The Presence of a Newly Identified Infectious Agent (SEN Virus) in Patients with Liver Diseases and in Blood Donors in Japan

Minoru Shibata,1 Richard Y.-H. Wang,1 Makoto Yoshida,2 J. Wai-Kuo Shih,3 Harvey J. Alter,3 and Keiji Mitamura1

1Second Department of Internal Medicine, Showa University School of Medicine, Tokyo, and 2Division of Gastroenterology, Showa University Fujigaoka Hospital, Kanagawa, Japan; 3Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland

The existence of the newly discovered SEN virus (SENV) was investigated in 379 Japanese patients with liver diseases and in 277 blood donors, to determine whether SENV is associated with non-A–E hepatitis. SENV DNA was detected by seminested polymerase chain reaction, with primers directed to 2 SENV strains: SENV-H and SENV-D. SENV was detected in 7 (32%) of 22 patients with fulminant hepatitis, in 15 (17%) of 86 patients with acute hepatitis, in 38 (27%) of 139 patients with chronic hepatitis, in 29 (31%) of 93 patients with liver cirrhosis, in 5 (33%) of 15 patients with autoimmune hepatitis, in 11 (46%) of 24 patients with primary biliary cirrhosis, and in 27 blood donors (10%). Infection occurred more frequently in patients with liver diseases than in blood donors; however, there were no significant differences in the SENV-positive rates between patients with non-A–C hepatitis and those with acute or chronic hepatitis due to known hepatitis virus or nonviral liver disease. This study did not suggest SENV as a possible causative agent of non-A–C hepatitis.

After the discovery of hepatitis C virus (HCV) [1], 10%–20% of persons with acute hepatitis [2–4], 24%–47% of those with fulminant hepatitis [5], and ~5% of those with chronic hepatitis [6] remained negative for all known hepatitis viruses and were classified as having non-A–E hepatitis. Investigators have continued to search for other infectious agents responsible for non-A–E hepatitis. As candidates for unknown hepatitis viruses, 2 novel isolates were identified from patients with non-A, non-B hepatitis and were designated hepatitis G virus (HGV) [7, 8] and TT virus (TTV) [9], respectively. When these viruses were discovered, they were expected to account for most of the residual cases of acute or chronic hepatitis that were unrelated to hepatitis A–E viruses. Although both HGV and TTV spread universally, there has been no confirmed demonstration of an etiologic association between these viruses and human diseases [10–13].

More recently, another novel virus, designated SEN virus (SENV), was described [14]. SENV is distantly related to the large TTV family [15]. SENV has a strong association with transfusion-transmitted non-A–E hepatitis [16]. SENV is a DNA virus that was discovered in the serum of an injection drug user (IDU) infected with human immunodeficiency virus (HIV). This virus was found subsequently in a large percentage of IDUs and polytransfused patients. Preliminary data showed that SENV accounts for a significant proportion of transfusion-associated hepatitis of unknown origin (92%) [17] and a variable proportion of chronic non-A–E hepatitis and that the incidence of infection in various control populations was low (0.9%–8%). It is not known whether SENV may be a causative agent of some cryptogenic hepatitis. This study determined the prevalence of SENV patients with various forms of liver disease and in healthy blood donors in Japan, to learn whether there is a significant association between SENV infection and non-A–E hepatitis.

Patients and Methods

Selection of patients and blood donors. We conducted a cross-sectional study by using stored serum samples of patients with liver disease diagnosed between 1995 and 1999. We studied 22 patients with fulminant hepatic failure, including 7 classified as non-A–C; 86 patients with acute hepatitis, including 26 classified as non-A–C; 139 patients with chronic hepatitis, including 15 classified as non-A–C; 93 patients with liver cirrhosis, including 15 classified as non-A–C; 15 patients with autoimmune hepatitis; 24 with primary biliary cirrhosis; and 277 nonremunerated blood donors (table 1). All subjects were Japanese and were studied at 2 institutions: 21 patients with fulminant hepatitis and 7 patients with acute hepatitis were treated at Showa University Fujigaoka Hospital (Kanagawa) and the rest were treated at Showa University Hospital (Tokyo).

None of the fulminant hepatitis and acute hepatitis patients had received blood transfusions or blood products before developing...
We initiated enrolled 30 patients with fulminant hepatitis but excluded 8, because they received 5–10 U of fresh frozen plasma or fresh whole blood in the referring hospital before being transferred to the study hospitals. International criteria were used to diagnose fulminant hepatitis [18]. Chronic hepatitis and liver cirrhosis were diagnosed histologically. International criteria were used to diagnose autoimmune hepatitis [19] and primary biliary cirrhosis [20]. We recorded clinical data for all patients. Serum samples were obtained at admission and were stored at −70°C before testing for SENV. All the blood donors were regular volunteers who met all standard eligibility criteria for donation (i.e., normal serum alanine aminotransferase levels and negative test results for hepatitis B surface antigen [HBsAg] and for antibodies to HCV and HIV).

Donor blood samples were obtained from Red Cross Blood Centers in Sapporo (n = 200) and Tokyo (n = 77) in January and February 2000.

Detection of SENV DNA by polymerase chain reaction (PCR). DNA was extracted from 100-μl patient samples with the QIAamp DNA extraction kit (Qiagen) and was eluted with 160 μl Tris-HCl and 0.5 mM EDTA (pH 9). We amplified 10 μl of the extract with Taq PCR mixture containing a common antisense primer, LUCKY2AS (5′-CCG TTG TT[G/T] CTGAA G[GTG] T[C/T] TGT GAT AGT-3′) and a sense primer D10S (5′-GTA ACT TTT AAC TAT AAC CCA-3′) or C BIOT (5′-CCG ATT GCA TGA AGA GTA TTA C-3′) for SENV-D or SENV-H, respectively. After 40 amplification cycles, the amplified products were detected by DNA EIA (DiaSorin) with coated biotinylated probes of D BIOT (5′-Biot-ATG ATA GGC TTC CC[C/T] TT TAA CAC TAT AAC CCA-3′) or C BIOT (5′-Biot-CCC CCT CCA GGT ATT GCA TGA AGA GTA TTA C-3′) for SENV-D or SENV-H, respectively. One SENV-D–positive serum sample, 1 SENV-H–positive serum sample, and 1 negative serum sample were extracted along with each batch of tested samples. Triplicates of ampiclon from known SENV-D– and SENV-H–positive samples at an estimated 10 copies each and reagent blank were tested in each PCR run. If 2 of the 3 replicates were negative, the PCR run was considered to be a failure and was repeated.

Detection of hepatitis viral markers. HBsAg, anti–hepatitis B core of IgM class, and anti–hepatitis A (anti-HA) of IgM class were measured by conventional methods (AxSYM Dinapack-II; Dainabot). Hepatitis B virus (HBV) DNA was detected by PCR by using a primer set deduced from the S gene [21]. Anti-HCV of the second generation was measured by EIA (AxSYM Dinapack-II; Dainabot), and HCV RNA was measured by PCR (Amplicor Monitor; Nippon Roche).

Statistical analysis. Categorical rates were compared by 2-tailed χ2 test. Logistic regression analyses were used to select independent variables related to SENV infection. P < .05 was considered to be statistically significant. We used the JMP 3.2.5 program (SAS Institute) for the statistical analyses.

Results

SENV prevalence in patients with liver diseases and in blood donors. We compared the SENV-positive rates of each disease group and of blood donors. SENV viremia was identified in 7 (32%) of 22 patients with fulminant hepatitis (95% confidence interval [CI], 12.4%–51.3%), in 15 (17%) of 86 patients with acute hepatitis (95% CI, 9.4%–25.5%), in 38 (27%) of 139 patients with chronic hepatitis (95% CI, 19.9%–34.8%), in 29 (31%) of 93 patients with liver cirrhosis (95% CI, 21.8%–40.6%), in 5 (33%) of 15 patients with autoimmune hepatitis (95% CI, 9.5%–57.2%), in 11 (46%) of 24 patients with primary biliary cirrhosis (95% CI, 25.9%–65.8%), and in 27 (10%) of 277 blood donors (95% CI, 6.3%–13.2%; table 2). Compared with blood donors, SENV infection was found more frequently in patients with fulminant hepatitis (P = .0017, Pearson’s product moment correlation), chronic hepatitis (P < .0001), liver cirrhosis (P < .0001), autoimmune hepatitis (P = .0044), and primary biliary cirrhosis (P < .0001). There was no significant difference between the SENV-positive rates of patients with acute hepatitis and blood donors (P = .0513). The prevalence of SENV did not significantly differ between patients with acute or chronic hepatitis and those with nonviral liver diseases. SENV-D infection was more prevalent than SENV-H infection in most types of liver disease and etiologies and in blood donors in Japan (table 2).

We did a subgroup analysis of the SENV-positive rates of
232 patients with chronic liver diseases—139 with chronic hepatitis and 93 with liver cirrhosis. Of these 232 patients, 55 had hepatocellular carcinoma and 177 did not. The SENV-positive rates for these groups were 31% and 28%, respectively (no significant difference).

**Association of SENV with non-A–C hepatitis.** We compared the frequency of SENV infection in patients with acute and chronic hepatitis of different etiologies (table 3). SENV was detected in 2 (29%) of 7 patients (95% CI, 0–6.2%) with non-A–C fulminant hepatitis, in 4 (15%) of 26 patients (95% CI, 1.52%–29.3%) with non-A–C acute hepatitis, in 6 (40%) of 15 patients (95% CI, 15.2%–64.8%) with non-B–non-C chronic hepatitis, and in 7 (47%) of 15 patients (95% CI, 21.4%–71.9%) with non-B–non-C cirrhosis. Although infection occurred more frequently in patients with chronic hepatitis and liver cirrhosis classified as non-B–non-C, compared with similar conditions due to HBV or HCV, the differences were not significant.

**Multivariate analysis.** We chose SENV status (positive or negative) as an outcome variable and age, sex, disease (fulminant, acute, or chronic hepatitis and liver cirrhosis), and the etiology of the diseases (known hepatitis viruses or non-A–C) as explanatory variables. We performed a logistic regression analysis, to select independent variables associated with the SENV infection in 340 patients with fulminant, acute, and chronic hepatitis and liver cirrhosis. None of these variables approached significance (age, \( P = .9079 \); sex, \( P = .7234 \); disease, \( P = .1930 \); and etiology, \( P = .2265 \)). The coefficient of determination of the statistical model was 0.0173 and did not reach a significant level.

**Discussion**

SENV appears to belong to a family of small, circular, non-enveloped, single-stranded DNA viruses that has been designated circoviruses. SENV is distantly related to the previously described TTV and other circoviruses, designated YONBAN and SANBAN, described in Japan. Although plant and animal circoviruses—such as the porcine circovirus, beak and feather disease virus, and the chicken anemia virus—are known to cause disease, none of the human circoviruses has an established disease association. Nonetheless, the members of the human circovirus family are so divergent that they might have very different pathogenic potential and disease associations and each will have to be investigated separately. In this study we examined whether the SENV variants were associated with acute or chronic liver disease that could not be attributed to hepatitis viruses A–C or to other established mechanisms.

SENV was found in 10% of volunteer Japanese blood donors, a rate ∼5-fold higher than that reported in Italy and the United States. TTV has also been found in much higher preva-
circulating in Japan than in Western nations. Although SENV prevalence has thus far been determined in a limited number of countries, it has been found in all countries tested, including Italy [14], the United States [16, 17], Canada [26, 27], and Japan [28], which suggests a global distribution. The 10% prevalence of SENV in Japanese blood donors is similar to the 12% prevalence originally reported for TTV [29]. However, because TTV was studied with additional primer sets, it was found that the prevalence could be as high as 82% [30]. The same increase in prevalence could be as high as 82% [30]. The same increase in prevalence was studied with additional primer sets, it was found that the prevalence could be as high as 82% [30]. The same increase in prevalence could be as high as 82% [30]. The same increase in prevalence could be as high as 82% [30]. The same increase in prevalence could be as high as 82% [30].

Fulminant hepatic failure is the end stage of many etiologic events [18], including drug-induced liver injury, other hepatotoxins, viral injury (especially hepatitis viruses A and B), and a variety of metabolic conditions. However, most fulminant hepatitis cases remain unexplained, and there has been a long-held suspicion that these cases are caused by an as yet unidentified viral agent. In general, it has been difficult to investigate the role of new viruses in fulminant hepatitis, because many patients receive transfusions before samples are drawn for viral detection, which confounds whether any detected agent was causative or simply was transferred passively at the time of transfusion therapy. In this study, we tested serum samples from 22 patients with fulminant hepatitis who had not received transfusions before testing. Of the 22, 7 were negative for hepatitis A virus (HAV) and HBV and had no other established etiology; only 2 of these 7 patients were SENV positive, and this frequency was not significantly different than that found in cases presumed to be due to HAV and HBV. These frequencies do not support a causal role for SENV in the development of fulminant hepatitis.

Although all patient groups (table 2) had higher rates of SENV than did the blood donor population, this difference does not imply an etiologic role for SENV in the causation of liver disease, because blood donors are not an appropriate control population. Blood donors are a highly selected population who, on average, are much younger than patients with liver disease and are less likely to have had blood exposures through transfusion or injection drug use. A more appropriate control group would be patients with nonviral liver disease. In this respect, we tested patients with autoimmune hepatitis and patients with primary biliary cirrhosis. The rate of SENV infection among these control patients was similar to the rate among patients with acute and chronic hepatitis and liver cirrhosis classified as non-ABC and those presumed to be due to HBV and HCV. This observation is further emphasized in table 3, where a comparison of patients with fulminant hepatic failure, acute hepatitis, chronic hepatitis, or liver cirrhosis classified as non-ABC showed no significant difference in SENV prevalence from patients with similar conditions related to HAV, HBV, or HCV. Thus, patients with cryptogenic cases of hepatitis or liver cirrhosis were no more likely to be infected with SENV than were control patients with other forms of viral hepatitis or control patients with nonviral liver disease.

Patients with primary biliary cirrhosis in this study had an exceptionally high prevalence of SENV (46%). Of 24 patients with primary biliary cirrhosis, 5 had received prior blood transfusion, and 3 of the 5 were positive for SENV DNA. We also compared the homology of the published amino acid sequence of the lypoyl-binding domain of pyruvate dehydrogenase complex E2 (PDC-E2) [31], which is the major reactive mitochondrial protein to serum antimitochondrial antibody (AMA) in primary biliary cirrhosis patients, with the sequences of SENV by using GENETYX-MAC (version 9; data not shown). However, no significant amino acid sequence homology was detected for the 2, including the immunodominant T cell epitope human PDC-E2 163–176 peptides [32]. Therefore, we believe that immunologic cross-reactivity due to this sequence homology is unlikely to occur. Confirming this supposition, SENV also was found in AMA-negative primary biliary cirrhosis patients.

However, we did not design the present study to clarify the association of SENV infection and primary biliary cirrhosis, so the sample size was small, and a error may have influenced the result. Further study of SENV prevalence in patients with primary biliary cirrhosis is required to clarify this issue.

By logistic regression analysis, neither age, sex, nor disease etiology correlated with SENV prevalence. Overall, the coefficient of determination was too low (0.0173) to implicate SENV strains D or H in the causation of hepatitis and liver cirrhosis of unknown origin. We did not examine the relation-

---

### Table 3. SENV virus (SENV)–positive rates for Japanese patients with fulminant hepatic failure, acute hepatitis, chronic hepatitis, and cirrhosis of different etiologies.

<table>
<thead>
<tr>
<th>Patient group, etiology type</th>
<th>SENV positive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulminant hepatic failure A, B, or C</td>
<td>33.3 (5/15)</td>
<td>.8233</td>
</tr>
<tr>
<td>Non-ABC</td>
<td>29 (27/27)</td>
<td></td>
</tr>
<tr>
<td>Acute hepatitis A, B, or C</td>
<td>18 (11/60)</td>
<td>.0713</td>
</tr>
<tr>
<td>Non-ABC</td>
<td>15 (4/26)</td>
<td></td>
</tr>
<tr>
<td>Chronic hepatitis B or C</td>
<td>26 (32/124)</td>
<td>.2440</td>
</tr>
<tr>
<td>Non-B–non-C</td>
<td>40 (6/15)</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis B or C</td>
<td>27 (21/78)</td>
<td>.1575</td>
</tr>
<tr>
<td>Non-B–non-C</td>
<td>47 (7/15)</td>
<td></td>
</tr>
</tbody>
</table>

a Types are as follows: A, hepatitis A virus positive; B, hepatitis B virus positive; C, hepatitis C virus positive, non-ABC, negative for hepatitis A, B, and C viruses; non-B–non-C, negative for hepatitis B and C viruses.

b Data are percentage of patients positive (no. with etiology type/total no. in group).
ship of other SENV variants, but, in prior studies [16, 17], only variants D and H had significant associations with transfusion-associated hepatitis. Even this significant association in the transfusion setting does not establish causality, and one must await additional epidemiologic studies in transfusion recipients, as well as proof that SENV both resides in liver cells and replicates in hepatocytes. It is probable, on the basis of the current findings, that SENV is not a causative agent of non-ABC (cryptogenic) hepatitis or liver cirrhosis.

Acknowledgments

We thank Christine Haley and Teresa Grandinetti for technical assistance, Toshio Morizane (Kanagawa Dental College) for statistical analyses, Hisami Ikeda and the Hokkaido Blood Center staff for collection of blood donor samples, and Hiroshi Miyakawa (4th Department of Internal Medicine, Teikyo University School of Medicine) for examining the homology of the published amino acid sequence of the lypoyl-binding domain of pyruvate dehydrogenase complex E2 and SEN virus.

References

24. Igik_FIFO, Takahashi K, Mihoshi S. Complete circular DNA genome of a TT virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. Virology 1999;260:17–22.