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The parallel measurement of serum antibodies to the hepatitis G virus (anti-HGV) and of viremia (HGV-RNA) should improve our understanding of HGV transmission by coagulation factor concentrates. The aim of this study was to assess the relationship between HGV, the type of concentrate infused, and liver disease in multitransfused hemophiliacs. To this end, anti-HGV and HGV-RNA were evaluated by an enzyme-linked immunosorbent assay and a nested-polymerase chain reaction assay in patients treated lifelong with nonvirus-inactivated plasma-derived concentrates (n = 128), virus-inactivated concentrates (n = 33), or recombinant factors (n = 7), and in 200 regular blood donors. The prevalence of serum HGV-RNA and anti-HGV was higher in the recipients of nonvirus-inactivated factors than in blood donors (HGV-RNA: 9% v 1.5%, P = .002; anti-HGV: 32% v 5%, P < .0001). In the recipients of virus-inactivated concentrates the prevalences of these markers were similar to those in blood donors (HGV-RNA: 3% v 1.5%; anti-HGV: 15% v 5%). The prevalence of either marker in the recipients of nonvirus-inactivated concentrates was higher than in the recipients of virus-inactivated factors (39% v 18%, P = .04). The former group had serum hepatitis C virus (HCV) RNA or anti-HCV more frequently than the latter group (HCV-RNA: 86% v 15%, P < .0001; anti-HCV: 96% v 18%, P < .0001). Serum alanine aminotransferase was persistently high in 83 (81%) patients with HCV-RNA alone, in 8 (89%) with HCV/HGV coinfection, and in none of the three patients with HGV-RNA only. Thus, HGV infection in hemophiliacs is more common than previous studies of HGV-RNA prevalence have suggested, but it resolved in most cases and caused chronic viremia only in a small number of patients, without biochemical evidence of persistent liver damage.

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THE GREAT MAJORITY of hemophilic patients treated with nonvirus-inactivated clotting factor concentrates prepared from pooled human plasma have chronic hepatitis as a consequence of infection with the blood-borne hepatitis C virus (HCV).1,2 The recently discovered hepatitis G virus (HGV), a flavivirus with a similar genome organization to HCV, is present in the serum of approximately 2% of volunteer blood donors and has been associated with transfusion-related and community-acquired cases of acute and chronic hepatitis.3-5 In recipients of nonvirus-inactivated clotting factor concentrates, relatively low rates (14%) of HGV infection were found with the polymerase chain reaction (PCR) for HGV-RNA despite frequent contamination of concentrate batches with HGV-RNA.6 These findings, while indicating that HGV is transmitted by pooled plasma products, suggest that HGV viremia may be time-limited, perhaps as a result of the activation of immunity against HGV.

Recently, an enzyme-linked immunosorbent assay (ELISA) detected IgG antibodies against the recombinant HGV envelope protein E2 (anti-HGV) in 9% of healthy donors and up to 41% of patients with parental risks, including those recovering from posttransfusion acute hepatitis.7 The findings that the presence of these antibodies and of HGV-RNA are usually mutually exclusive and that antibodies correlate with the length of infection8 corroborate the views that serum anti-HGV marks recovery from HGV infection. Seroprevalence studies of anti-HGV should improve our understanding of the epidemiology and natural history of HGV in hemophiliacs, the epitome of multitransfused patients lifelong exposed to blood-borne viruses. The goals of this study were to assess exposure of hemophiliacs to HGV, the relationships between type of treatment and HGV transmission, and the role of HGV in the liver disease of hemophiliacs. Therefore, HGV-RNA and anti-HGV were measured in parallel in the plasma of 168 hemophiliacs who were followed with periodical liver exams at our center and who had received different types of concentrates prepared from plasma treated with different procedures for virus inactivation or obtained with recombinant technologies.

MATERIALS AND METHODS

Patients. Between September 1995 and June 1996, fresh serum samples were obtained from 168 consecutive hemophiliacs (mean age 29 years; range 2 to 71 years) attending the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center. The only criterion used for selection was that all patients had been prospectively followed with annual liver function tests since their first treatment at the Center (mean follow-up, 16 years; range 0.7 to 27 years). The patients’ main epidemiological and clinical features are shown in Table 1.

The majority of patients (n = 128) had been treated with unmodified concentrates until 1985 and with virally inactivated concentrates thereafter; 33 were given only virus-inactivated concentrates. The virus-inactivation methods reflect the strategies implemented by manufacturers through the years 1984 to 1987 to improve safety. Until 1987, virus inactivation was performed using dry heating at temperatures between 60 and 68°C, which avoided the transmission of human immunodeficiency virus (HIV) but not of HCV.9 Since 1987 more effective methods, such as dry heating at 80°C or higher, vapor heating, solvent/detergent, and pasteurization, were adopted. Seven patients were treated exclusively with recombinant factor VIII.

Two hundred consecutive unpaid blood donors (153 men, 47 women; mean age 40 years) from the hospital blood bank served as
controls. All had persistently normal alanine aminotransferase (ALT) and no serum hepatitis B antigen (HBsAg), anti-HCV, or antibody to HIV (anti-HIV).

Methods. HBsAg, anti-HBs, and anti-HIV were tested in serum, with immunoenzymatic assays (Abbott Laboratories, North Chicago, IL). A second generation immunosassay was used to detect antibodies against HCV (anti-HCV, HCV ELISA; Ortho Diagnostic Systems, Raritan, NJ). Serum HCV-RNA was detected, as previously described, by a nested reverse transcription (RT)-PCR, using primers of the 5′ noncoding region of the viral genome. Serum ALT was measured with conventional colorimetric methods.

HGV-RNA nested PCR. Nucelic acids were isolated from 200 μL of serum by acid guanidinium-thiocyanate-phenol-chloroform and were converted into cDNA using random hexamers. cDNAs were amplified using a nested PCR protocol with degenerated primers derived from the 5′ encoding region of the genome. The sequences of the sense and antisense primers used for the first round of the nested PCR were, respectively: 5′-dGGCCAAAAGGTGGTGGATG-3′ and 5′-dGTGGGCGTCGTTTGCCCAGG-3′. PCR was done with Taq DNA Polymerase for 35 cycles, consisting of denaturation at 96°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The second round of PCR was performed for 25 cycles (consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute) with nested primers: sense (5′-dJTTGGTAGCCACATATAGGTTG-3′) and antisense (5′-dGTAGGACCAAACATCGTGG-3′). PCR products of the expected size (140 bp) were resolved on 3% agarose gel. To avoid PCR product carry-over, all recommended precautions were observed and appropriate controls were used.

Anti-HGV ELISA. An ELISA was used for the qualitative determination of IgG antibodies to the HGV Env-antigen. The Env-transmembrane protein was bound onto streptavidin-coated microtiter plates, which were incubated with the diluted specimen, and the antibodies directed against Env protein were detected using an anti-human IgG-peroxidase conjugate and 2,2′ azino di-[3 ethylbenzothiazoline sulfonate (6)] (ABST) as peroxidase substrate. Extinction was measured at 405 nm (Anti-HGenv, Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. The prevalence of serum markers of HGV, HCV, and HIV and their relation to other variables were analyzed by the chi-squared test or Fisher’s exact test.

RESULTS

HGV. Table 2 shows the results of HGV markers in hemophilic patients and blood donors. The prevalence of serum HGV-RNA and anti-HGV was higher in the recipients of unmodified concentrates than in blood donors (HGV-RNA: 9% v 1.5%, P = .002; anti-HGV: 32% v 5%, P < .0001). On the other hand, the corresponding prevalence in the recipients of virus-inactivated concentrates was similar to those in donors (HGV-RNA: 3% v 1.5%; anti-HGV: 15% v 5%). None of the 7 patients treated exclusively with recombinant factor had HGV-RNA or anti-HGV. Only two patients, who had received nonvirus-inactivated concentrates, circulated both HGV-RNA and anti-HGV (Table 2). The prevalence of either marker in the 128 recipients of nonvirus-inactivated concentrates was higher than in the 33 recipients of virus-inactivated factors (39% v 18%, P = .04).

Other viruses. HCV-RNA and anti-HCV were more frequent in the recipients of nonvirus-inactivated concentrates than in those given virus-inactivated concentrates (HCV-RNA: 86% v 15%, P < .0001; anti-HCV: 98% v 18%, P < .0001; Table 2). Both markers were absent in patients treated exclusively with recombinant factors. Anti-HIV was detected in 30 recipients (23%) of noninactivated concentrates but in none of those given virus-inactivated or recombinant factors (Table 2). In the recipients of noninactivated concentrates, exposure to HGV was less frequent than exposure to HCV (32% anti-HGV v 98% anti-HCV, P < .0001) and as frequent as exposure to HIV (32% anti-HGV v 23% anti-HIV, P = .08). Nine (7%) recipients of unmodified concentrates concurrently circulated HGV-RNA and HCV-RNA (Table 3).

Liver disease. Serum ALT was persistently high in 83 patients with HCV-RNA alone, in eight with HCV/HGV coinfection, but in none with HGV-RNA only (Table 3).

DISCUSSION

The first goal of this study was to evaluate the prevalence of HGV infection in hemophiliacs treated with large-pool coagulation factor concentrates by using a new serological method that measures antibodies to this virus. Previous findings of a relatively low prevalence of HGV viremia (6% to 14%) in recipients of nonvirus-inactivated clotting factor concentrates, despite frequent contamination of HGV-RNA sequences in batches, led us to surmise that HGV is not efficiently transmitted with concentrates or that, unlike HCV, it does not establish long-term viremia in the majority of infected patients. Our findings of low rates (9%) of viremia but a higher prevalence (32%) of anti-HGV in the recipients of nonvirus-inactivated concentrates favor the latter interpretation. Because anti-HGV may mark recovery from infection, perhaps these findings indicate that HGV was transmitted with concentrates but that most patients recovered from infection. Because the rates of anti-HGV seroconversion in high-risk patients increase with time, perhaps currently viremic hemophiliacs are those who have been more recently infected with HGV. It must be recognized, however, that the biological and clinical significance of anti-HGV is not fully understood. The anti-HGV assay used in this study seems to be specific for the envelope protein E2 of HGV, because it does not detect cross-reacting anti-HCV E2 antibodies. However, the probability of cross-reaction with other related viruses cannot be ruled out. Except in two patients, serum

| Table 1. Epidemiologic and Clinical Characteristics of Hemophilic Patients |
|-----------------------------|----------|----------|
| Number of patients         | 188      |
| Age, yrs                   | 29 (2-71)|          |
| Type of hemophilia         |          |
| A                          | 143 (85%)|
| B                          | 25 (15%) |
| Severity of hemophilia     |          |
| Severe                     | 112 (67%)|
| Moderate                   | 16 (9%)  |
| Mild                       | 40 (24%) |
| Type of concentrates       |          |
| Nonvirus-inactivated (until 1985) | 128 (75%) |
| Virus-inactivated only     | 33 (20%) |
| Recombinant                | 7 (4%)   |

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HGV-RNA and anti-HGV were mutually exclusive. In a previous study, 4% of drug users and 18% of patients with transfusion-associated hepatitis had both HGV antibody and HGV-RNA. More studies are necessary to establish whether the coexistence of HGV-RNA and anti-HGV heralds recovery from infection or whether it signals infection with different strains of HGV.

The second goal of this study was to evaluate the effect of virus-inactivation procedures on the rate of HGV transmission by concentrates. We found a relatively high rate (18%) of either HGV marker in the recipients of virus-inactivated concentrates, albeit lower than in the recipients of nonvirus-inactivated concentrates (39%). Therefore, HGV infection was not completely prevented by the use of virucidal methods. Like HCV, HGV may have been transmitted with concentrates inactivated by the early dry-heating methods (temperatures of 60°C to 68°C), known to be less efficient than virucidal methods developed later, such as dry heating at temperatures of 80°C or higher, pasteurization, vapor heating, and solvent detergent. Because all of our patients had received more than one type of virus-inactivated concentrate, we could not directly show that early dry-heated concentrates were responsible for HGV transmission.

The third goal of this study was to evaluate whether a relationship exists between HGV infection and liver disease in hemophiliacs. A recent study in patients with transfusion-associated hepatitis failed to establish a causal relation between HGV and liver disease. This study in multitransfused hemophiliacs is consistent with these views. Of a total of 91 hemophiliacs with elevated ALT, a rough biochemical index of chronic liver disease, none had HGV alone, 8 had HGV plus HCV, and 83 had HCV alone. These findings would suggest that HGV is not a cause of chronic liver disease in hemophiliacs.

In conclusion, HGV infected a large number of recipients of plasma-derived concentrates but spontaneously resolved in the majority of them, causing chronic viremia only in a few without current signs of persistent liver damage.

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High Rates of Hepatitis G Virus Infection in Multitransfused Patients With Hemophilia

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