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Frequent Detection of Hepatitis C Virus US Strain in Japanese Hemophiliacs

Makoto Hijikata,[1] Nobuyuki Kato,[1] Shigehisa Mori,[1] Yuko Ootsuyama,[1] Masanori Nakagawa,[1] Takashi Sugimura,[1] Showgo Ohkoshi,[2] Hideo Kojima,[2] Takashi Meguro,[3] Masashi Taki,[3] Shigenobu Takayama,[3] Kaneo Yamada[3] and Kunitada Shimotohno[1,4]

[1]*Virology Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104,* [2]*Third Department of Internal Medicine, Niigata University School of Medicine, Asahimachi, Niigata 951, and* [3]*Department of Pediatrics and Blood Transfusion Center, St. Marianna University School of Medicine, Sugao, Miyamae, Kawasaki 213*

Hemophiliacs have been found to be at high risk of hepatitis. Hemophiliacs in Japan receive imported clotting factors as well as domestic ones. Recently we found that hepatitis C virus (HCV) could be classified into at least two types, HCV-US and HCV-J, depending on the nucleotide sequence. We analyzed the nucleotide sequences of virus RNAs from the plasma of patients with hemophilia A or B and found HCV-US in 3 of 4 Japanese hemophiliacs examined.

Key words: Non-A, non-B hepatitis — Hepatitis C virus — Hemophilia — Transfusion — Clotting factors

Non-A, non-B hepatitis associated with blood transfusion is mainly caused by infectious viral agents. It was recently proposed that hepatitis C virus (HCV) is one of the most probable etiological agents of non-A, non-B hepatitis.[1)] Serum antibody against a protein (C100-3) produced in yeast by a recombinant plasmid that expresses an HCV gene was detected frequently in patients with chronic hepatitis.[2)] The prevalence rate of the HCV antibody in healthy people is between 1 and 2% in Japan.[3)] Significant higher percentages (78% and 15%) of patients with chronic and acute post-transfusion non-A, non-B hepatitis were shown to be positive for the viral antibody.[2)] A high incidence of the antibody in patients with hepatocellular carcinoma was also reported.[4–6)]

The HCV genome was detected with high frequency by means of the polymerase chain reaction (PCR) in sera from patients with chronic hepatitis.[7–9)] HCV isolates (HCV-J) from Japanese patients with chronic hepatitis were shown to have significantly different nucleotide sequences (about 20% difference) from the original isolate in the USA, which we called HCV-US,[9)] whereas much less variation (less than 10%) was found in the nucleotide sequences of the HCV-J isolates. Hence, HCV could be classified into at least two strains according to the nucleotide sequence. Previously we showed that it was possible to amplify certain regions of the virus genome by the PCR using primers whose sequences were conserved in the two strains.[9)]

Hemophiliacs are at risk of non-A, non-B hepatitis through infusion of clotting factor concentrates. Patients with hemophilia A and B and von Willebrand's disease have a high incidence (70%) of anti-HCV antibody[10, 11)] in their serum.

In Japan these patients receive imported or domestic blood products. Patients who have received imported clotting factors are suspected of having a high incidence of infection by HCV other than the Japanese strain. To test this possibility, we examined the HCV genome from hemophiliacs who have been receiving imported as well as domestic blood products and showed a high level of serum alanine aminotransferase activity. The histories of the patients are as follows.

Patient 1: A male, 19 years old, with hemophilia B. He received about 500 units of an imported clotting factor IX before an operation for chronic sinusitis in August, 1981. One month later he was hospitalized with acute viral hepatitis and was then diagonized as having chronic hepatitis.

Patient 2: A male, 17 years old, with hemophilia A. He received more than 8300 units of a domestic clotting factor from 1976 to 1979 and an imported factor from 1980 to 1985. He has been receiving another imported factor since 1986.

Patient 3: A male, 21 years old, with hemophilia A. He received about 150,000 units of two domestic clotting factors from 1974 to 1979 and received 330,000 units of imported factor from 1979 to 1989.

Patient 4: A male, 24 years old, with hemophilia A. He received more than 100,000 units of two domestic clot-

[4] To whom correspondence should be addressed.

ting factors from 1966 to 1981 and two imported factors from 1982 to 1987. He is now receiving an imported factor.

All these hemophiliacs are positive for HCV RNA, detected with reverse transcriptase followed by the PCR, and antibody against C100, measured by ELISA (Ortho Diagnostic Systems).

Samples of 0.5 ml of plasma from each patient were used for analysis. RNA was extracted and subjected to reverse transcription with Molony murine leukemia reverse transcriptase using the primer, HCV 36R (5′-CACGGGTGAGGGAGTAGACCCT-3′), the sequence of which is complementary to that of nucleotides 7,050 to 7,071 of the published sequence of the Japanese strain of HCV (HCV-J).[9] Then the cDNA was amplified by the PCR in a DNA thermal cycler (Perkin-Elmer, Cetus) for 35 cycles as described previously.[9] The primers used for the PCR were HCV 36R and HCV 42 (5′-AAGTGGCGTGCTGACGACTA-3′), the sequence of which corresponds to that of nucleotides 6,755 to 6,774 of HCV-J.[9] The amplified DNA fragments were cloned into the HincII site of pTZ19R vector. Nucleotide sequences were determined directly by the dideoxynucleotide chain-termination method (Sequenase DNA sequencing kit, USB Corp., OH).

By the method used, 275 nucleotides in the amplified DNA fragments could be analyzed. The nucleotide sequences of patients 1, 2 and 3 showed no more than 8% variation from that of HCV-US, whereas the sequence of patient 4 showed 27% difference from that of HCV-US, but less than 3% variation from that of HCV-J. Parts of the nucleotide sequences from these specimens are shown in Fig. 1. The sequence of HCV-US was taken from data by Houghton *et al.*[12] The sequence of HCV-J is the concensus sequence obtained from analysis of 19 isolates of HCV-J.[13] The nucleotide sequences of the HCVs of patients 1, 2 and 3 are similar to that of HCV-US, whereas the sequence in patient 4 is similar to that of HCV-J. We detected HCV-US in 3 of 4 patients with hemophilia. Two of the patients with HCV-US have been using factor VIII from different sources for many years and one had received an infusion of factor IX once before operation. In previous analyses of the nucleotide sequences of the same region of HCV, we found that all 19 Japanese patients with chronic hepatitis or hepatocellular carcinoma examined had the sequences of HCV-J.[13] Therefore, HCV-US is rare in patients with hepatitis or hepatocellular carcinoma in Japan. The finding of HCV-US in 3 of 4 hemophiliacs suggests that the virus was transmitted in clotting factors which were prepared from several

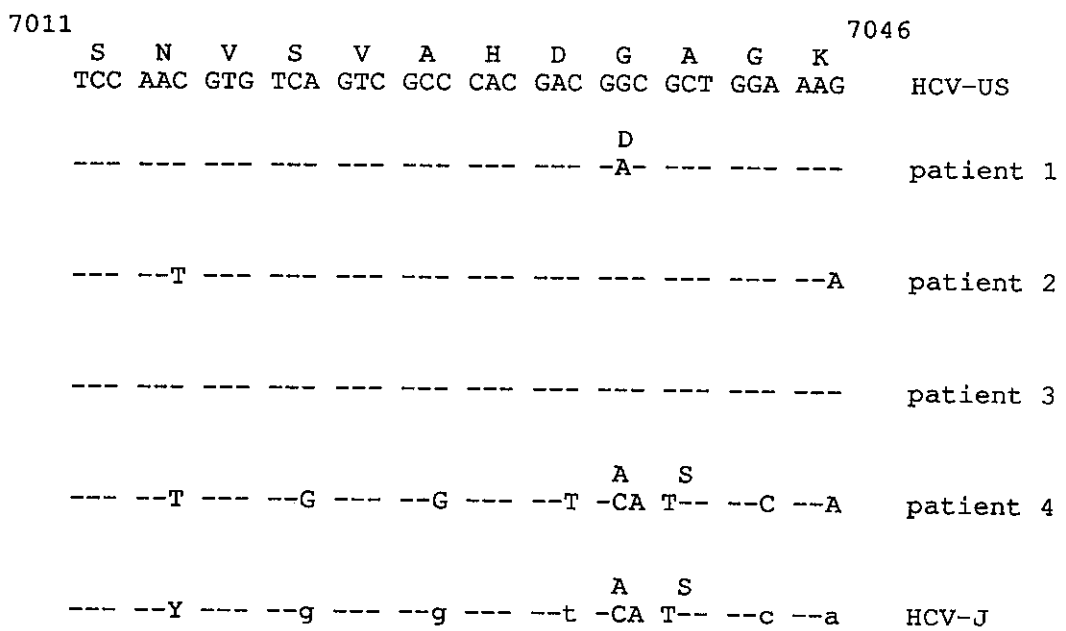

Fig. 1. Nucleotide sequences of part of the HCV genome. Nucleotide numbers from 7011 to 7046 are according to Houghton *et al.*[12] Letters above nucleotide sequences are those of amino acids according to the single letter system. Capital and small letters in the sequence of HCV-J indicate identical and nearly identical nucleotides, respectively, in 19 isolates. Y indicates C or T. Dashes indicate the presence of the same nucleotides as those in HCV-US. To eliminate the possible misreading by Taq polymerase, we determined the nucleotide sequences of more than 3 independent clones from each patient.

thousand individual donors and so could have been contaminated with HCV-US. This is likely, since the seroprevalence rate in blood donors has been estimated as 0.5 to 1.5% in various countries. Patient 4 was found to be infected with HCV-J. He has received many different batches of concentrate including a domestic product. However, the possibility that this patient was infected through the community cannot be ruled out.

These patients have received infusion of several different kinds of clotting factors for many years, so it is difficult to identify the clotting factors responsible for the virus infection. However, it is noteworthy in this connection that non-heat-treated factor VIII/IX concentrates were reported to contain the viral RNA[14] and to be able to cause viral infection.[15]

Patients who have used clotting factors from geographically different sources may harbor more than two HCV strains. However, we only detected sequences derived from two HCV strains, HCV-J and HCV-US, in this study. This may have been because there are only two major strains of HCV in the world or because the primers used in this work cannot anneal to the sequences of other strains and so these were not detectable by reverse transcription and amplification with the PCR.

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