Hepatitis E Virus Reinfections in Solid-Organ-Transplant Recipients Can Evolve Into Chronic Infections

Florence Abravanel,1,2 Sebastien Lhomme,1,2 Sabine Chapuy-Regaud,1,2 Jean-Michel Mansuy,2 Fabrice Muscari,4 Federico Sallusto,3 Lionel Rostaing,1,3 Nassim Kamar,1,3 and Jacques Izopet1,2

1Centre de Physiopathologie de Toulouse Purpan, INSERM U1043, and 2National Reference Center for Hepatitis E, Laboratoire de virologie, Institut fédératif de biologie, Hôpital Purpan, and 3Service de Néphrologie, Dialyse et Transplantation multi-organe and 4Service de chirurgie viscérale et digestive, Hôpital Rangueil, CHU Toulouse, France

Background. Hepatitis E virus (HEV) infections are a major cause of acute hepatitis in developing and industrialized countries. Little is known about anti-HEV immunity in solid-organ recipients.

Methods. We screened 263 solid-organ recipients for anti-HEV immunoglobulin G (IgG) at transplantation. They were followed up for 1 year and tested for HEV RNA and anti-HEV antibodies 1 year after transplantation and if their liver enzyme activities increased.

Results. A total of 38.4% had anti-HEV IgG at transplantation. The mean concentrations (±SD) of anti-HEV IgG at transplantation (8 ± 17.5 U/mL) and 1 year later (6.4 ± 12.0 U/mL, \(P = .4\)) were similar. There were 3 de novo HEV infections during the 1-year follow-up among patients who were HEV seronegative before transplantation, giving an annual incidence of 2.1%. We also identified 3 HEV reinfections among patients who were seropositive before transplantation through detection of HEV RNA, for an annual incidence of 3.3%. Their anti-HEV IgG concentrations were 0.3, 2.1, and 6.2 World Health Organization (WHO) units/mL before transplantation. Reinfection of the patient with the lowest IgG concentration at transplantation had evolved to a chronic infection.

Conclusions. Low anti-HEV antibodies (<7 WHO units/mL) seemed not to protect solid-organ recipients. HEV reinfection in immunocompromised patients can lead to chronic infection, as in primary infections.

Keywords. Hepatitis E virus; solid organ transplant recipients; reinfection; incidence.

Hepatitis E virus (HEV) is a small, nonenveloped virus with a single-stranded RNA genome. The virus has 4 main genotypes but only 1 serotype. HEV1 and HEV2 infect humans, whereas HEV3 and HEV4 also infect pigs and several other mammalian species [1]. In developing countries, the transmission of HEV1 and HEV2 occurs by the fecal-oral route. An HEV infection can lead to fulminant hepatic failure in pregnant women and patients with chronic liver diseases [2, 3]. The transmission of HEV3 and HEV4 is zoonotic in industrialized countries [4]. There is growing concern about HEV3 infections in immunosuppressed patients. An HEV3 infection can become chronic in solid-organ-transplant recipients [5, 6], in patients with hematological diseases who are given chemotherapy [7, 8], and in those with human immunodeficiency virus infection and a low CD4+ T-cell count [9, 10]. Chronic HEV infection can be defined as persisting HEV replication beyond 3 months after infection [11]. Liver fibrosis can develop very rapidly in immunocompromised patients, leading to liver cirrhosis just a few years after infection [5, 12, 13].

Hepatitis E is commonly diagnosed on the basis of the presence of anti-HEV immunoglobulin M (IgM) in the blood or by detecting virus RNA in the serum and/or feces during the acute phase of the infection [14]. Unfortunately, the sensitivities of serologic tests and molecular tests vary greatly, making the diagnosis of HEV less reliable than that of the other human
hepatitis viruses [15–17]. Serological testing may also be unreliable in immunosuppressed patients, as anti-HEV immunoglobulin G (IgG) and IgM are infrequently detected at the acute phase [9, 18]. It is therefore essential to use polymerase chain reaction (PCR)–based detection of HEV RNA for diagnosis.

The risk of HEV reinfection is unclear. Studies in humans and primates have reported that anti-HEV IgG antibodies are protective [19, 20]. Although the minimum protective concentration of antibodies has not been determined, a vaccine study suggests that an antibody concentration of 2.5 World Health Organization (WHO) units/mL (hereafter, “U/mL”) is protective [21]. But studies of individuals who were HEV RNA positive have suggested that reinfection can occur, based on a high IgG avidity index [22, 23] or a low ratio of HEV IgM to total immunoglobulin [24]. No HEV reinfections of immunocompromised patients have yet been described. Furthermore, the risk of a reinfection becoming chronic in this population is unknown.

We therefore determined the incidence of HEV infection in solid-organ-transplant recipients and correlated it with their anti-HEV antibody status before transplantation. The transplant recipients were followed for 1 year, and their liver enzyme activities and serological and molecular HEV markers were monitored.

PATIENTS AND METHODS

Patients
A total of 263 adult patients underwent kidney (n = 211) or liver (n = 52) transplantations in 2010 and 2011 at the Toulouse University Hospital (Toulouse, France). They were tested for anti-HEV antibodies (IgG and IgM) for HEV RNA on the day of transplantation and then followed for 1 year. Their liver enzyme activities (alanine aminotransferase [ALT] level, aspartate aminotransferase [AST] level, alkaline phosphatase level, and gamma-glutamyl transeptidase level) were measured twice per week for the first month after transplantation, once per week for the next 2 months, twice per month for the next 3 months, and once per month for the last 6 months. Patients were tested for anti-HEV antibodies and HEV RNA if their liver enzyme activity increased. They were also tested for anti-HEV IgG and IgM and HEV RNA at the annual visit, 1 year after transplantation. This noninterventional study involved no additional procedures. Biological material and clinical data were obtained only for standard virus diagnosis following physicians’ orders (no specific sampling, no modification of the sampling protocol, and no questions in addition to the national standardized questionnaire). Data were analyzed using an anonymized database. Such a protocol does not require written informed consent, according to French Public Health law (CSP Art L 1121–1.1).

Laboratory Investigation for HEV Infection
We used the Wantai HEV IgG enzyme immunoassay (EIA) kit and the Wantai HEV IgM EIA kit according to the manufacturer’s instructions (Wantai Biologic Pharmacy Enterprise, Beijing, People’s Republic of China) to detect anti-HEV IgG and IgM. The ratios of the anti-HEV antibodies were calculated as follows: sample OD/cutoff OD. A result was considered to be positive if the sample ratio was ≥1. The WHO anti-HEV IgG reference material, 95/584, (supplied by the National Institute for Biological Standards and Control, South Mimms, United Kingdom), was used to determine the concentrations of anti-HEV antibodies. The limit of detection was 0.25 U/mL. The Wantai assay is linear from 0.25 to 5 U/mL. We have validated the calibration curve by testing 10 replicates of the WHO standard diluted from 0.25 to 5 U/mL. The linearity was checked with a linear regression test. Blood samples with an anti-HEV IgG level of >5 U/mL were retested after dilution. We used a validated real-time PCR technique based on the ORF3 region of the HEV genome to detect HEV RNA, with a detection limit of 100 copies/mL [25]. The genotype was determined by sequencing a 189-nucleotide fragment within the ORF2 gene and performing phylogenetic analyses based on reference sequences [26].

Case Definitions
Patients who tested negative for anti-HEV IgG and IgM antibodies at transplantation and then developed anti-HEV IgG or tested positive for HEV RNA during follow-up were considered to have a de novo HEV infection. Patients who tested positive for anti-HEV IgG, with or without detection of anti-HEV IgM at transplantation, and tested positive for HEV RNA during follow-up were considered to have become reinfeeted.

Statistical Analysis
Analyses were performed using Stata, version 9.2 (Stata). The $\chi^2$ test or Fisher exact test was used to compare proportions. The Wilcoxon signed rank test was used to analyze repeated measures of anti-HEV IgG concentrations. A P value of <.05 was considered to be statistically significant.

RESULTS

HEV Seroprevalence Before Transplantation
Blood samples from the 263 solid-organ-transplant recipients were screened on the day of the transplantation (Figure 1). The median age of the patients at transplantation was 53 years (range, 19–76 years), and 169 patients (64.3%) were men. Anti-HEV IgG was found in 101 patients (38.4%; Table 1). The lack of correlation with age was due to the narrow range of the transplant recipients’ ages. The mean anti-HEV IgG concentration (±SD) in the 101 anti-HEV–positive samples was 7.6 ± 15.3 U/mL. Four of the IgG anti-HEV–positive patients
but none of the 162 IgG anti-HEV–negative patients had anti-HEV IgM. In addition, none of the 263 serum samples were reactive for HEV RNA. These results suggest that 4 patients (1.5% of the total) were recovering from acute HEV infection but that none had chronic hepatitis E.

**Incidence of HEV Infection Among Anti-HEV–Negative Patients at Transplantation**

Of the 162 patients who were seronegative before transplantation, 13 died, 7 lost their graft, and 2 moved from our area during the 1-year follow-up. The liver enzyme activities of 62 patients did not become elevated during the 1-year follow-up, but there were 93 episodes of hepatic cytolysis in the other 78 patients. We tested for HEV RNA in blood samples collected during all episodes of hepatic cytolysis. An acute HEV infection was diagnosed in 3 patients with elevated liver enzyme activities by detecting HEV RNA (Table 2). Two of these primary HEV infections have become chronic, as defined by the persistence of HEV RNA in the blood for >3 months; the other patient cleared the virus 2 months after the acute phase. Thus, we used molecular tools to identify 3 cases of de novo HEV infection, corresponding to an annual incidence of 2.1% (95% confidence interval 95% [CI], 0.44%–6.13%).

**Incidence of HEV Infection Among Anti-HEV–Positive Patients at Transplantation**

Of the 101 patients who were seropositive before transplantation, 5 died, 3 lost their graft, and 2 moved from our area during the 1-year follow-up. The 91 remaining patients were still anti-HEV IgG positive, with a mean anti-HEV IgG concentration (±SD) of 6.9 ± 13.5 U/mL. The concentrations of anti-HEV IgG at transplantation and 1 year after transplantation were similar (P = .6) (Figure 2).

The liver enzyme activities of 37 patients did not become elevated during the 1-year follow-up, but there were 63 episodes of hepatic cytolysis in the other 54 patients. No HEV RNA was detected in 28 patients with hepatic cytolysis the first 4 months following transplantation, indicating that reactivation had not occurred. The 4 patients who were anti-HEV IgG and IgM positive at transplantation showed no increase in their liver enzyme activities and did not become HEV RNA positive during follow-up. But HEV RNA was detected in the blood of 3 patients with elevated ALT values 5, 11, and 12 months after transplantation (Table 2). They had anti-HEV IgG concentrations of 2.1, 6.2, and 0.3 U/mL, respectively, at the time of transplantation. One of these patients had no anti-HEV IgM at diagnosis. One of these HEV reinfections (occurring in the patient with the

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**Table 1. Seroprevalence of Hepatitis E Virus Immunoglobulin G (IgG) Before Solid-Organ Transplantation, Overall and by Age, Sex, and Transplant Type**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. Positive for IgG/No. Tested (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>101/263 (38.4)</td>
<td>.42</td>
</tr>
<tr>
<td>Age at transplantation</td>
<td>44/124 (35.5)</td>
<td>≥53 y 57/139 (41)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>.22</td>
</tr>
<tr>
<td>Male</td>
<td>70/169 (41.4)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>31/94 (32.9)</td>
<td></td>
</tr>
<tr>
<td>Transplant type</td>
<td></td>
<td>.51</td>
</tr>
<tr>
<td>Kidney</td>
<td>22/52 (42.3)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>79/211 (37.4)</td>
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lowest anti-HEV IgG concentration at baseline (0.3 U/mL) became chronic, as defined by the persistence of HEV RNA in the blood for >4 months. Thus, we identified 3 cases of reinfection, corresponding to an annual incidence of 3.3% (95% CI, 0.69–9.33), an incidence similar to that of de novo infection (P = .68).

**Epidemiological Characteristics of HEV Cases**

The 6 patients who had HEV infections or reinfections completed a questionnaire that examined their living conditions and food and drink intakes. None of the patients had traveled abroad before the onset of the disease, indicating that their hepatitis E was autochthonous. None of them were hunters or declared having had any direct contact with a potential animal reservoir (ie, pigs, wild boar, deer, or rabbits). However, all of them consumed food products containing pork (eg, ham, bacon, or sausage). One of them occasionally consumed deer meat bought at the supermarket, and another ate mussels. None of them drank water from a well or spring.

**DISCUSSION**

We found that 38.4% of the recipients of solid-organ transplants monitored in southwestern France had anti-HEV IgG. We followed these patients for 1 year and identified 3 cases of de novo HEV infection and 3 cases of HEV reinfection.

The high prevalence of anti-HEV antibodies (38.4%) in solid-organ-transplant recipients was determined using the Wantai EIA HEV IgG assay. This prevalence is 3 times higher than our previous estimate (14.1%) in the transplant population, which was made using a less sensitive assay [27]. These data...
are in keeping with the high seroprevalence (52%) found among blood donors [28] and among patients undergoing stem cell transplantation (36%) [29] from our area when the Wantai assay was used to detect anti-HEV IgG. Our present results confirm the high exposure to HEV in southwestern France.

This prospective study identified 3 cases of de novo HEV infection among the seronegative patients, corresponding to an incidence of 2.1%. This annual incidence appears to be higher than those found in other areas of the world, such as 0.2% in the United Kingdom [30] and 0.7% in the United States [31]. However, the seroconversion rate of anti-HEV antibodies was 4.3% per year in China [32].

Anti-HEV IgG was still detected in all seropositive patients 1 year after transplantation, with a mean concentration similar to that measured at transplantation. Therefore, the immunosuppressive therapy given to prevent graft rejection does not seem to influence the humoral anti-HEV response, at least in the short term. But little is known about anti-HEV immunity in immunosuppressed patients. It was demonstrated recently that chronic hepatitis E is associated with impaired HEV-specific T-cell responses in solid-organ-transplant recipients [33]. T-cell responses became detectable after spontaneous or treatment-induced clearance of HEV [33]. Immunocompromised patients are at considerable risk (up to 65.9%) of an infection becoming chronic [18, 34]. Importantly, 1 of the reinfections detected in our study became chronic. Thus, the risk of a reinfection evolving into a chronic infection has to be considered carefully.

Previous studies have used an IgG avidity test to identify possible HEV reinfections in immunocompetent patients in regions where HEV genotype 3 is predominant [22, 23]. Reinfec-
tion was suspected in HEV RNA–positive immunocompetent patients with a high IgG avidity index. However, no earlier serum samples were available to confirm this hypothesis. Similarly, in Nepal where genotype 1 is predominant, Seriwatana et al have suspected secondary infections when the ratio of anti-HEV IgM to total immunoglobulins was low, occurring in <5% of 200 cases of acute hepatitis E [24]. A recent large-scale study in China suggested that 83% of the symptomatic infections in immunocompetent individuals were probably caused by primary infection and 17% by reinfection [35]. We identified 3 cases of HEV reinfection in our cohort of immunocompromised patients, for an incidence rate similar to that of primary infections.

The concentration of anti-HEV antibodies needed to protect against an HEV infection is still unknown. Huang et al used the Rhesus macaque model to show that a previous HEV infection may give cross-genotype protection, although HEV RNA was detected in rechallenged monkeys without hepatic cytolysis [36]. In this model, complete protection was defined as a normal serum ALT activity and no detectable fecal HEV RNA in the monkeys that were challenged, while partial protection was defined as a normal serum ALT activity but detectable fecal HEV RNA. Monkeys whose prechallenge anti-HEV IgG concentrations were relatively high (mean, 41.8 U/mL) were completely protected. Monkeys whose prechallenge anti-HEV IgG concentrations were lower (mean, 7.1 U/mL) were partially protected [36]. In vitro studies have demonstrated that HEV strains found in serum samples can replicate efficiently in cultured cells despite the presence of anti-HEV antibodies in the serum [37]. A phase 3 HEV vaccine trial found that both the virus breakthrough in vaccinated individuals and the reinfection in individuals with naturally acquired immunity were associated with lower anti-HEV IgG concentrations at baseline [35]. This suggests that the concentration of anti-HEV IgG is correlated with the degree of protection. But the precise concentra-
tion of anti-HEV IgG that can protect an immunocompromised patient against infection remains to be determined. We found that a low anti-HEV antibody concentration (<7 U/mL) did not appear to protect our patients.
We have also identified cases of reinfection by a retrospective investigation of our previous cohort of solid-organ-transplant recipients infected with HEV who were screened before transplantation by means of a less sensitive assay [18]. The 42 infected patients included 4 (9.5%) who were anti-HEV IgG positive at transplantation or 6 months before the acute phase, using the Wantai assay, with anti-HEV concentrations of 2.5–10 U/mL (data not shown). Only 1 patient, whose anti-HEV IgG concentration was 2.5 U/mL at transplantation, became reinfected with HEV 8 years later, and this reinfection became chronic.

Le Coutre et al reported that HEV infection of a German patient with acute lymphoblastic leukemia became reactivated 14 weeks after an allogeneic stem cell transplantation [7]. A second case of reactivation was recently suspected in a hematological patient after an allogeneic stem cell transplantation [38]. This raises the question of the persistence of HEV and its reactivation in immunosuppressed patients. We detected no HEV RNA in any of our patients during the first 4 months following transplantation, including the 4 patients who had tested positive for anti-HEV IgM at transplantation. This agrees well with our study of patients who underwent hematopoietic stem cell transplantation in our area; none of them experienced HEV reactivation after transplantation [29]. This suggests that HEV does not persist in the liver or any other cell compartment after clearance.

In conclusion, we have shown that solid-organ-transplant recipients can become reinfected with HEV. Importantly, HEV reinfecion can lead to chronic infection. Further studies on more patients are required to estimate the risk of an HEV reactivation becoming chronic in immunocompromised patients.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


