A Prospective Study of Transfusion-Transmitted GB Virus C Infection: Similar Frequency but Different Clinical Presentation Compared With Hepatitis C Virus

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To study the incidence and outcome of GB virus C (GBV-C) infection in blood recipients. Serum samples collected in a prospective study were examined for GBV-C RNA by a nested polymerase chain reaction assay. Among the 400 adults who underwent cardiac surgery, 40 were positive for GBV-C RNA, including six whose pretransfusion sera were already positive and seven coinfected with hepatitis C virus (HCV) during transfusion. The risk of transmission was estimated to be ~0.46% per donor. GBV-C viremia was detectable 1 week after transfusion and could persist for 8 years. However, no evident symptoms or signs were noted in the 25 patients infected by GBV-C alone, and the average peak serum alanine aminotransferase activity was 31 IU/L only (range, 12 to 123), with persistently normal levels in 26 patients. In the seven patients coinfected with HCV, the clinical courses of posttransfusion hepatitis were similar to those infected by HCV alone. In eight patients with posttransfusion non-A-E hepatitis, only one was positive for GBV-C RNA. Sixty samples were chosen to test hepatitis G virus (HGV) RNA in contrast to none of the 30 GBV-C negative samples. In conclusion, GBV-C can be transmitted by transfusion in ~9% of patients who underwent cardiac surgery. Nevertheless, this virus does not seem to cause classic hepatitis in most instances.

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Molecular cloning of the hepatitis viruses and rapid progress in serologic assays have enabled us to diagnose the etiology in the majority of posttransfusion hepatitis (PTH) cases. However, after screening for the antibody to hepatitis C virus (anti-HCV) in blood donors, there are still 2% to 3% of patients whose posttransfusion hepatitis cannot be attributed to hepatitis A, B, C, D, or E viruses. Among the candidate non-A-E hepatitis viruses, a well-known one that has been characterized extensively in animal models, is the GB hepatitis agent. Recently two GB hepatitis agent-like viruses, GBV-A and GBV-B, have been identified and isolated from the inoculated tamarins, but both seem absent in humans. After further study, novel GBV-like sequences, designated GBV-C, were isolated from serum samples of some non-A-E hepatitis patients in western Africa and North America. Preliminary data have shown viremia in humans. However, the modes of transmission and the clinical significance of GBV-C infection remain unknown.

As we have conducted a prospective study of posttransfusion hepatitis in Taiwan since 1987, the serial serum samples collected from these prospectively followed recipients can be used to study the risk of GBV-C transmission through blood transfusion, as well as the clinical course of those who are infected. Accordingly, we investigated the presence of GBV-C genome in these recipients by using a nested polymerase chain reaction (PCR) assay, and the clinical correlation of hepatitis was analyzed. After testing of GBV-C RNA were completed in our study, a closely related agent, hepatitis G virus (HGV) was identified. We, therefore, also tested HGV RNA in some of our samples.

MATERIALS AND METHODS

Blood recipients. From June 1987, we conducted a prospective study of posttransfusion hepatitis in the National Taiwan University Hospital. Interim results for hepatitis virus infection and human immunodeficiency virus (HIV) antibody were reported in detail before. Briefly, patients who received blood transfusion and met the following criteria were recruited: normal liver function tests before transfusion; no transfusion in the past year; no previous history of liver disease; alcoholism, drug addiction, or exposure to hepatotoxic drugs. After transfusion, the recipients were followed-up every 2 to 4 weeks for 6 months. Patients with elevated alanine transaminase (ALT) during this 6-month period were followed every month for 1 year and then every 3 months for as long as possible. Blood samples were obtained during each visit and kept frozen at ~80°C until tested. Additional samples beside the follow-up schedule were obtained in some patients who were checked for blood tests due to their underlying disease. The samples were carefully handled and aliquoted to avoid contamination and degradation of viral nucleic acid. As of June 1994, a total of 1,038 patients were enrolled. Of them, 910 recipients completed the 6-month follow-up, while 128 patients were lost to follow-up at the 6-month period. Among the 910 recipients, 665 received transfusion when anti-HCV was not screened in blood donors, and 245 patients were transfused after anti-HCV screening that was initiated on July 1, 1992. Most of the blood recipients were patients receiving cardiac surgery and had a mean donor number of 18.6 ± 14.6 (range, 1 to 97). For the GBV-C study, 400 recipients were enrolled. They included the first 250 consecutive patients transfused since 1987 and before anti-HCV screening was implemented in blood donors and the first 150 patients transfused after the anti-HCV screening. Of the 400 recipients tested for GBV-C, 24 were infected by HCV and eight were diagnosed as posttransfusion non-A-E hepatitis by serologic assays and PCR for hepatitis B and C viruses as previously described.

All the 24 HCV infections were detected before anti-HCV screening. After testing of GBV-C RNA, 30 GBV-C positive recipients and 30 GBV-C negative recipients were selected and tested for HGV RNA by PCR including: (1) the pretransfusion sample and two posttransfusion samples of all the eight non-A-E hepatitis patients (24 samples,
two of them were GBV-C RNA positive), (2) two posttransfusion samples of 18 randomly selected patients without hepatitis (36 samples; 28 of them were GBV-C RNA positive).

Donors. The blood or blood components used were donated by volunteers negative for hepatitis B surface antigen, antibody to human immunodeficiency virus, and with SER ALT activities <45 IU/L (normal, <31 IU/L). Antibody to HCV by a second generation assay was added to the screening list in July 1992. Plasma samples of donors were also collected from transfused fresh frozen plasma or whole blood before transfusion as completely as possible.

PCR assay. To screen GBV-C viral RNA, the pretransfusion, and the 6-month posttransfusion serum samples from these 400 recipients were tested by a nested PCR. We had tested GBV-C in 104 patients in both first and 6-month posttransfusion samples. Of them, seven were positive at both first and 6-month posttransfusion samples, three were positive at 6 months, and one was positive at the first posttransfusion samples only. Therefore, to avoid including recipients with carry-over rather than true infection and to save the resources and time of so many PCR experiments, we tested the 6-month posttransfusion sample in the subsequent patients. Serial posttransfusion samples of a given recipient were tested if the 6-month posttransfusion sample was positive and the pretransfusion sample was negative. Plasma samples from 200 volunteer blood donors unrelated to this study were tested as controls. The initial PCR screening was done by pooling 10 to 15 μL of serum specimens from seven to 10 patients. The outer primer pair was GBV-C-s1 and GBV-C-a1, inner primers were GBV-C-4-s1 and GBV-C-4-a1, or GBV-C-5-s1 and GBV-C-5-a1. Nucleic acid was extracted from 100 μL of serum, reverse transcribed and subjected to a nested PCR, as described previously. Ten microliters of the PCR products were electrophoresed in a 2% agarose gel and stained with ethidium bromide. Positive samples were then broken down by testing GBV-C RNA in each individual sample by the same method. Negative samples were tested again by adding a GBV-C RNA positive serum sample to exclude the presence of reaction inhibitor. The PCR assay has been proven to be specific for detecting serum GBV-C RNA after sequencing the PCR products from more than 10 patients. For each PCR assay, a weak positive serum sample, which was positive only after nested PCR by both inner primers, was used as positive control. Serum samples from healthy subjects that were collected during a routine physical check-up and were proven to be negative for GBV-C RNA with all of the above-mentioned GBV-C primers (they also tested negative for HGV RNA after HGV sequences were available) were used as negative controls. For each experiment, a negative serum sample, as well as reagents without template, were used as negative control. To detect HGV RNA by PCR, two primer pairs from the HGV genome were used: the outer primer pair was HGV-F1 5'agggcttcacagccgccgag; HGV-R1 5'tcaagggctccgacgtagatgct; the inner primer pair was HGV-F2 5'ggacctggatagctgaaat; HGV-R2 5'gagcaccagagcgtggc. The procedures were the same as those for GBV-C RNA.

DNA sequencing. DNA sequences were determined in PCR products from recipients positive for GBV-C RNA to confirm the specificity. For sequencing reaction, 40 μL of the specific biotinylated amplification product (amplified by primer GBV-4s1 and 4a1 or GBV-5s1 and 5a1) were used to generate single-stranded template for sequencing by Dynabeads M280 streptavidin (Dynal AS, Oslo, Norway). The single-stranded, biotinylated PCR products were directly sequenced using a cycle sequencing protocol and reagents supplied with the Taq Dye Terminator Cycle Sequencing Kit (ABI, Foster City, CA). The thermal cycling condition of the specific biotinylated amplification product was 35 cycles at 94°C for 20 seconds 60°C for 20 seconds and 72°C for 50 seconds after the initial denaturation step at 94°C for 5 minutes. After PCR, the reaction mixtures were extracted with phenol/chloroform (4:1) twice and precipitated with 95% (vol/vol) ethanol and 3 mol/L sodium acetate, pH 5.2. Following centrifugation for 25 minutes, each pellet was dried in a speed-Vac (Savant, NY) and stored at -20°C until electrophoresis. Dried pellets were resuspended in 4 mL of loading buffer [5:1 (vol/vol) deionized formamide/50 mmol/L EDTA pH 8.0] and were heated at 95°C for 3 minutes to loading onto a 6% (wt/vol) polyacrylamide gel containing 7 mol/L urea. Electrophoresis was performed on an ABI 373 automated sequencer (ABI).

Southern blot. To further confirm the specificity of the nested PCR, 30 random samples were checked by southern blot. The PCR products from a sequenced sample amplified by primer pair GBV-4s1 and 4a1 were purified and used as the probe. The probe was labeled using a enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK) according to the manufacturer's instructions. Southern hybridization was performed as previously described. Briefly, the PCR products were electrophoresed and transferred to a nylon membrane (Oncor, Gaithersburg, MD). The filters were prehybridized with a rapid hybridization buffer (Amersham) at 65°C for 1 to 2 hours and hybridized with the probe at 65°C for 2 hours. Filters were then washed with 0.1% sodium dodecyl sulfate (SDS) and 0.5× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate) at 42°C for 20 minutes twice and then washed with 0.1% SDS and 0.1× SSC for 20 minutes. The film was exposed for 1 minute after treating with the substrate.

Statistical methods. Student's t-test and Chi-square test were used for statistical analysis.

RESULTS

None of the pooled negative samples showed reaction inhibitor after repeat testing. On repeat tests for each individual after initial screening, 39 of the posttransfusion samples (39 of 400 = 8.6%, 95% confidence intervals = 6.8 to 12.7), six of the pretransfusion samples of the 400 recipients (6 of 400 = 1.5%, 95% confidence intervals = 0.3 to 2.7) and four of the 200 donors were positive for serum GBV-C RNA, respectively. All six pretransfusion samples and 34 of the posttransfusion samples were positive for both primers (Fig 1A). All of the samples checked by Southern blot specifically hybridized to the probe (Fig 1B). Four of the five patients were positive by GBV-5s1/5a1 primer, while one was positive by GBV-4s1/4a1 only. Samples positive for only one primer pair were considered positive if more than two of the serial samples were positive. Two of these samples were sequenced and confirmed to be GBV-C RNA by a homology of 85% and 87% in 82 nucleotides with the reported sequences, respectively. Five of the six patients positive for GBV-C RNA in pretransfusion samples were still positive on the posttransfusion samples. Therefore, 40 recipients were positive on either pre- or posttransfusion samples. The positive rates for the recipients and donors were 40 of 400 and 4 of 200, respectively. Of the 34 recipients (34 of 394 = 8.6%, 95% confidence intervals = 5.8 to 11.4) with new posttransfusion GBV-C viremia, 22 (8.8%) were transfused before, while 12 (8.0%) were transfused after anti-HCV screening (22 of 250 v 12 of 150, P > .1). Seven (20.6%) of the 34 recipients were coinfect with HCV during blood transfusion. The number of donors in these 34 patients was significantly higher than those not infected with GBV-C (24.2 v 17.6, P < .01). Two recipients, each carrying HCV and HTLV-I, were superinfected by GBV-C during transfusion. Therefore, 25 recipients were
infected by GBV-C alone. These 25 recipients had a mean peak serum ALT of 31 IU/L (Table 1), 20 did not have any ALT elevation during the 6-month follow-up (Fig 2A), three had mild ALT elevation (less than twofold the upper limit of normal), and two were found to have elevated ALT levels more than twofold the upper limit (peak ALT value 101 and 123 IU/L, respectively). Among these five recipients with elevated serum ALT activities, the elevation was so mild that only one fit the criteria of posttransfusion hepatitis.9

Except for those coinfected with HCV, all the GBV-C infected subjects were asymptomatic during the acute stage. In the follow-up period of 1 to 8 years, they appeared clinically well, and there was no evidence of hepatitis or other systemic diseases. The clinical course and laboratory data in the seven HCV coinfected recipients was similar to those infected by HCV only (Table 1). All seven patients developed chronic hepatitis, two of them died 6 years after transfusion due to underlying heart disease, and another patient was found to have unexplained iron deficiency anemia (Hb = 9.2 to 9.8 gm/dL) 7 years after transfusion. The results of serial testings showed that GBV-C viremia appeared as early as 1 week after transfusion and remained detectable for as long as 8 years. In three patients coinfected by GBV-C and HCV, HCV RNA could be detected in most of their serial serum samples, while GBV-C RNA was detectable intermittently during the follow-up period (Fig 2B).

Of 22 patients (13 GBV-C infected alone, seven with HCV coinfection, one HCV carrier, and one HTLV-1 carrier) who had two or more serum samples available 6 months after transfusion, eight had GBV-C RNA detectable. The number of samples tested for persistent infection in each patient was 5.6 (range 2 to 20) and the time of sampling ranged from 9 months to 8 years after transfusion. Therefore, if defined by the presence of GBV-C RNA for >6 months after infection, the persistent infection rate of acute GBV-C infection acquired after blood transfusion was estimated to be 36% (8 of 22).

In the eight patients with posttransfusion non-A-E hepatitis, only one (12.5%) had serum GBV-C RNA. The patient with acute non-A-E PTH became GBV-C positive 1 month after transfusion (Fig 2C). The GBV-C viremia persisted for 2 years, while ALT became normal 3 months after transfusion.

In the 30 GBV-C positive samples, 26 were positive for HGV, while none of the 30 GBV-C negative samples were positive for HGV sequences. The patient with acute non-A-E PTH and GBV-C positivity was positive for HGV sequences.

**DISCUSSION**

Blood transfusion is a vital therapeutic intervention in daily clinical practice, but it always carries the risk of blood-transmitted viral infections, including human hepatitis viruses and retroviruses.14,15 The recently cloned human GB-hepatitis like virus, GBV-C,7 prompted our concern. Because GBV-C viremia has been demonstrated in man, it may be transmitted through blood-borne infection and studying recipients of blood transfusion is the most likely way to document this possibility.

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**Table 1. Clinical Data of Patients With Posttransfusion GBV-C Infection Compared With HCV Infection**

<table>
<thead>
<tr>
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<th>GBV-C Alone</th>
<th>With HCV Coinfection</th>
<th>HCV Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>25</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td><strong>Mean age</strong></td>
<td>44.1</td>
<td>55.6</td>
<td>46.2</td>
</tr>
<tr>
<td><strong>Sex (male:female)</strong></td>
<td>16:9</td>
<td>3:4</td>
<td>11:6</td>
</tr>
<tr>
<td><strong>Mean donor number</strong></td>
<td>21.2</td>
<td>28.4</td>
<td>20.4</td>
</tr>
<tr>
<td><strong>Mean peak ALT, IU/L</strong></td>
<td>31</td>
<td>442</td>
<td>501</td>
</tr>
<tr>
<td><strong>Chronicity rate</strong></td>
<td>38% (5/13)</td>
<td>100% (7/7)</td>
<td>88% (15/17)</td>
</tr>
<tr>
<td><strong>Jaundice</strong></td>
<td>0</td>
<td>28% (2/7)</td>
<td>35% (6/17)</td>
</tr>
</tbody>
</table>

Excluding one HCV carrier and one HTLV-1 carrier, both had mild ALT elevation.

* Significantly larger than recipients without GBV-C infection ($P < .01$, Student's t-test).

† For HCV infection.
The recently cloned HGV is closely related to GBV-C by the high nucleotide and amino acid homology between these two viruses. In the same series of serum samples, our data also showed a high consistency of viremia of these two viruses in different patients. These results suggested GBV-C and HGV are probably identical.

When clinical presentations of posttransfusion GBV-C infection were compared with those of HCV, GBV-C seemed not to cause evident hepatitis as that by HCV. The majority (20 of 25, 80%) of posttransfusion GBV-C infected recipients had persistently normal levels of serum ALT, and most (7 of 8, 87.5%) patients who had documented non-A-E hepatitis in our posttransfusion hepatitis study were negative for serum GBV-C RNA. In the one patient with non-A-E hepatitis and posttransfusion GBV-C/HGV infection, the hepatitis was mild, and ALT did not elevate during a follow-up period of 2 years. Taken together, the results suggested that GBV-C/HGV may not be a classic hepatotropic agent, like hepatitis viruses A–E. However, caution must be taken in interpreting these data because the cases infected by GBV-C/HGV in our series were limited in number, and GBV-C/HGV may cause severe hepatitis in only a small proportion of infected patients, as was shown in a recent study that this virus has been claimed to be associated with fulminant hepatitis of unknown etiology. Another important implication of the low GBV-C/HGV positivity rate in posttransfusion non-A-E hepatitis is that perhaps there exists another non-A-E, non-GBV-C/HGV hepatitis agent(s) that can be transmitted through blood transfusion.

In our study, we found a prevalence of GBV-C viremia in ~10% of the blood recipients. Because currently no serologic tests are available, emergence of GBV-C viremia is the only clue for acute infection of this virus. Although there could be subjects with chronic intermittent viremia as shown in the case depicted in Fig 2B and thus ostensibly fulfilled the criterion, the low PCR positivity rate before transfusion (1.5% vs 9.8% posttransfusion, P < .01) suggested that most of them were indeed infected through blood transfusion. On the other hand, defining acute GBV-C infection by screening the 6-month posttransfusion samples could miss some patients whose viremia were cleared within this short period. However, the proportion seemed small as shown by our preliminary results, and detection of viremia at an early stage could include GBV-C RNA carried over from the donor without true infection in the recipients. Taken together, the infection rate in our study should be close to the real incidence, although serological testing is necessary to clarify it in the future.

In those with posttransfusion GBV-C infection, the viral genome was detected in the first serum sample 1 week posttransfusion and could then persist for as long as 8 years.

**Fig 2.** Viremia and serum ALT levels of three patients with posttransfusion GBV-C infection. (A) A 43-year-old woman infected with GBV-C after blood transfusion. Note the persistently normal ALT levels (<31 IU/L) despite GBV-C viremia for up to 7 years. (B) A 68-year-old woman coinfected with GBV-C and HCV after blood transfusion. Note the intermittent GBV-C viremia and persistent HCV viremia. The serum ALT levels are similar to those infected by HCV alone showing biphasic peaks. (C) A 49-year-old male with non-A-E hepatitis and GBV-C infection alone. Note the transient mild ALT elevations of serum ALT levels that decrease to normal range despite persistent GBV-C viremia.
This indicates that GBV-C may cause a chronic infection. The chronicity rate was estimated to be 36% by the presence of viremia for more than 6 months. However, the actual rate might be lower if seroconversion instead of viremia at the 6-month sample after transfusion was adopted to diagnose acute infection. The only risk factor associated with GBV-C infection in our study was the number of blood donors. Screening of anti-HCV in blood donors did not seem to reduce the risk of GBV-C infection (8.8% vs 8.0%, \( P > .1 \)), although many were coinfected by HCV and GBV-C before the anti-HCV screening. We also found a 1.5% and 2% GBV-C prevalence rate of viremia in the pretransfusion samples of the recipients and volunteer blood donors, respectively. The prevalence of GBV-C carriage in the Taiwanese adult population should be close to this range. The risk of transfusion-associated transmission in Taiwan was estimated to be approximately 0.46% per donor [34/(400 × 18.6) = 0.0046]. However, because there could be a recipient receiving blood or its products from multiple GBV-C viremic donors, the actual risk could have been higher than this estimate. Because only donors from transfused plasma and whole blood were collected and the PCR assay might be more sensitive than in vivo infectivity, we did not calculate the risk of transmission from the prevalence rate of viremic donors.

The inapparent clinical symptoms and signs in the acute GBV-C infected patients did not preclude the necessity for further screening for this virus, as serious sequelae could happen only in a small proportion of patients with chronic viral infections. For example, the risk of anemia happened in only some of those infected with parvovirus B19. The risk of the development of a tropical spastic paraparesis/HTLV-I–associated myelopathy and adult T-cell leukemia has been well documented in patients with HTLV-I infection, yet only a small proportion of those with long-standing infections had these complications.

In summary, we have documented the transmission of GBV-C/HGV through blood transfusion. Although we could not find definite associations of this viral infection with hepatitis, we have shown chronicity of transfusion-acquired acute GBV-C/HGV infections. Some might develop significant sequelae after prolonged carriage of the virus. Therefore, a longer follow-up in more GBV-C/HGV infected subjects is warranted. On the other hand, GBV-C/HGV infection in patients with different hepatic and nonhepatic diseases should also be studied to clarify other possible clinical implications of this infection. In the meantime, serologic assays for markers of GBV-C/HGV infection should also be developed to ravel the true frequencies of GBV-C/HGV infection, as well as for clinical diagnosis and, perhaps, also for donor screening in the future.

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A prospective study of transfusion-transmitted GB virus C infection: similar frequency but different clinical presentation compared with hepatitis C virus

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