JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2001, p. 1536–1539 Vol. 39, No. 4
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.4.1536–1539.2001

Molecular Characteristic-Based Epidemiology of Hepatitis B, C, and E Viruses and GB Virus C/Hepatitis G Virus in Myanmar

KAZUHIKO NAKAI,[1,2] KHIN MAUNG WIN,[3] SAN SAN OO,[4] YASUYUKI ARAKAWA,[2] AND KENJI ABE[1]*

Department of Pathology, National Institute of Infectious Diseases,[1] and 3rd Department of Internal Medicine, Nihon University School of Medicine,[2] Tokyo, Japan, and Department of Hepatology, Yangon General Hospital,[3] and Department of Medical Research,[4] Yangon, Myanmar

Received 28 August 2000/Returned for modification 8 January 2001/Accepted 6 February 2001

We carried out a molecular characteristic-based epidemiological survey of various hepatitis viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), and GB virus C (GBV-C)/hepatitis G virus (HGV), in Myanmar. The study population of 403 subjects consisted of 213 healthy individuals residing in the city of Yangon, Myanmar, and the surrounding suburbs and 190 liver disease patients (155 virus-related liver disease patients and 35 nonviral disease patients). The infection rates of the viruses among the 213 healthy subjects were as follows: 8% for HBV (16 patients), 2% for HCV (4 patients), and 8% for GBV-C/HGV (17 patients). In contrast, for 155 patients with acute hepatitis, chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma, the infection rates were 30% for HBV (46 patients), 27% for HCV (41 patients), and 11% for GBV-C/HGV (17 patients). In the nonviral liver disease group of 35 patients with alcoholic liver disease, fatty liver, liver abscess, and biliary disease, the infection rates were 6% for HBV (2 patients), 20% for HCV (7 patients), and 26% for GBV-C/HGV (9 patients). The most common viral genotypes were type C of HBV (77%), type 3b of HCV (67%), and type 2 of GBV-C/HGV (67%). Moreover, testing for HEV among 371 subjects resulted in the detection of anti-HEV immunoglobulin G (IgG) in 117 patients (32%). The age prevalence of anti-HEV IgG was 3% for patients younger than 20 years and 30% or more for patients 20 years of age or older. Furthermore, a high prevalence of anti-HEV IgG (24%) was also found in swine living together with humans in Yangon. These results suggest that these hepatitis virus infections are widespread in Myanmar and have led to a high incidence of acute and chronic liver disease patients in the region.

Viral hepatitis exists throughout the world and is a major global public health problem. Although sensitive and specific tests for the detection of known hepatitis viruses are available, other as-yet-unidentified hepatitis viruses may also be responsible for acute and chronic hepatitis. These viruses may or may not be related to known agents of hepatitis virus types A through E. In 1996, novel RNA viruses were identified from the sera of patients with liver disease by two American groups: these possible agents have been named hepatitis GB virus type C (GBV-C) and hepatitis G virus (HGV), respectively (8, 25). Molecular characterization of these two agents has shown them to be different isolates of the same virus (26). Although there have been extensive investigations of GBV-C/HGV since its discovery, the nature of GBV-C/HGV and its real pathogenic role remain controversial. To approach these problems, we are working on the molecular characteristic-based epidemiology of hepatitis viruses and their pathogenesis in different geographic regions. Here we report the prevalence of hepatitis viruses, including types B, C, and E, and GBV-C/HGV, in Myanmar, where there has been no detailed epidemiological information obtained on these hepatitis virus infections in the past. In addition, the prevalence of antibody to hepatitis E virus (HEV) was also determined for swine to gain an understanding of the modes of HEV transmission in Myanmar.

* Corresponding author. Mailing address: Department of Pathology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan. Phone: (81) 3-5285-1111, ext. 2624. Fax: (81) 3-5285-1189. E-mail: kenjiabe@nih.go.jp.

MATERIALS AND METHODS

Study population. We used serum samples obtained from 213 healthy blood donors and 190 liver disease patients in Myanmar. All were Myanmarese ranging in age from 7 to 80 years. The male/female ratio was 2.5:1. Most individual blood donors who received a health checkup did not appear to have any serious health problems. The patients with liver diseases were examined at the Yangon General Hospital, Yangon, Myanmar. The clinical diagnosis for these patients was based on the findings of ultrasound, serology, and liver histopathology. They were residents of Yangon, Myanmar, or its suburbs. Informed consent for participation in this study was obtained from each individual. The serum samples were collected from 1998 to 2000 and stored at −40°C or below until analysis.

Serum samples from swine. We collected serum samples for anti-HEV assay from 86 swine livestock housed in a slaughterhouse located in Yangon.

Extraction of nucleic acids and detection of HBV DNA, HCV RNA, and GBV-C/HGV RNA by multiplex PCR method. Both DNA and RNA were extracted simultaneously from 100-μl volumes of serum by using the SepaGene RV-R kit (Sanko Junyaku Co., Ltd., Tokyo, Japan), precipitated with isopropanol, and washed in ethanol. The resulting pellet was resuspended in 50 μl of RNase-free water. The sequences of PCR primers were as follows: (i) for hepatitis B virus (HBV) (X region), 5′-TGCCAACTGGATCCTTCGCGGGACG TCCTT-3′ (MD24, sense primer, nucleotide [nt] 1392 to 1421) and 5′-GTTCA CGGTGGTCTCCATG-3′ (MD26, antisense primer, nt 1625 to 1607) for the outer primer pairs (233 bases) and 5′-GTCCCCTTCTTCATCTGCCGT-3′ (HBx1, sense primer, nt 1487 to 1507) and 5′-ACGTGCAGAGGTGAAGCGA AG-3′ (HBx2, antisense primer, nt 1604 to 1584) for the inner primer pairs (118 bases); (ii) hepatitis C virus (HCV) (5′-untranslated region), 5′-GCGACACTC CACCATAGAT-3′ (19, sense primer, nt 2 to 20) and 5′-GCTCATGGTGCAC GGTCTA-3′ (20, antisense primer, nt 312 to 330) for the outer primer pairs (329 bases) and 5′-CTGTGAGGAACTACTGTCT-3′ (21, sense primer, nt 28 to 46) and 5′-ACTCGCAAGCACCCTATCA-3′ (22, antisense primer, nt 277 to 295) for the inner primer pairs (268 bases); and (iii) for GBV-C/HGV (5′-untranslated region), 5′-GGTCGTAAATCCCGGTCACC-3′ (HG1, sense, nt 139 to 158) and 5′-CCCACTGGTCCTTGTCAACT-3′ (HG1R, antisense, nt 381 to 400) for the outer primer pairs (262 bases) and 5′-TAGCCACTATAGGTGG GTCT-3′ (HG2, sense, nt 163 to 182) and 5′-ATTGAAGGGCGACGTGGAC C-3′ (HG2R, antisense; nt 331 to 350) for the inner primer pairs (188 bases). The

TABLE 1. Prevalence of HBV, HCV, and GBV-C/HGV in Myanmar

Subject group and disease	*n*	No. of subjects with[a]:			
		HBV DNA	HCV RNA	HGV RNA	Coinfection
Patients with:					
Acute hepatitis	21	3 (14)	2 (10)	1 (5)	1 (5)
Chronic hepatitis	28	9 (32)	4 (14)	3 (11)	2 (7)
Liver cirrhosis	81	20 (25)	29 (36)	9 (11)	6 (7)
Hepatocellular carcinoma	25	14 (56)	6 (24)	4 (16)	3 (12)
Subtotal	155	46 (30)	41 (27)	17 (11)	12 (8)
Patients with:					
Alcoholic liver disease	13	1 (8)	4 (31)	3 (23)	3 (23)
Fatty liver	11	0	1 (9)	0	0
Liver abscess	7	1 (14)	2 (29)	2 (29)	0
Biliary system disease	4	0	0	4 (100)	0
Subtotal	35	2 (6)	7 (20)	9 (26)	3 (9)
Healthy individuals	213	16 (8)	4 (2)	17 (8)	0
Total	403	64 (16)	52 (13)	43 (11)	15 (4)

[a] Values in parentheses indicate percentages.

nucleotide positions were deduced from HBVadr4 isolate (5) for HBV, HC-J1 isolate (21) for HCV, and HGV-PNF2161 isolate (8) for GBV-C/HGV.

To obtain simultaneous detection of hepatitis B and C and GBV-C/HGV viral genomes, we used the multiplex PCR method as described previously (6). In fact, the multiplex PCR was performed in a one-step process that combines cDNA synthesis and PCR in a single tube. That is, for HCV and GBV-C/HGV RNA, the first PCR was combined with the reverse transcriptase (RT) step in one tube containing 50 μl of a reaction buffer containing 10 U of RNase inhibitor (Promega, Madison, Wis.), 100 U of Moloney murine leukemia virus RT (Promega), 40 ng of outer primer for (each) HBV, HCV, and GBV-C/HGV, a 300 μM concentration of each of the four deoxynucleotides, 2 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, Conn.), and 1× reaction buffer containing 1.5 mM $MgCl_2$. To obtain an automatic hot-start reaction, we used AmpliTaq Gold DNA polymerase instead of regular thermostable DNA polymerase. The thermocycler was programmed to incubate the samples for 50 min at 37°C for the initial RT step and then preheat at 95°C for 10 min to activate the AmpliTaq Gold polymerase; these preliminary steps were followed by 40 cycles of 94°C for 30 s, 50°C for 45 s, and 72°C for 1 min using a model no. 2400 or 9700 thermal cycler (Perkin-Elmer). For the second reaction, 2μl (1/25 volume) of the first PCR product was added to a tube containing the inner primer, deoxynucleotides, AmpliTaq Gold DNA polymerase, and PCR buffer as described for the first reaction mixture, but the RT was omitted, as was the initial 50 min of incubation at 37°C. Amplification was performed for 40 cycles with the following parameters: preheating at 95°C for 10 min; 20 cycles of amplification at 94°C for 30 s, annealing at 53°C for 45 s, and extension at 72°C for 1 min; and an additional 20 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min. The PCR products were electrophoresed on a 3% agarose gel, stained with ethidium bromide, and evaluated under UV light. The sizes of the PCR products were estimated according to the migration pattern of a 50-bp DNA ladder (Pharmacia Biotech, Piscataway, N.J.). To avoid the risk of false-positive results, PCR assays were done with strict precautions against cross-contaminations. Furthermore, all PCR assays were performed in duplicate to confirm reproducibility.

Genotyping by PCR assay. The genotyping of each virus was determined by PCR method using type-specific primers as reported previously (15, 18, 20).

Assay for antibody to HEV in human and swine. Immunoglobulin G (IgG) and IgM antibodies to HEV were measured by enzyme-linked immunosorbent assay. The enzyme-linked immunosorbent assay to detect anti-HEV using virus-like particles expressed by a recombinant baculovirus was performed as reported previously (7), except that the secondary antibody was replaced with alkaline phosphatase-conjugated goat anti-swine IgG and IgM (Bethyl Laboratories, Inc., Montgomery, Tex.).

RESULTS

Prevalence of HBV, HCV, and GBV-C/HGV infections. Among 213 healthy individuals, HBV DNA, HCV RNA, and GBV-C/HGV RNA were detected in 16 (8%), 4 (2%), and 17 (8%) subjects, respectively (Table 1). In contrast, these viruses were detected in 46 (30%), 41 (27%), and 17 (11%) subjects, respectively, among 155 liver disease patients with acute hepatitis, chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma. In particular, hepatocellular carcinoma patients were most often infected with HBV (56%), followed by HCV (24%) and GBV-C/HGV (16%). On the other hand, these viruses were present in 2 (6%), 7 (20%), and 9 (26%) patients, respectively, among nonviral liver diseases patients, including alcoholic liver disease, fatty liver, liver abscess, and biliary disease. In particular, HCV RNA was detected in 31% of patients (4 of 13 tested patients) who were diagnosed clinically as having alcoholic liver diseases.

Prevalence of anti-HEV antibodies in humans and swine. The overall seroprevalences of anti-HEV antibodies were 31.5% (117 of 371 persons) for IgG and 1% (4 of 371 persons) for IgM among tested human individuals. The age-specific prevalence reached 32% (49 of 151 persons) among persons 21 to 40 years of age, 33% (45 of 138 persons) among persons 41 to 60 years of age, and 42% (22 of 52 persons) among persons over 61 years old but was only 3% (1 of 30 persons) among those under 20 years old. On the other hand, anti-HEV IgG and anti-HEV IgM were also detected in 21 (24%) and in 5 (6%) of 86 tested swine, respectively.

Genotypic distribution of each virus. As shown in Table 2, genotype C of HBV (77%) was the most prevalent, followed by type A (8%), among 64 patients tested. For HCV, the most common genotype among the 24 patients examined was type

TABLE 2. Genotypic distribution of HBV, HCV, and GBV-C/HGV in Myanmar

Virus (no. of persons tested) and genotype	No. of subjects[a]
HBV (64)	
A	5 (8)
B	0 (0)
C	49 (77)
D	0 (0)
E	0 (0)
F	0 (0)
B + C[b]	2 (3)
Unclassified	8 (13)
HCV (24)	
1a	3 (13)
1b	1 (4)
3a	2 (8)
3b	16 (67)
Unclassified	2 (8)
GBV-C/HGV (43)	
1	0 (0)
2	29 (67)
3	5 (12)
4	7 (16)
2 + 3[b]	2 (5)

[a] Values in parentheses are percentages.
[b] Coinfection of more than one genotype.

3b (67%), followed by types 1a (13%) and 3a (8%). For GBV-C/HGV, type 2 (67%) was the most widespread, followed by type 4 (16%) and type 3 (12%), for the 43 patients tested. Interestingly, 13% of the HBV cases and 8% of the HCV cases were unclassified for these populations.

DISCUSSION

Viral hepatitis is an important public health and economic problem. However, information on infectious diseases in developing countries, and particularly in isolated communities, is scarce. In Myanmar, an adequate level of information on the epidemiology of HBV, HCV, HEV, and GBV-C/HGV infections has not been available so far. In this study, we performed molecular characteristic-based seroepidemiology of these virus infections in Myanmar and found that these hepatitis viruses are widespread in Myanmar and have led to a high incidence of acute and chronic liver disease patients in this region. However, the prevalence of GBV-C/HGV among healthy individuals (8%) was not significantly different from that found for the viral liver disease group (11%) including acute or chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma patients. Genotype C of HBV was previously found to be the most widespread in Asia (9), but no information on HCV genotypes in Myanmar has been available until now. Our investigation (this study) showed that the genotypes prevailing in this country were type C for HBV and type 3b for HCV. In addition, 13% of HBV cases and 8% of HCV cases were unclassified in these populations. These findings suggest that there are some variants of HBV and HCV in Myanmar. In fact, we obtained several HBV isolates (from Myanmarese subjects) recently that have a deletion mutant in the pre-S gene (data not shown). Characterization of the genome and genotyping of HBV are important, because a vaccination plan against HBV is to be advanced in the future in Myanmar. A relevant study is in progress, and we plan to report it elsewhere. In general, the route of viral infection in tropical areas is not clear. In fact, the routes and factors involved were not identified in the present study. There was only a little evidence of the massive use of intramuscular and/or intravenous injection or blood transfusion. We are presently conducting an investigation to clarify the transmission route of these viruses.

GBV-C/HGV, a recently discovered human RNA virus (8, 25), which may be transmitted by transfusion of blood or blood products, is distributed worldwide. The presence of GBV-C/HGV has been detected in the blood of asymptomatic individuals and in the blood of patients with liver diseases (1, 8, 12, 19). On the other hand, it has also been reported that GBV-C/HGV infection does not induce significant liver damage; hence, the nature of GBV-C/HGV and its real pathogenic role remain controversial (2, 3). GBV-C/HGV is not characterized by a great genome variability as great as that of HCV, but several studies have suggested the existence of three different genotypes (13, 14, 22, 24). Recently, we reported the existence of a novel genotype of GBV-C/HGV in Southeast Asian countries and designated it genotype 4 (16, 17). Therefore, GBV-C/HGV can be classified now into four different genotypes corresponding to geographic distribution. Based on this classification, we found that the major genotype of GBV-C/HGV in infected Myanmarese is genotype 2, followed by genotype 4.

HEV, previously referred to as enterically transmitted non-A, non-B hepatitis, is a major cause of epidemic hepatitis and of acute, sporadic hepatitis in developing countries (23). Many outbreaks of HEV-induced hepatitis have been reported in India, Southeast and Central Asia, Africa, and Mexico (4). Our results indicated that although there was a high prevalence of anti-HEV antibodies among persons over 21 years old, the prevalence of these antibodies was very low among persons younger than 20 years old. This suggests that a mass outbreak of HEV infection has not occurred in the last 20 years in Myanmar. It is noteworthy that we also found a high prevalence of anti-HEV antibodies in swine living together with humans in Yangon. Recently, Meng et al. (10, 11) reported isolating, from swine, a novel virus closely related to the human HEV, which they named swine HEV. Although the sequence analysis of swine HEV remains to be investigated, our findings may help to provide an understanding of the modes of HEV transmission and may also raise potential public health concerns for zoonosis in Myanmar.

In conclusion, hepatitis virus infections are widespread in Myanmar and have led to a high incidence of acute and chronic liver disease in the region. In particular, more than half of the hepatocellular carcinoma patients in Myanmar were found to be infected with HBV, followed in frequency by HCV. Establishment of defense measures against hepatitis virus infection is an important and urgent matter for Myanmar.

ACKNOWLEDGMENTS

We thank Tetsutaro Sata for his continuous encouragement during this study and Nami Konomi, Tomoko Inami, Hideo Naito, Naoto Aiba, Tian-Cheng Li, Naokazu Takeda, Yutaka Takebe, the National Institute of Infectious Diseases, and the staff of the Department of Hepatology, Yangon General Hospital, for their kind cooperation during this study.

This study was supported in part by grants-in-aid for science research from the Ministry of Education, Science and Culture of Japan, by the Ministry of Health and Welfare of Japan, and by an International Medical Cooperation Research grant for Japan.

REFERENCES

1. **Abe, K., M. Moriyama, S. Hayashi, K. Nakai, I. Miyauchi, Y. Edamoto, T. Saito, S. Fukushima, T. Shimizu, H. Matsumura, and Y. Arakawa.** 1997. Prevalence of hepatitis G virus infection among patients with liver diseases in Japan. Int. Hepatol. Commun. **6:**239–248.
2. **Alter, H. J., Y. Nakatsuji, J. Melpolder, J. Wages, R. Wesley, W.-K. Shih, and J. P. Kim.** 1997. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. N. Engl. J. Med. **336:**747–754.
3. **Alter, M. J., M. Gallagher, T. T. Morris, L. A. Moyer, E. L. Meeks, K. Krawczynski, J. P. Kim, and H. S. Margolis.** 1997. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. N. Engl. J. Med. **336:**741–746.
4. **Bradley, D. W.** 1992. Hepatitis E: epidemiology, aetiology, and molecular biology. Rev. Med. Virol. **2:**19–28.
5. **Fujiyama, A., A. Miyanohara, C. Nozaki, T. Yoneyama, N. Ohtomo, and K. Matsubara.** 1983. Cloning and structural analyses of hepatitis B virus DNAs, subtype adr. Nucleic Acids Res. **11:**4601–4610.
6. **Konomi, N., M. Yamaguchi, H. Naito, N. Aiba, T. Saito, Y. Arakawa, and K. Abe.** 2000. Simultaneous detection of hepatitis B, C and G viral genomes by multiplex PCR method. Jpn. J. Infect. Dis. **53:**70–72.
7. **Li, T. C., Y. Yamanaka, K. Suzuki, M. Tatsumi, M. A. A. Razak, T. Uchida, N. Takeda, and T. Miyamura.** 1997. Expression and self-assembly of empty virus-like particles of hepatitis E virus. J. Virol. **71:**7207–7213.
8. **Linnen, J., J. Wages, Z.-Y. Zhang-Keck, K. Fry, K. Krawczynski, H. Alter, E. Koonin, M. Gallagher, M. Alter, S. Hadziyannis, P. Karayiannis, K. Fung, Y. Nakatsuji, J. W. K. Shin, L. Young, M. Piatak, C. Hoover, J. Fernandez, S. Chen, J-C. Zou, T. Morris, K. C. Hyams, S. Ismay, J. D. Lifson, G. Hess, S. K. H. Foung, H. Thomas, D. Bradley, H. Margolis, and J. P. Kim.** 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science **271:**505–508.

9. **Magnius, L. O., and H. Norder.** 1995. Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. Intervirology **38:**24–34.
10. **Meng, X. J., P. G. Halbur, M. S. Shapiro, S. Govindarajan, J. D. Bruna, I. K. Mushahwar, R. H. Purcell, and S. U. Emerson.** 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J. Virol. **72:**9714–9721.
11. **Meng, X. J., R. H. Purcell, P. G. Halbur, J. R. Lehman, D. M. Webb, T. S. Tsareva, J. S. Haynes, B. J. Thacker, and S. U. Emerson.** 1997. A novel virus in swine is closely related to the human hepatitis E virus. Proc. Natl. Acad. Sci. USA **94:**9860–9865.
12. **Miyakawa, Y., and M. Mayumi.** 1997. Hepatitis G virus—a true hepatitis virus or an accidental tourist? N. Engl. J. Med. **336:**795–796.
13. **Muerhoff, A. S., D. B. Smith, T. P. Leary, J. C. Erker, S. M. Desai, and I. K. Mushahwar.** 1997. Identification of GB virus C variants by phylogenetic analysis of 5′-untranslated and coding region sequence. J. Virol. **71:**6501–6508.
14. **Muerhoff, A. S., J. N. Simons, T. P. Leary, J. C. Erker, M. L. Chalmers, T. J. Pilot-Matias, G. J. Dawson, S. M. Desai, and I. K. Mushahwar.** 1996. Sequence heterogeneity within the 5′-terminal region of the hepatitis GB virus C genome and evidence for genotypes. J. Hepatol. **25:**379–384.
15. **Naito, H., and K. Abe.** 2001. Genotyping system of GBV-C/HGV type 1 through type 4 by polymerase chain reaction using type-specific primers and geographic distribution of viral genotypes. J. Virol. Methods **91:**3–9.
16. **Naito, H., K. M. Win, and K. Abe.** 1999. Identification of a novel genotype of hepatitis G virus in Southeast Asia. J. Clin. Microbiol. **37:**1217–1220.
17. **Naito, H., S. Hayashi, and K. Abe.** 2000. The entire nucleotide sequence of two hepatitis G virus isolates belonging to a novel genotype: isolation in Myanmar and Vietnam. J. Gen. Virol. **81:**189–194.
18. **Naito, H., S. Hayashi, and K. Abe.** 2001. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by polymerase chain reaction using type-specific primers. J. Clin. Microbiol. **39:**362–364.
19. **Nakatsuji, Y., J. W.-K. Shih, E. Tanaka, K. Kiyosawa, J. Wages, Jr., J. P. Kim, and H. J. Alter.** 1996. Prevalence and disease association of hepatitis G virus infection in Japan. J. Viral Hepat. **3:**307–316.
20. **Ohno, O., M. Mizokami, R.-R. Wu, M. G. Saleh, K.-I. Ohba, E. Orito, M. Mukaide, R. Williams, and J. Y. N. Lau.** 1997. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J. Clin. Microbiol. **35:**201–207.
21. **Okamoto, H., S. Okada, Y. Sugiyama, S. Yotsumoto, T. Tanaka, H. Yoshizawa, F. Tsuda, Y. Miyakawa, and M. Mayumi.** 1990. The 5′-terminal sequence of the hepatitis C virus genome. Jpn. J. Exp. Med. **60:**167–177.
22. **Orito, E., M. Mizokami, T. Nakano, R. R. Wu, K. Cao, K. Ohba, R. Ueda, M. Mukaide, K. Hikiji, Y. Matsumoto, and S. Iino.** 1996. GB virus C/hepatitis G virus infection among Japanese patients with chronic liver diseases and blood donors. Virus Res. **46:**89–93.
23. **Pawlotsky, J. M., L. Belec, G. Cresenguet, L. Deforges, M. Bouvier, J. Duval, and D. Dhumeaux.** 1995. High prevalence of hepatitis B, C, and E in young sexually active adults from the Central African Republic. J. Med. Virol. **46:**269–273.
24. **Saito, T., T. Shiino, Y. Arakawa, S. Hayashi, and K. Abe.** 1998. Geographical characterization of hepatitis G virus genome: evidence for HGV genotypes based on phylogenetic analysis. Hepatol. Res. **10:**121–130.
25. **Simons, J. N., T. P. Leary, G. J. Dawson, T. J. Pilot-Matias, A. S. Muerhoff, G. G. Shclauder, S. M. Desai, and I. K. Mushahwar.** 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat. Med. **1:**564–569.
26. **Zuckerman, A. J.** 1996. Alphabet of hepatitis viruses. Lancet **347:**558–559.