Molecular Epidemiology of a Hepatitis C Virus Outbreak in a Leprosy Sanatorium in Japan[▽]

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The hepatitis C virus (HCV) outbreak that occurred between 1940 and 1999 in a closed leprosy sanatorium located on a small island in Japan was analyzed. The analysis of 318 nucleotides in the NS5B region of HCV allowed us to establish the existence of at least three different HCV strains in this sanatorium.

Since 1938, the National Sanatorium Oku-Komyo-En has been one of 13 national leprosy sanatoriums in Japan. Because of the leprosy isolation policy in Japan (Leprosy Prevention Law of 1907) in the early years, leprosy patients were forced to live in a closed sanatorium, and once confined, patients were strictly isolated from the general public, even after death. The Leprosy Prevention Law remained in force until the end of 1996; autopsies were routinely performed on almost all patients who had died in the sanatoriums, and the tissue samples were usually formalin fixed and archived. In a review of the samples (Table 1), the pathological data showed a sharp rise in deaths between 1960 and 1969 due to liver cirrhosis in patients. We also noticed a sharp rise in deaths between 1980 and 1999 from hepatocellular carcinoma (HCC) with cirrhosis with statistical significance (Fig. 1). As 75% of all HCC cases in Japan are now reported to be from hepatitis C virus (HCV) infection (4), we examined the frequency of HCV infection in these archived tissue samples from leprosy patients, by using PCR type-specific primers (7) to detect HCV RNA (9).

In this study, we investigated the possibility of nosocomial

infection in more detail, and we examined the sequence similarity of HCVs that were detected in these archived samples. Our study plan conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committees of National Sanatorium Oku-Komyo-En, Kojin Hospital, and Fujita Health University, School of Medicine, respectively. Tissues of 30 patients with leprosy (HCV genotype 1b) (Table 1) kept at the National Sanatorium Oku-Komyo-En (between 1940 and 1999) were freshly embedded in paraffin, followed by RNA extraction. Using the RNA as template for reverse transcription-PCR (RT-PCR), we first planned to sequence the HVR1 (3) and NS5 (8) regions of HCV, which have been used before for analogous studies. However, while the amplification of the HVR region was unsuccessful (data not shown), the NS5B region was readily amplified in 12 of the samples (Table 1). NS5B RT-PCR was performed with primers Pr2 (5'-GGCGGAATTCCTGGTCATAGCCTCCGTGAA-3') and Pr3 (5'-TATGAYACCCGCTGYTTTGACTC-3') (8). Part of the amplified products were analyzed by electrophoresis, and the products were purified with the GeneClean II kit

TABLE 1. Summary data for the National Sanatorium Oku-Komyo-En (1940 to 1999)

Yr	Mean no. of resident patients/yr (no. male/no. female)	No. of patient deaths (no. male/no. female)	No. (%) of deaths due to disease (no. male/no. female) ^a :		No. of tissue samples showing HCV genotype (no. from males/no. from females):	
			Cirrhosis	HCC plus cirrhosis	1b	2a
1940–1949	918.5 (668.1/250.4)	604 (471/133)	8 (1.3) (8/0)	0 (0) (0/0)	6 (6/0)	0 (0/0)
1950–1959	937.4 (617.9/319.5)	62 (44/18)	0 (0) (0/0)	0 (0) (0/0)	0 (0/0)	0 (0/0)
1960–1969	915.5 (592/323.5)	114 (87/27)	12 (10.5) (9/3)*	1 (0.9) (1/0)	7 (6/1)	1 (1/0)
1970–1979	740.9 (461.8/279.1)	46 (29/17)	3 (6.5) (3/0)*	2 (4.3) (1/1)*	4 (4/0)	0 (0/0)
1980–1989	592 (363/229)	111 (77/34)	9 (8.1) (6/3)*	9 (8.1) (7/2)*	8 (7/1)	3 (2/1)
1990–1999	431.3 (251.7/179.6)	59 (45/14)	4 (6.8) (2/2)*	5 (8.5) (3/2)*	5 (4/1)	0 (0/0)
Total	755.9 (492.4/263.5)	996 (753/243)	36 (28/8)	17 (12/5)	30 (27/3)	4 (3/1)

^a *, $P < 0.05$ in Fisher's exact test (two-sided), in comparison of value with that for 1940 to 1949.

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FIG. 1. Prevalence of cirrhosis of the liver and HCC in autopsy samples archived at the National Sanatorium Oku-Komyo-En (1940 to 1999).

(MP Biomedicals, LLC), followed by cloning into the pTAC-1 vector (TA PCR cloning kit; BioDynamics Laboratory Inc.). Recombinant clones were then sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and the Applied Biosystems ABI 3100 Genetic Analyzer. Furthermore, phylogenetic analysis of NS5 sequences was carried out using the neighbor-joining method (MEGA 5 software) (10) by comparing the sequences obtained from the 12 Oku-Komyo-En samples with the 38 reported sequences of the HCV NS5 region in GenBank (Fig. 2). EF032892, EF032893, and EF032894 are samples from the same patient at 1, 5.5, and 14 months after infection, respectively (5). Even though the samples are identical in the NS5B region of the sequence, the distances are different (0, 0.00010, and 0.00074, respectively) in the full sequences. Furthermore, EF032891 is the donor of EF032892, EF032893, and EF032894, and the different distances of 0.00629 for the NS5B sequences (316 bases/318 bases, 99% similarity) and 0.00658 are 8,740 bp by comparison (EF03891 is reported to be 8,740 bp for partial sequence). Our data may suggest that at least three strains of HCV existed in this sanatorium. The first group is Oku1967M, Oku1944M, and Oku1984M (group 1); the second group is Oku1981M, Oku1943M, Oku1947M, Oku1965M, and Oku1961M (group 2); and the third group is Oku1964M, Oku1964F, Oku1948M, and Oku1987F (group 3). In this phylogenetic tree, the difference in sequences of Oku1987F and EF032893 (United States) is 96%. These similarities may be because the sequence alignment is based on a small, 318-bp region of NS5B that was amplified compared to the 9,500-bp full sequence (1).

It is generally accepted that the intervals between initial HCV infection and the development of cirrhosis and of HCC are 20 and 30 years, respectively (2). Taken together, we can assume that horizontal transmission of the HCV occurred between 1940 and 1949 and 20 and 30 years before cirrhosis and HCC, respectively, because three groups contained samples from the 1940s. This period is the same time frame as the establishment of the National Sanatorium Oku-Komyo-En. Most of the patients in the sanatorium had received regular intravenous drugs for treatment of pain and subcutaneous injection of chaulmoogra oil for the treatment of leprosy using nondisposable syringes and needles. Furthermore, leprosy is a

FIG. 2. Phylogenetic analysis of the NS5B region (318 bp) of HCV (genotype 1b). Twelve isolates from the National Sanatorium Oku-Komyo-En (Oku1943M [cirrhosis, 1943, male], Oku1944M [cirrhosis, 1944, male], Oku1947M [cirrhosis, 1947, male], Oku1948M [cirrhosis, 1948, male], Oku1961M [cirrhosis, 1961, male], Oku1964M [cirrhosis, 1964, male], Oku1964F [cirrhosis, 1964, female], Oku1965M [cirrhosis, 1965, male], Oku1967M [cirrhosis, 1967, male], Oku1981M [cirrhosis, 1981, male], Oku1984M [cirrhosis, 1984, male], and Oku1987F [cirrhosis, 1987, female]) and 38 unrelated sequences from GenBank (AB249644, AB442221, AF165058, AF165061, AF165062, AF207753, AF207754, AF207756, AF207761, AF207765, AF207767, AF207769, AF207770, AF207774, AY045702, D10934, D63857, EF032892, EF032893, EF032894, EU155362, EU155364, EU155366, EU155374, EU155382, EU234062, EU239714, EU255960, EU256045, EU256059, EU256065, EU256076, EU256077, EU256078, EU256088, EU256089, EU256090, and EU256099).

dermatological disease, and patients' skin was cared for with reusable sharpeners and bandages. Thus, there were many chances for staff and patients to come into contact with blood without adequate sterilization. Consequently, a study by Kiyosawa et al. (4) reported that in Japan, sharp rises in death rates from primary liver cancer in men were observed around 1975, and for women this rise was more gradual and occurred much later, in 1980. However, in this leprosy sanatorium, we could not conclude that there was a sex difference, primarily because the female samples are too few for drawing conclusions (Table 1). The reason for the male predominance in this sanatorium is that males are more at risk for contracting leprosy than are females (12), and the male/female ratio of infection is similar to the male/female ratio of the average number of resident patients (Table 1). In this leprosy sanatorium, HCV genotypes 1b and 2a in Oku-Komyo-En *in toto* were 85.7% and 14.3%, respectively (9), while the subtypes of HCV in present-day Japan include 1b (69.4%), 2a (14.8%), and 2b (5.5%) (6). HCV genotype 1b was predominant, but statistically it is not prominent compared with genotypes outside present-day Japan. HCV genotype 1b was believed to have been introduced into Japan in 1882 and started to spread exponentially in the 1920s and the 1930s according to the molecular clock theory (11). This sanatorium may show an example of the HCV spread phenomenon in Japan.

Here we report evidence of transmission of HCV by constructing a phylogenetic tree of multiple NS5B sequences isolated from archived tissues from leprosy patients who were confined to the National Sanatorium Oku-Komyo-En of Japan. This observation strongly suggests the horizontal transmission of HCV in the past 70 years in this leprosy sanatorium.

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