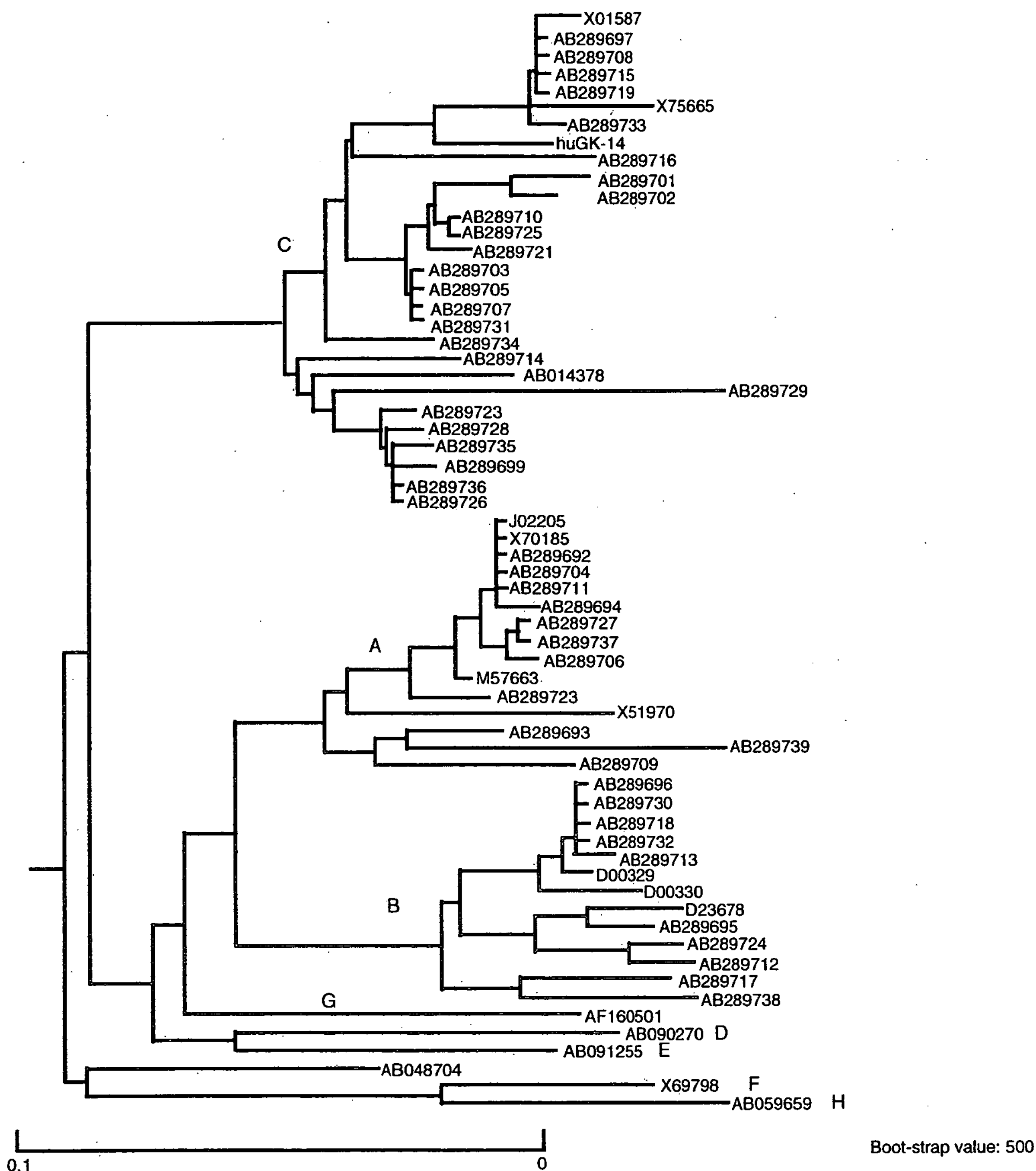

Figure 1 Phylogenetic tree constructed using hepatitis B virus (HBV)-DNA sequences of the *S* gene. The sequences include four with genotype A, four with genotype B, three with genotype C, and those recovered from the serum of 48 patients with acute hepatitis B. J02205 (genotype A) is used for the production of Heptavax and X01587 (genotype C) is used for the production of Bimmugen. The horizontal bar indicates the number of nucleotide substitutions per site. Accession numbers are shown for the isolates that have been deposited in the DDBJ/EMBL/GenBank databases. The accession numbers for the HBV sequences from the 48 patients are also shown.

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          101                                                           163
J02205    QGMLPVCPLIPGSTTTSTGPCKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAKYLW  (Heptavax)
X01587    ---------L--TS-----------I----T-----------S----------------RF--  (Bimmugen)
huGK-14   ---------L--T------------------T-----------S----------------RF--  (Meinyu)
AB014378  ---------L--TS-----------I----T-----------S----------------RF--  Genotype C
X75665    ---------L--TS-----------I----T-----------S---------------VRF--
AB048704  ---------L-----------R---IT---T-----------S-------------G---F--
AB289719  ---------L--TS-----------I----T-----------S----------------RF--
AB289715  ---------L--TS-----------I----T-----------S----------------RF--
AB289708  ---------L--TS-----------I----T-----------S----------------RF--
AB289697  ---------L--TS-----------I----T-----------S----------------RF--
AB289716  ---------L--TS-----------I----T-----------S----------------RF--
AB289698  ---------L--TS-----------I----T-----------S----------------RF--
AB289700  ---------L--TS-----------I----T-----------S----------------RF--
AB289735  ---------L--TS-----------I----T-----------S----------------RF--
AB289729  ---------L--TS-----------I----T-----------S----------------RF--
AB289722  ---------L--TS-----------I----T-----------S----------------RF--
AB289699  ---------L--TS-----------I----T-----------S----------------RF--
AB289726  ---------L--TS-----------I----T-----------S----------------RF--
AB289737  ---------L--TS-----------I----T-----------S----------------RF--
AB289702  ---------L--TS-----------I----T-----------S----------------RF--
AB289734  ---------L--TS-----------I----T-----------S----------------RF--
AB289721  ---------L--TS-----------I----T-----------S----------------RF--
AB289710  ---------L--TS-----------I----T-----------S----------------RF--
AB289725  ---------L--TS-----------I----T-----------S----------------RF--
AB289732  ---------L--TS-----------I----T-----------S----------------RF--
AB289707  ---------L--TS-----------I----T-----------S----------------RF--
AB289703  ---------L--TS-----------I----T-----------S----------------RF--
AB289705  ---------L--TS-----------I----T-----------S----------------RF--
AB289720  ---------L--TS-----------I----P-----------S----------------RF--
AB289714  ---------L--TS-----------I----P-----------S----------------RF--
AB289736  ---------L--TS-----------I----P-----------S----------------RF--
AB289701  ---------L--TS-----------I----A-----------S----------------RF--
M57663    ---------------------------------------------------------------  Genotype A
X70185    ---------------------------------------------------------------
X51970    ---------L-----------------------------------------------------
AB289739  ---------------------------------------------------------------
AB289723  ---------------------------------------------------------------
AB289709  ---------------------------------------------------------------
AB289694  ---------------------------------------------------------------
AB289693  ---------------------------------------------------------------
AB289711  ---------------------------------------------------------------
AB289704  ---------------------------------------------------------------
AB289692  ---------------------------------------------------------------
AB289706  ---------------------------------------------------------------
AB289727  ------------------------------------------------------------F--
AB289728  ------------------------------------------------------------F--
D00329    -------------S----------------T-----------M--------------------  Genotype B
D00330    -------------S----------------T--------------------------------
D23678    -------------S----------------T--------------------------------
AB289717  -------------S----------------T--------------------------------
AB289738  -----------R-S----------------T--------------------------------
AB289695  -------------S----------------T--------------------------------
AB289713  -------------S----------------T--------------------------------
AB289718  -------------S----------------T--------------------------------
AB289733  -------------S----------------T--------------------------------
AB289696  -------------S----------------T--------------------------------
AB289731  -------------S----------------T--------------------------------
AB289712  -------------S----------------T--------------------------------
AB289724  -------------S----------------T--------------------------------
AB289730  -------------S----------------T--------------------------------
AB090270  -------------S---V---R----TV--T--Y--------S---------------G-F--  (Genotype D)
AB091255  -------------S-------R----L---T--------S--S---------------G-F--  (Genotype E)
X69798    ---------L----------------L---T--------S--S--------------LG-F--  (Genotype F)
AF160501  -------------S-------------------Y--------S--------------------  (Genotype G)
AB059659  ---------L----------------L---T-----------S---------------G----  (Genotype H)
```

Figure 2 Comparison of amino acid sequences of the major hydrophilic region (MHR) of the *S* gene.

countries with a low prevalence of HBV infection, vaccination is very important for preventing mother-to-child transmission as well as patient-to-staff transmission.

HBV is classified into several genotypes that differ from one another in nucleotide sequence by more than 8% of the entire genome. The aa sequences of their phenotypes also differ among genotypes. The difference in the aa sequence of the 'a' determinant region may alter the three-dimensional structure and antigenicity, and may reduce the protectivity of HBV vaccines.

As mentioned above, the aa sequences of currently available recombinant vaccines differ from each other. J02205 and X01578 differ in eight amino acids (i.e. aa110, aa113, aa114, aa126, aa131, aa143, aa160 and aa161) between aa101 and aa163. Three (i.e. aa126, aa131 and aa143) of them are included in the MHR and may alter the hydropathy and three-dimensional

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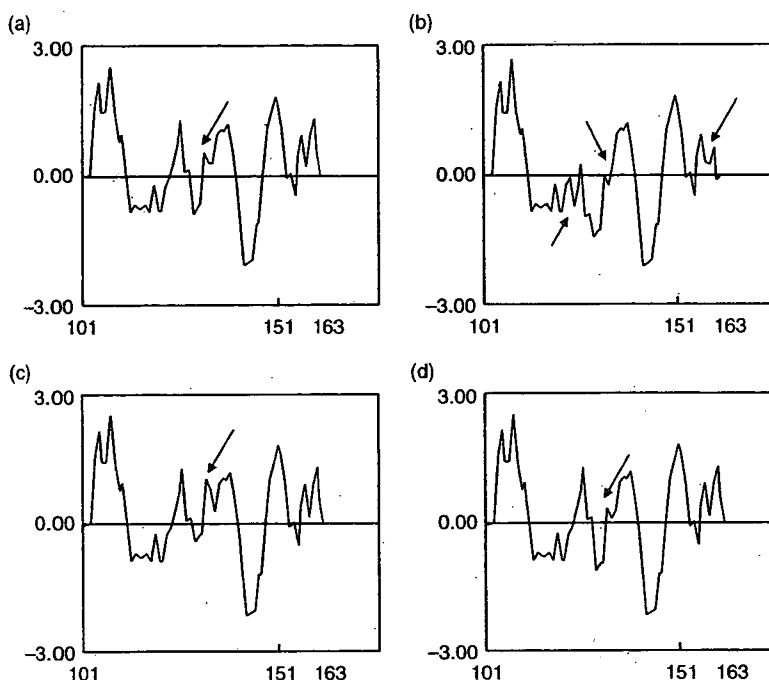

Figure 3 Hydropathy profile of the major hydrophilic region (MHR) of the *S* gene elaborated using the Kyte-Doolittle hydropathy index. Arrows show the positions of amino acids which are different among X01587, J02205, AB289701 (alanine-131) and AB289720 (proline-131). (a) X01587, (b) J02205, (c) AB289701 (alanine-131), (d) AB289720 (proline-131).

structure of the region. Therefore, the antibody produced against J02205 vaccines may not completely neutralize X01578 and vice versa. Indeed, previous studies showed that antibody profiles induced by recombinant vaccines produced from different genotypes are not identical with each other,[12] which suggests that antibodies produced by recombinant vaccines might not protect viral infection with different genotypes.

As shown in Figure 2, the aa sequences of our isolates classified into genotype A are very close to the aa sequence of J02205. Therefore, the transmission of genotype A HBV is prevented by Heptavax which is made from J02205.

The aa sequences of our isolates classified into genotype B are the same as the aa sequence of J02205 except for one substitution at aa131. This aa, which is asparagine and is located in the first stem loop structure of the MHR, was substituted with threonine in our genotype B isolates. Because asparagine and threonine have an uncharged side chain and similar polarity, genotype B HBV infection may be prevented effectively by Heptavax.

The aa sequences of our isolates classified into genotype C were the same as that of X01587 except for four isolates having a substitution at aa131. Bimmugen, which is produced from X01587, may be effective for preventing genotype C HBV infections caused by those four isolates. However, Heptavax may not be effective for preventing genotype C HBV infection because of the difference in eight amino acids as described above.

The four isolates have proline or alanine instead of threonine-131, which has never been reported before. The polarities of threonine and proline/alanine are quite different. The Kyte-Doolittle hydropathy analysis suggests that substituting threonine at aa131 with alanine or proline would increase hydrophobicity, which may then lead to a change in antigenicity. Hou *et al.* reported that some blood donors who were tested negative for serum HBsAg had a substitution of isoleucine for threonine at aa131 in the S region.[24] They suggested that the structure and antigenicity of HBV may be altered by this substitution.

The secondary structure of our isolate with alanine-131 predicted by Chou-Fasman analysis suggested an α-helix configuration instead of a β-configuration in the region from aa126 to aa135. The secondary structure of our isolate with proline-131 predicted by Robson analysis suggested that this change causes the loss of a turn structure between aa131 and aa134. Some changes in the secondary structure can affect the three-dimensional structure of the protein and thus affect antigenicity. These results suggest that the transmission of the four

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isolates with an aa substitution at aa131 may not be prevented by either Heptavax or Bimmugen.

However, the protective immunity elicited by HBV vaccines, which is usually polyclonal in nature, may not be totally lost or severely affected *in vivo* by the alteration of only a single amino acid in the 'a' determinant region.[25] Also, antibodies against regions outside the 'a' determinant region may be protective.[26] The protectivity of current vaccines may be elucidated by *in vitro* binding studies using polyclonal antibodies.

It was reported that some individuals immunized with recombinant vaccines are infected with HBV with or without mutations in the 'a' determinant region.[11,27,28] HBV isolates with amino acid substitutions at aa144[29–31] or 145[11,27,28] are known to be transmitted despite vaccination. Indeed, some chronic HBV carriers are reported to have HBV with such amino acid substitutions.[32,33] We were unable to find patients who had these substitutions in the present study. However, large-scale studies are necessary to elucidate the prevalence of 'vaccine-escape mutants' in patients with acute hepatitis B.

In conclusion, we have shown that the aa sequence of the MHR in the *S* gene of HBV is different among isolates from patients with acute HBV infection. Current vaccination may prevent the transmission of these HBV isolates, which should be further investigated.

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REFERENCES

1 Manno M, Camma C, Schepis F *et al.* Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology* 2004; 127: 756–63.
2 Liaw YF, Lin DY, Chen TJ, Chu CM. Natural course after the development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Liver* 1989; 9: 235–41.
3 Fattovich G, Brollo L, Giustina G *et al.* Natural history and prognostic factors for chronic hepatitis type B. *Gut* 1991; 32: 294–8.
4 Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology* 1988; 8: 493–6.
5 Chang MH, Chen CJ, Lai MS *et al.* Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997; 336: 1855–9.
6 Ni YH, Chang MH, Huang LM *et al.* Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med* 2001; 135: 796–800.
7 Tanaka J, Kumagai J, Katayama K *et al.* Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3 485 648 first-time blood donors during 1995–2000. *Intervirology* 2004; 47: 32–40.
8 Kobayashi M, Suzuki F, Arase Y *et al.* Infection with hepatitis B virus genotype A in Tokyo, Japan during 1976 through 2001. *J Gastroenterol* 2004; 39: 844–50.
9 Yotsuyanagi H, Okuse C, Yasuda K *et al.* Distinct geographic distributions of hepatitis B virus genotypes in patients with acute infection in Japan. *J Med Virol* 2005; 77: 39–46.
10 Suzuki Y, Kobayashi M, Ikeda K *et al.* Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. *J Med Virol* 2005; 76: 33–9.
11 Water JA, Kennedy M, Voet P *et al.* Loss of the common 'A' determinant of hepatitis B surface antigen by a vaccine-induced escape mutant. *J Clin Invest* 1992; 90: 2543–7.
12 Heijtink RA, Schneeberger PM, Postma B, Crombach W. Anti-HBs levels after hepatitis B immunisation depend on test reagents: routinely determined 10 and 100 IU/L seroprotection levels unreliable. *Vaccine* 2002; 20: 2899–905.
13 Hieu NT, Kim KH, Janowicz Z, Timmermans I. Comparative efficacy, safety and immunogenicity of Hepavax-Gene and Engerix-B, recombinant hepatitis B vaccines, in infants born to HBsAg and HBeAg positive mothers in Vietnam: an assessment at 2 years. *Vaccine* 2002; 20: 1803–8.
14 Kamisango K, Kamogawa C, Sumi M *et al.* Quantitative detection of hepatitis B virus by transcription-mediated amplification and hybridization protection assay. *J Clin Microbiol* 1999; 37: 310–14.
15 Kato H, Orito E, Sugauchi F *et al.* Frequent coinfection with hepatitis B virus strains of distinct genotypes detected by hybridization with type-specific probes immobilized on a solid-phase support. *J Virol Methods* 2003; 110: 29–35.
16 Koike K, Yoshida E, Katagiri K *et al.* Production of hepatitis B virus surface antigen particles by the human hepatoma cell line huGK-14 in a serum-free medium. *Jpn J Cancer Res* 1987; 78: 1341–6.
17 McAleer WJ, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. *Nature* 1984; 307: 178–80.
18 Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* 1982; 298: 347–50.
19 Sugita S, Yoshioka Y, Itamura S *et al.* Molecular evolution of hemagglutinin genes of H1N1 swine and human influenza A viruses. *J Mol Evol* 1991; 32: 16–23.

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20 Kyte J, Doolittle F. A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 1982; 157: 105–32.
21 Garnier J, Osguthorpe DJ, Robson B. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J Mol Biol* 1978; 120: 97–120.
22 Chou PY, Fasman GD. Conformational parameters for amino acids in helical, β-sheet, and random coil regions calculated from proteins. *Biochemistry* 1974; 13: 211–12.
23 Ni YH, Chang MH, Huang LM *et al.* Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med* 2001; 135: 796–80.
24 Hou J, Wang Z, Cheng J *et al.* Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. *Hepatology* 2001; 34: 1027–34.
25 Ogata N, Cote PJ, Zanetti AR *et al.* Licensed recombinant hepatitis B vaccines protect chimpanzees against infection with the prototype surface gene mutant of hepatitis B virus. *Hepatology* 1999; 30: 779–86.
26 Paulij WP, de Wit PL, Sunnen CM *et al.* Localization of a unique hepatitis B virus epitope sheds new light on the structure of hepatitis B virus surface antigen. *J Gen Virol* 1999; 80: 2121–6.
27 Carman WF, Zanetti AR, Karayiannis P *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; 336: 325–9.
28 Carman WF, Korula J, Wallace L, MacPhee R, Mimms L, Decker R. Fulminant reactivation of hepatitis B due to envelope protein mutant that escaped detection by monoclonal HBsAg ELISA. *Lancet* 1995; 345: 1406–7.
29 Lee PI, Chang LY, Lee CY, Huang LM, Chang MH. Detection of hepatitis B surface gene mutation in carrier children with or without immunoprophylaxis at birth. *J Infect Dis* 1997; 176: 427–30.
30 Protzer-Knolle U, Naumann U, Bartenschlager R *et al.* Hepatitis B virus with antigenically altered hepatitis B surface antigen is selected by high-dose hepatitis B immune globulin after liver transplantation. *Hepatology* 1998; 27: 254–63.
31 Shields PL, Owsianka A, Carman WF *et al.* Selection of hepatitis B surface 'escape' mutants during passive immune prophylaxis following liver transplantation: potential impact of genetic changes on polymerase protein function. *Gut* 1999; 45: 306–9.
32 Yamamoto K, Horikita M, Tsuda F *et al.* Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol* 1994; 68: 2671–6.
33 Kato J, Hasegawa K, Torii N, Yamauchi K, Hayashi N. A molecular analysis of viral persistence in surface antigen-negative chronic hepatitis B. *Hepatology* 1996; 23: 389–95.

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Impact of New Methicillin-Resistant *Staphylococcus aureus* Carriage Postoperatively After Living Donor Liver Transplantation

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ABSTRACT

Background. Preoperative carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with an increased risk of MRSA infection after liver transplantation. It is not known, however, whether new MRSA carriage postoperatively also increases the risk of MRSA infection after liver transplantation.

Methods. We retrospectively reviewed the data from 242 adult patients who underwent living donor liver transplantation (LDLT) including microbiological and medical records from admission to 3 months after LDLT. Uni and multivariate analyses were performed to identify independent risk factors for postoperative MRSA infection among preoperative noncarriers of MRSA.

Results. Postoperative MRSA infection occurred in 18 of 219 preoperative noncarriers of MRSA by median postoperative day 26. Operation time of at least 16 hours and postoperative colonization with MRSA independently predicted postoperative MRSA infection.

Conclusion. Postoperative surveillance cultures should be performed periodically after liver transplantation to identify high-risk candidates for postoperative MRSA infection, even among preoperative noncarriers of MRSA.

STAPHYLOCOCCUS AUREUS is a major cause of bacterial infection after liver transplantation.[1,2] Isolates of *S aureus* causing clinical nosocomial infection can be divided into two groups: methicillin-susceptible *S aureus* and methicillin-resistant *S aureus* (MRSA). MRSA infection frequently complicates the postoperative course after deceased donor liver transplantation (DDLT).[1,3–5] Among several centers, 91% (45 of 49 isolates) of all *S aureus* infections after DDLT were caused by MRSA.[4]

Preoperative MRSA carriage is associated with an increased risk for MRSA infection after DDLT.[1,3–5] In addition, postoperative MRSA colonization is prevalent in DDLT.[6] Positive MRSA cultures on both postoperative and preoperative surveillance is considered important because increased MRSA colonization in a patient during hospitalization increases the risk of MRSA infection.[7] In a prospective study,[7] the relative risk of developing an MRSA infection among patients with MRSA colonization was greater than among patients who were not colonized with *S aureus*. In this particular study, 12 of 394 patients had MRSA colonization during hospitalization, and 4 of the 12 later developed MRSA infection.

It is not known, however, whether new MRSA carriage postoperatively following liver transplantation also increases the risk of MRSA infection. Moreover, MRSA in cases of living donor liver transplantation (LDLT), in which operations are scheduled in a more selective manner, is not well described. The aim of the present study was to assess the details of postoperative MRSA infection among preoperative noncarriers of MRSA and to analyze whether new MRSA carriage postoperatively increased the risk of MRSA infection after LDLT using multivariate analysis.

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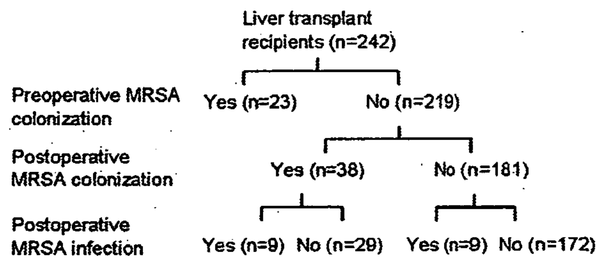

Fig 1. The patient profile of postoperative MRSA colonization and infection. MRSA, methicillin-resistant *S aureus*.

PATIENTS AND METHODS

Patients

We reviewed the 242 patients who underwent LDLT between 1996 and 2004, including 23 colonized with MRSA preoperatively, who were excluded from the study. Of the remaining patients, 119 were men and 100 were women of median age 50 years (range, 19 to 67). The indications included hepatitis C ($n = 62$), followed by primary biliary cirrhosis ($n = 48$) and hepatitis B ($n = 31$). The median Child-Pugh and model for end-stage liver disease (MELD) scores of those patients were 10 (range, 5 to 14) and 13 (range, −3 to 48), respectively. Our donor selection criteria[8] and surgical techniques for recipient and donor operations have been described elsewhere.[9]

Perioperative Management

Antimicrobial prophylaxis consisted of intravenous cefotaxim (1.0 g just before surgery, followed by 1.0 g every 6 hours intraoperatively and thereafter), ampicillin/sulbactam (1.0 g just before surgery, followed by 1.5 g every 12 hours intraoperatively and thereafter), and gentamicin, 60 mg every 12 hours after surgery) for 5 days. Fluconazole (200 mg every 24 hours) was administered intravenously for 7 days after surgery. All patients received the same immunosuppressive regimens using tacrolimus (Prograf, Fujisawa Pharmaceutical Corporation, Tokyo, Japan) and methylprednisolone (Solu-Medrol, Pfizer Inc, New York, NY, USA).[10]

Microbiological Data Collection

All patients were screened preoperatively for *S aureus* after admission for LDLT. Follow-up specimens were collected twice a week during the first month after LDLT and thereafter once a week during the hospital stay. Screened specimens consisted of swabs of the anterior nares, pharynx, sputum, urine, stool, swabs of wound or skin lesions, bile, and abdominal cavity discharge. A catheter or blood sample was also submitted when infection was suspected.

Specimens were plated onto mannitol-salt agar or sheep blood agar. *S aureus* was identified using standard microbiological methods. Methicillin resistance was determined using a disk diffusion test performed on Mueller-Hinton agar after incubation for 24 to 48 hours at 30°C. The strains with an oxacillin minimum inhibitory concentration value of at least 4 μg/mL were defined as MRSA colonization. Patients colonized with *S aureus* at any site and at any time during the hospital stay were considered carriers, and contact precautions were taken in cases with MRSA.

Definition of MRSA Infection

The medical and microbiological records of the patients were reviewed for the occurrence of MRSA infection in the 3 months following LDLT. Only the first MRSA infection was recorded for each patient.

Nosocomial infections were defined according to the reports from the Centers for Disease Control and Prevention in 1988 and in 1992, as described elsewhere.[11] Surgical site infection included superficial incisional, deep incisional, and organ/space infections that occurred within 30 days after surgery. Wound and intra-abdominal cavity infections that occurred more than 1 month after the operation were defined as a gastrointestinal system infection. When an organism isolated from blood culture was compatible with a related nosocomial infection at another site, the bloodstream infection was classified as a secondary bloodstream infection. When MRSA was isolated from culture samples in the presence of nosocomial infection including surgical site infection and other pathogenic organisms were absent, MRSA infection was diagnosed. An MRSA-positive culture sample without the presence of clinical symptoms was diagnosed as MRSA colonization.

Statistical Analysis

Background and clinical data collected for each patient included preoperative, surgical, and postoperative variables. Quantitative variables are presented as medians and ranges. Categorical variables are presented as absolute counts. Univariate analysis was used to identify associations between each of the variables and postoperative MRSA infection. Chi-square test or Fisher exact test was used to compare categorical data.

For multivariate analysis, only variables with a $P < .20$ in the univariate analysis were entered into a logistic regression model by the backward-elimination procedure. The final regression model included covariates associated with a likelihood ratio of $P < .1$. The results of the logistic regression were reported as odds ratios with 95% confidence intervals. A P value of less than .05 was considered statistically significant. All statistical analyses were performed using the JMP5.1 software package (SAS institute Inc, Cary, NC, USA).

RESULTS

Postoperative MRSA Colonization and Infection (Fig 1)

Postoperative MRSA infection occurred in 18 patients among the preoperative noncarriers of MRSA: nine patients were new MRSA carriers postoperatively, and nine

Table 1. Postoperative MRSA Infection in 18 Patients

	Colonized with MRSA (n = 9)	Noncarriers with MRSA (n = 9)	Total (n = 18)
Onset of MRSA infection (postoperative day)	16 (7–54)	40 (9–64)	26 (7–64)
Duration between colonization and infection	13 (2–21)	0	1 (0–21)
During hospitalization infection	9	8	17
SSI	6	3	9
Deep incisonal SSI	6	0	6
Organ/space SSI	0	3	3
Gastrointestinal system infection	2*	4	6
Intra-abdominal infection	2	4	6
Pneumonia	0	1	1
Lower respiratory infection	1	0	1
Primary BSI	0	1	1
Laboratory-confirmed BSI	0	1	1

*One patient had secondary surgical site infection. SSI, surgical site infection; BSI, bloodstream infection.

Table 2. Association Between Postoperative MRSA Infection and Preoperative, Surgical, and Postoperative Variables

Variables	MRSA Infection (−) (*n* = 201)	MRSA Infection (+) (*n* = 18)	*P* Value
Preoperative variables			
Age (y) ≥50	51 (19–67)	48 (24–62)	
	111	8	.46
Gender (male/female)	106/95	13/5	.14
Underlying liver disease			
Hepatitis C	55	7	
Primary biliary cirrhosis	46	2	
Hepatitis B	30	1	
Fulminant hepatitis	19	3	
Biliary atresia	10	1	
Autoimmune hepatitis	8	1	
Primary sclerosing cholangitis	8	1	
Metabolic disease	9	0	
Cryptogenic cirrhosis	6	0	
Alcoholic cirrhosis	2	2	
Others	8	0	
Hepatocellular carcinoma	59	5	1.0
Child-Pugh score	10 (5–14)	11 (5–12)	
≥10	105	13	.14
MELD score	12.9 (−3.4–48.2)	14.6 (4.3–29.4)	
≥15	67	6	1.0
Ascites	95	12	.14
Use of diuretics	109	12	.34
Encephalopathy	32	4	.51
Preoperative apheresis	38	4	.76
PT-INR	1.61 (0.89–7.48)	1.60 (1.23–2.35)	
≥1.7	80	6	.80
Serum bilirubin (mg/dL)	4.1 (0.3–38.6)	7.3 (1.2–32.4)	
>3.0	134	13	.80
Serum albumin (g/dL)	2.9 (1.5–4.4)	2.8 (1.8–3.8)	
>2.8	71	9	.31
Serum creatinine (mg/dL)	0.71 (0.2–7.7)	0.62 (0.4–2.4)	
≥1.5	11	2	.29
Steroid pulse therapy	23	2	1.0
Use of antimicrobials	46	8	.08
Beta lactam	37	7	.06
Glycopeptide	2	0	1.0
Fluroquinolone	13	3	.13
Amynoglycoside	5	1	.41
Others	2	0	1.0
History of abdominal surgery	93	8	1.0
Diabetus mellitus	24	2	1.0
MSSA colonization	100	10	.81
Surgical variables			
Operation time (h)	14.9 (10.7–33.2)	16.3 (12.2–19.3)	
≥16	64	11	.02
Blood loss (mL)	5240 (830–53835)	4415 (2590–34800)	
≥5000	106	8	.62
Blood transfusion (mL)	6970 (900–42890)	6385 (4240–26240)	
≥8000	83	6	.62
GV/SLV ratio (%)	46 (25–88)	42 (36–66)	
≥40	160	15	1.0
Duct to duct biliary reconstruction	144	14	.78
Postoperative variables			
ICU stay (d)	5 (3–46)	5 (4–26)	
≥10	18	4	.09
Apheresis	23	6	.02
Reoperation	72	4	.31
Acute rejection	58	5	1.0

Table 2. (continued)

Variables	MRSA Infection (−) (*n* = 201)	MRSA Infection (+) (*n* = 18)	*P* Value
Cytomegalovirus infection	87	5	.22
Fungal infection	6	1	.46
Colonization with MRSA	29	9	.001

PT-INR, the international normalized ratio of prothrombin time; MSSA, methicillin-susuceptible *S aureus*; MRSA, methicillin-resistant *S aureus*; GV, graft volume; SLV, standard liver volume; ICU, intensive care unit.

patients were MRSA noncarriers until the onset of infection. During the study period, 29 patients were asymptomatic carriers of MRSA. Among the nine patients who were colonized with MRSA postoperatively and subsequently developed infection, the MRSA-colonized sites before the onset of infection were sputum in six, stool in six, nares in five, pharynx in five, urine in two, discharge from an abdominal drain in two, and ascites in one patient.

Details of Postoperative MRSA Infection (Table 1)

The median days to onset of MRSA infection in all the patients with infection, in patients colonized with MRSA before infection, and in patients colonized concurrently with infection were postoperative days 26, 16, and 40, respectively. Among patients who were colonized with MRSA before infection, the median duration between the onset of colonization and infection was 13 days. During the study period, median length of hospital stay after LDLT was 50 (range, 6 to 90) days for patients without MRSA infection and 68 (range, 46 to 90) days for those with MRSA infection. MRSA infection occurred during hospitalization in 17 patients and after discharge in one patient.

Surgical site infection was detected in nine patients. One patient with gastrointestinal system infection had a secondary bloodstream infection. We treated MRSA infection with intravenous vancomycin in 12 patients, reoperation and intravenous vancomycin in two, reoperation alone in two, lavage of the intra-abdominal cavity through the surgical drain in one, and debridement of the wound in one. None of the 18 patients with MRSA infection died during the 3 months after LDLT.

Risk Factors for Postoperative MRSA Infection (Tables 2, 3)

Postoperative MRSA infection was significantly associated with operation time (≥16 hours; $P = .02$), postoperative apheresis ($P = .02$), and postoperative colonization with MRSA ($P = .001$, Table 2). In the multivariate analyses (Table 3), 10 risk factors with *P* values of less than .20 were entered into a logistic regression model using the backward-elimination procedure. In the final model, operation time (≥16 hours; odds ratio, 3.27) and postoperative colonization with MRSA (odds ratio, 7.13) independently predicted postoperative MRSA infection.

Table 3. Multivariate Analysis of Risk Factors for MRSA Infection After LDLT

Variable	Odds Ratio (95% Confidence Interval)	*P* Value
Preoperative use of beta lactam	3.03 (0.95–9.37)	.06
Operation time (h) ≥16	3.27 (1.15–9.89)	.03
Colonization with MRSA	7.13 (2.43–21.65)	.0004

MRSA, methicillin-resistant *S aureus*.

DISCUSSION

We have shown the impact of postoperative colonization with MRSA on subsequent MRSA infection after LDLT. Among patients with MRSA infection, 9 of 18 (50%) in the present study were colonized with MRSA before the onset of infection. MRSA infection occurred soon after the operation in patients who were new MRSA carriers postoperatively. Of 18 patients with MRSA infection, 10 developed the infection within 1 month after LDLT, among whom seven were colonized with MRSA before the onset of infection. In addition, patients who were colonized with MRSA developed MRSA infection soon after colonization with MRSA. Of nine patients with MRSA colonization and subsequent infection, all developed infections within 3 weeks after colonization with MRSA.

Postoperative surveillance cultures should be performed at multiple sites, including the nares, after LDLT. Although the anterior nares is the most frequent carriage site for *S aureus*,[12] other extranasal sites such as skin, perineum, pharynx, gastrointestinal tract, vagina, and axillae can also harbor the organism.[5,12] Among nine patients who were colonized with MRSA postoperatively and subsequently developed infection, nasal colonization was detected in 5 (56%). If surveillance culture is performed for only the nares as reported in previous studies in DDLT, new postoperative carriers of MRSA at sites other than the nares[1,6] might be overlooked, thereby delaying the administration of appropriate antimicrobials such as vancomycin in patients suspected of MRSA infection.

The results of the present study indicated that postoperative MRSA colonization and prolonged operative time independently increased the risk of postoperative MRSA infection. MRSA infection is well described in previous studies of DDLT.[1,3–5] Most studies have reported that preoperative MRSA carriage increased the risk of MRSA infection, but these studies[2,3,5] were not focused on the impact of new postoperative MRSA carriage on subsequent infection. Of 38 patients, 9 (24%) who were colonized with MRSA subsequently developed MRSA infection in the present study. This rate is comparable to that of the previous reports [around 30%].[7,13] In one recent retrospec-

tive study, 60 of 209 (29%) patients developed subsequent MRSA infection in the 18-month period after the initial MRSA-positive culture.[13] Postoperative surveillance culture should be performed periodically after LDLT to identify new MRSA carriers who are high-risk candidates for subsequent MRSA infection.

Prolonged operative time increased the risk of MRSA infection in the present study. Prolonged surgical duration indicates technically more difficult surgical procedures in which the risk of complication is increased.[14] George et al[15] used multivariate analysis to demonstrate that prolonged surgery duration increased the risk of bacterial infection among in liver transplant recipients. In contrast, Singh et al[1] reported that there was no such association.

Intense antimicrobial use, measured by the administration of preoperative antimicrobials, during the month before LDLT did not correlate with postoperative MRSA infection among preoperative noncarriers of MRSA in the present study. Although there is little doubt that widespread use of antimicrobials provides multidrug-resistant strains of MRSA with a selective survival advantage,[16] the relationship between MRSA and antimicrobials seemed more complex in the current series. Some studies using multivariate analysis have failed to show such an association.[17] In other studies, exposure to specific antimicrobials, such as third-generation cephalosporins, amoxicillin with clavulanic acid, quinolones, and broad-spectrum antibiotics, increased the risk of MRSA infection or colonization.[18] Crowcroft et al[19] found no association between total antimicrobial use and MRSA colonization or infection, suggesting that the problem was inappropriate rather than excessive use of antimicrobials. This discrepancy is probably due to the fact that in the present study, all patients received multiple antimicrobials, resulting in broad coverage as perioperative prophylaxis, per protocol, and it is difficult to detect the effect of a specific antimicrobial.

One limitation to the present study is that we could not differentiate specific MRSA strains. Pulsed-field gel electrophoresis analysis was not accessible. Therefore, we could not analyze the impact of MRSA transmission, such as patient-to-patient transmission by transient carriage on the hands of the medical staff. Similarly, it was not possible to determine whether infection was due to the same strain as that of the colonization or to a newly acquired strain when the infection occurred. Chang et al[4] analyzed isolates from infected sites and those from the anterior nares in seven patients with MRSA infection, reporting detection of the same isolates. Such a detailed analysis might yield further information to elucidate the relationship between new postoperative MRSA carriage and subsequent infection following LDLT.

REFERENCES

1. Singh N, Paterson DL, Chang FY, et al: Methicillin-resistant Staphylococcus aureus: The other emerging resistant gram-positive coccus among livertransplant recipients. Clin Infect Dis 20:322, 2000

2. Bert F, Bellier C, Lassel L, et al: Risk factors for Staphylococcus aureus infection in liver transplant recipients. Liver Transpl 11:1093, 2005

3. Bert F, Galdbart JO, Zarrouk V, et al: Association between nasal carriage of Staphylococcus aureus and infection in liver transplant recipients. Clin Infect Dis 31:1295, 2000

4. Chang FY, Singh N, Gayowski T, et al: Staphylococcus aureus nasal colonization and association with infections in liver transplant recipients. Transplantation 65:1169, 1998

5. Desai D, Desai N, Nightingale P, et al: Carriage of methicillin-resistant Staphylococcus aureus is associated with an increased risk of infection after liver transplantation. Liver Transpl 9:754, 2003

6. Santro-Lopes G, de Gouvea EF, Monterio RC, et al: Colonization with methicillin-resistant Staphylococcus aureus after liver transplantation. Liver Transpl 11:203, 2005

7. Davis KA, Stewart JJ, Crouch HK, et al: Methicillin-resistant Staphylococcus aureus (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clin Infect Dis 39:776, 2004

8. Kokudo N, Sugawara Y, Imamura H, et al: Tailoring the type of donor hepatectomy for adult living donor liver transplantation. Am J Transplant 5:1694, 2005

9. Sugawara Y, Makuuchi M: Technical advancement in living-related liver transplantation. J Hepatobiliary Pancreat Surg 16:245, 1999

10. Sugawara Y, Makuuchi M, Kaneko J, et al: Correlation between optimal tacrolimus doses and the graft weight in living donor liver transplantation. Clin Transplant 16:102, 2002

11. Horan TC, Gaynes RP, Martone WJ, et al: CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definition of surgical wound infections. Infect Control Hosp Epidemiol 13:606, 1992

12. Williams RE: Healthy carriage of Staphylococcus aureus: its prevalence and importance. Bacteriol Rev 27:56, 1963

13. Huang SS, Platt R: Risk of methicillin-resistant Staphylococcus aureus infection after previous infection or colonization. Clin Infect Dis 36:281, 2003

14. Arnow PM: Infection following orthotopic liver transplantation. HPB Surg 3:221, 1991

15. George DL, Arnow PM, Fox AS, et al: Bacterial infection as a complication of liver transplantation: epidemiology and risk factors. Rev Infect Dis 13:387, 1991

16. Boyce JM: Understanding and controlling methicillin-resistant Staphylococcus aureus infections. Infect Control Hosp Epidemiol 23:485, 2002

17. Topeli A, Unal S, Akalin HF: Risk factors influencing clinical outcome in Staphylococcus aureus bacteraemia in a Turkish University Hospital. Int J Antimicrob Agents 14:57, 2000

18. Weber SG, Gold HS, Hooper DC, et al: Fluoroquinolones and the risk for methicillin-resistant Staphylococcus aureus in hospitalized patients. Emerg Infect Dis 9:1415, 2003

19. Crowcroft NS, Ronveaux O, Monnet DL, et al: Methicillin-resistant Staphylococcus aureus and antimicrobial use in Belgian hospitals. Infect Control Hosp Epidemiol 20:31, 1999

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Original Article

Prevalence of coinfection with human immunodeficiency virus and hepatitis C virus in Japan

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People with human immunodeficiency virus (HIV) infection are frequently infected with hepatitis C virus (HCV), because of the common transmission routes. Since the dissemination of hyperactive antiretrovirus therapy (HAART), the morbidity and mortality associated with HIV infection have declined. However, the reduction in mortality due to opportunistic infection has made HCV-associated liver diseases the leading cause of mortality in Western countries. A similar situation is assumed in Japan, but the status of coinfection with HIV and HCV is unclear. We conducted a nationwide survey to determine the prevalence of coinfection with HIV and HCV by distributing a questionnaire to the hospitals in the HIV/AIDS Network of Japan. Among 4877 patients reported to be HIV-positive, 935 (19.2%) were also positive for the anti-HCV antibody. Most (84.1%) of the patients coinfected with HIV and HCV were recipients of blood products. These data, for the first time, show the current status of coinfection with HIV and HCV in Japan. A detailed analysis of the progression and severity of liver diseases in the coinfected patients is expected.

Key words: coinfection, hepatitis C, HIV, liver disease

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection and human immunodeficiency virus (HIV) infection are major public health problems worldwide. In the USA, the estimated prevalence of the anti-HCV antibody is 1.8%, with 2.7 million people having HCV-RNA detected in their blood, indicative of ongoing HCV infection.[1] The prevalence of HIV is <1%, and the virus is estimated to have infected approximately 800 000 people.[2] Because of the common transmission routes, that is, parenteral ones, many people with HIV infection are also infected with HCV.[3] Before the introduction of hyperactive antiretroviral treatment (HAART) in 1996, most people with HIV infection died of HIV-associated opportunistic infections such as *Pneumocystis carinii* (currently called *P. jiroveci*) pneumonia and cytomegaloviral infection. Since the dissemination of HAART, the morbidity and mortality associated with HIV infection have declined. However, the reduction in mortality due to opportunistic infection has made patients coinfected with HIV and HCV faced with the menace of progressive liver diseases due to HCV infection in the United States and Europe.[4,5]

Coinfection with HIV has been shown to increase the HCV load in HCV infection,[6] being a negative prognostic factor for clearance of HCV in anti-HCV therapy using interferon.[7,8] It also accelerates the development of cirrhosis and, eventually, hepatocellular carcinoma. Although still controversial, coinfection with HIV and HCV yields a more rapid progression to acquired immunodeficiency syndrome (AIDS) in some cases.[9,10] Importantly, coinfection with HIV and HCV will increase the morbidity and mortality of HIV-infected patients also in Japan, where the prevalence of HIV infection is increasing in a linear fashion, exceptionally among developed countries.[11] There are more than 10 000 HIV-positive people in Japan as of the end of 2004, according to the AIDS National Survey in Japan,[12] and approximately 1.8 million chronic HCV carriers, according to the estimation by the Ministry of Health, Labor and Welfare (MHLW) of Japan. However, unfortunately, the prevalence of coinfection with HIV and HCV in Japan has been unclarified to date. Therefore, we conducted a nationwide study by distributing an

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email-based questionnaire to the hospitals in the HIV/AIDS Network of Japan.

METHODS

IN THE QUESTIONNAIRE, the following information was obtained from hospitals regarding the number of patients who visited the hospitals at least once between January and December 2003: (1) the number of HIV-positive patients; (2) the number of anti-HCV-positive patients among (1); (3) the number of HCV-RNA-positive patients among (2); (4) the number of HIV-positive patients who contracted HIV from blood products; (5) the number of anti-HCV-positive patients among (4); (6) the number of HCV-RNA-positive patients among (5); (7) the number of HIV-positive patients among men who have sex with men (MSM); (8) the number of anti-HCV-positive patients among (7); (9) the number of HCV-RNA-positive patients among (8); (10) the number of HIV-positive patients who contracted HIV through intravenous drug use; (11) the number of anti-HCV-positive patients among (10); (12) the number of HCV-RNA-positive patients among (11); (13) the number of HIV-positive patients whose transmission routes were classified as 'others'; (14) the number of anti-HCV-positive patients among (13); and (15) the number of HCV-RNA-positive patients among (14).

The questionnaire was sent to the 366 hospitals in the HIV/AIDS Network of Japan by email. When emails were returned with a failure of delivery, the questionnaire was forwarded by post. Answers were mostly returned by email, and in some cases by fax. The list of the hospitals in the HIV/AIDS Network of Japan can be browsed at: http://www.acc.go.jp/mLhw/mLhw_frame.htm.

RESULTS

THE QUESTIONNAIRE WAS sent to all 366 hospitals that were on the list of hospitals in the HIV/AIDS Network of Japan in January 2004. One hundred and seventy-six hospitals (48.1%) responded within the indicated period. A collection rate of 47.8% may appear rather low, particularly considering the number of reported HIV-positive people, 10 000, in 2004 according to the statistics of the MHLW of Japan.[12] However, not all the HIV-positive cases are visiting hospitals, and answers to the questionnaire were obtained from most of the major hospitals in the HIV/AIDS Network in big cities around Japan. These factors suggest that not all but a majority of HIV-positive patients in Japan were enrolled in the study.

Table 1 Number of hospitals categorized by the number of patients infected with HIV and those coinfected with HIV and HCV

No. of HIV(+)/HCV(+)	No. of HIV(+)				
	0	1–19	20–49	50+	Total
0	43	52	5	1	101
1–9	0	45	9	3	57
10+	0	2	4	12	18
Total	43	99	18	16	176

There were one or more HIV-positive patients in 133 of 176 (75.6%) hospitals; there were no HIV-positive patients in the remaining 43 hospitals (Table 1). Eighteen of 176 (10.2%) hospitals had 20–49 HIV-positive patients, and 16 (9.1%) hospitals had 50 or more HIV-positive patients. On the other hand, there were one or more patients who were coinfected with HIV and HCV in 75 (42.6%) of 176 hospitals, and there were 10 or more HIV/HCV coinfected patients in 18 (10.2%) hospitals. HIV/HCV coinfected patients were concentrated in specific hospitals in big cities around Japan. In particular, in the Kanto area, HIV/HCV coinfected patients were concentrated in the HIV/AIDS Network hospitals in the Tokyo city area (Fig. 1). Of the 16 hospitals with 50 or more HIV-positive patients and of the 18 hospitals with 10 or more HIV/HCV coinfected patients, 12 were the same hospitals (Table 1). Hospitals with 10 or more HIV/HCV coinfected patients, but with less than 50 HIV-positive patients had the characteristic that most HIV-positive patients contracted HIV from blood products.

In total, 4877 patients were reported to be HIV-positive. Among these, 935 (19.2%) were positive for anti-HCV (Table 2). Of these 935 patients, 780 were HCV-RNA-positive, although it should be noted that not all the patients underwent HCV-RNA testing.

HCV prevalence when fractionated by routes of transmission was as follows. Among 811 HIV-positive patients who contracted HIV from blood products such as unheated concentrated coagulation factors, 786 (96.9%) were anti-HCV-antibody-positive. Of 20 intravenous drug users, nine (45.0%) were anti-HCV-antibody-positive. Among 2730 HIV-positive patients who were MSM (men who have sex with men), 114 (4.2%) were anti-HCV positive. In the remaining 1316 HIV-positive patients whose routes of HIV transmission

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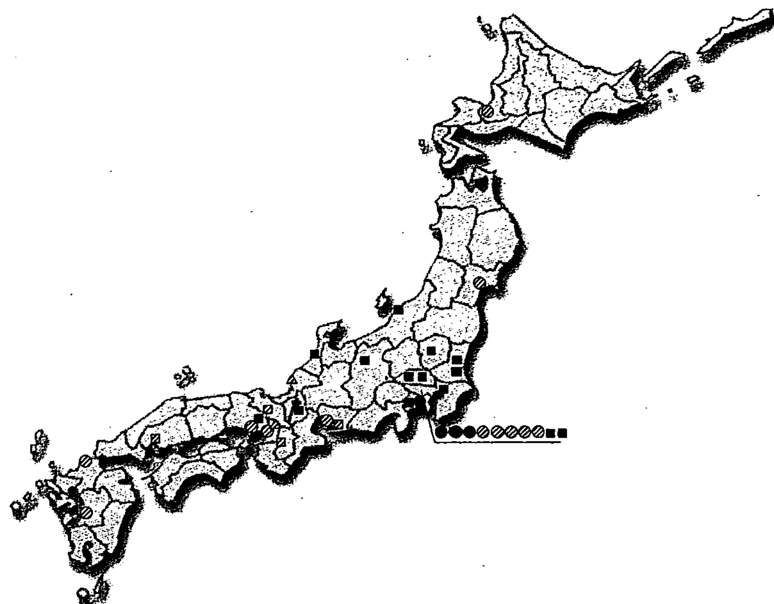

Figure 1 Nationwide distribution of hospitals in the HIV/AIDS Network of Japan that a number of HIV-positive or HIV/HCV coinfected patients are visiting regularly. Note that in the Kanto area, HIV/HCV coinfected patients were concentrated in the HIV/AIDS Network hospitals in the Tokyo city area. (△) hospitals with 1–19 HIV-positive patients; (□) hospitals with 20–49 HIV-positive patients; (○) hospitals with 50+ HIV-positive patients. Hatched figures: hospitals with 10 or more HIV/HCV coinfected patients. Closed figures: hospitals with less than 10 HIV/HCV coinfected patients. For easier visual comprehension, hospitals with 19 or less HIV-positive patients and 9 or less HIV/HCV coinfected patients are omitted from the figure.

were classified as "others", most of whom contracted HIV heterosexually, 26 (2.0%) were anti-HCV-antibody-positive. On the other hand, in HIV/HCV coinfected patients, 786 (84.1%) of 935 patients were recipients of blood products. Thus, the majority of HIV/HCV coinfected patients in Japan are those who contracted HIV, and most likely also HCV, from blood products.

DISCUSSION

ACCORDING TO THE statistics of the MHLW of Japan, the number of reported HIV-positive people was just over 10 000 in 2004.[12] The total number of HIV-positive patients in the current study is approximately half of that. By a simple calculation, there would be about 1900 HIV/HCV coinfected patients in Japan. However, because HIV-positive patients who contracted HIV from blood products are almost all registered in Japan and most of them should have been enrolled in this survey, the number of HIV/HCV coinfected patients is likely smaller than 1900. It is regrettable that not all the patients underwent HCV-RNA testing, but it is unavoidable in this type of questionnaire-based study. In some cases, the existence of a positive anti-HCV antibody indicates a memory of a remote HCV infection.

Almost all of the patients who contracted HIV through blood products were also anti-HCV-antibody-positive, suggesting that both viruses were transmitted through the same route. In MSM patients who were HIV-positive, approximately 4% were anti-HCV-antibody-positive, which is about threefold higher than the prevalence of HCV in Japan.[13] In people aging from 40 to 50 years old in the general Japanese population, whose ages are similar to those of the MSM patients in the current study, the prevalence of HCV is less than 0.5%.[13] Therefore, an HCV prevalence of 4% in MSM

Table 2 Prevalence of HCV infection in HIV-positive patients

Routes of transmission	No. of patients	Anti-HCV-positive	HCV-RNA-positive†
Blood products	811	786 (96.9%)	667
MSM‡	2730	114 (4.2%)	98
Drug addicts	20	9 (45.0%)	8
Others (heterosexual etc.)	1316	26 (2.0%)	7
Total	4877	935 (19.2%)	780

†Not all patients were subjected to HCV-RNA test. ‡MSM, men who have sex with men.

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HIV-positive patients is quite high, suggesting the same route of the transmission of HIV and HCV, and a more intensive exposure to HCV or more susceptibility to HCV in these HIV-positive patients. Similarly, an HCV prevalence of 1.4% in heterosexually transmitted HIV-positive patients is higher than that of the general Japanese population of the same age.

To establish measures that decrease the morbidity and mortality of HIV/HCV coinfected patients, it is essential to recognize the current status of the coinfection. In the present study, the number and transmission routes of HIV/HCV coinfected patients in Japan were first described, although detailed information on the progression of HCV-associated liver diseases in HIV/HCV coinfected patients has not yet been obtained. Undoubtedly, this will be the first step for improving the prognosis and quality of life of patients coinfected with HIV and HCV in Japan. A detailed analysis of the progression and severity of HCV-associated liver diseases is expected.

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REFERENCES

1 Alter MJ. Hepatitis C virus infection in the United States. *J Hepatol* 1999; 31: 88–91.
2 Weinbaum CM, Sabin KM, Santibanez SS. Hepatitis B, hepatitis C, and HIV in correctional populations: a review of epidemiology and prevention. *AIDS* 2005; 19: S41–6.
3 Sulkowski MS. Hepatitis C virus infection in HIV-infected patients. *Curr HIV/AIDS Rep* 2004; 1: 128–35.
4 Bica I, McGovern B, Dhar R *et al.* Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; 32: 492–7.
5 HCV in HIV. Challenges and opportunities. *The PRN NOTEBOOK* 2001; 6: 14–18. [Cited 15 July 2004.] Available from URL: http//: www.prn.org.
6 Picchio GR, Nakatsuno M, Boggiano C *et al.* Hepatitis C (HCV) genotype and viral titer distribution among Argentinean hemophilic patients in the presence or absence of human immunodeficiency virus (HIV) co-infection. *J Med Virol* 1997; 52: 219–25.
7 Benhamou Y, Bochet M, Di Martino V *et al.* Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. *The Multivirc Group Hepatology* 1999; 30: 1054–8.
8 Marsen AH, Easterbrook PJ, Taylor C *et al.* Impact of human immunodeficiency virus (HIV) infection on the progression of liver fibrosis in hepatitis C virus infected patients. *Gut* 2003; 52: 1035–40.
9 Staples CT Jr, Rimland D, Dudas D. Hepatitis C in the HIV (human immunodeficiency virus) Atlanta V.A. (Veterans Affairs Medical Center) Cohort Study (HAVACS): the effect of coinfection on survival. *Clin Infect Dis* 1999; 29: 150–4.
10 Monga HK, Rodriguez-Barradas MC, Breaux K *et al.* Hepatitis C virus infection-related morbidity and mortality among patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; 33: 240–7.
11 Tatsunami S, Taki M, Shirahata A, Mimaya J, Yamada K. Increasing incidence of critical liver disease among causes of death in Japanese hemophiliacs with HIV-1. *Acta Haematol* 2004; 111: 181–4.
12 National AIDS Survey Report. *The Ministry of Health, Welfare and Labor of Japan.* [Cited 10 July 2006.] Available from URL: http://www.wam.go.jp/wamappl/bb14GS50.nsf/vAdmPBigcategory40/73F664B9EA8D63D4492570290023D454?OpenDocument.
13 Tanaka J, Kumagai J, Katayama K *et al.* Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3 485 648 first-time blood donors during 1995–2000. *Intervirology* 2004; 47: 32–40.

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