Chronic Hepatitis E Virus Infection Is Specifically Associated With an Interferon-Related Transcriptional Program

Valérie Moal,1,4,5 Julien Textoris,4,5 Amira Ben Amara,4,5 Vikram Mehraj,4,5 Yvon Berland,1,2,5 Philippe Colson,3,4,5 and Jean-Louis Mege4,5

1Centre de Néphrologie et Transplantation Rénale, 2Centre d’Investigation Clinique, 3Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bacteriologie-Virologie-Hygéne, Assistance Publique - Hôpitaux de Marseille, 4URMITE, UMR CNRS 7278, INSERM U1095, Méditerranée Infection, and 5Aix-Marseille Université, Marseille, France

Background. Hepatitis E virus (HEV) is a new causative agent of chronic hepatitis in solid organ transplant recipients. Clinical studies suggest that the occurrence and persistence of chronic HEV infection are related to the immunological status of patients.

Methods. We used whole-genome microarray and quantitative reverse transcription polymerase chain reaction (qRT-PCR) to compare the transcriptional profiles of whole blood from 8 kidney transplant recipients with chronic HEV infection and 8 matched kidney transplant recipients without HEV infection.

Results. We found that 30 genes in HEV-infected patients were upregulated, compared with those in control patients, as determined by microarray analysis. In contrast, no genes were downregulated. The 30 upregulated genes included 25 interferon-stimulated genes. Increased expression of the genes that encode IFIT1, IFI44L, RSAD2, EPSTI1, and ISG15 was confirmed by qRT-PCR. Interestingly, the expression levels of these genes were associated with the persistence of HEV infection.

Conclusions. Increased expression of interferon-stimulated genes may favor the persistence of an HEV infection. Whether the expression of interferon-stimulated genes is a marker of ongoing viremia or independent prognostic marker of HEV clearance needs further investigations.

Clinical Trials Registration. NCT01090232.

Keywords. Hepatitis E virus; chronic hepatitis; kidney transplantation; microarray; interferon-stimulated genes; IFIT1; IFI44L; RSAD2; EPSTI1; ISG15.

Hepatitis E virus (HEV) is a small nonenveloped virus, and its genome is a single-stranded, linear, positive-sense RNA [1]. In developing countries, HEV is a leading cause of waterborne acute hepatitis in adults [2, 3]. In Europe, HEV causes a porcine zoonosis and is an emerging causative agent of autochthonous acute hepatitis, which is caused by HEV genotype 3. HEV genotypes 1 and 2 are involved in sporadic cases and major outbreaks of HEV infection in Asia, Africa, and Central America [2, 3]. Although HEV hepatitis is an acute, self-limiting infection, the disease exhibits a severity varying from unapparent infection to fulminant hepatitis. Chronic HEV infections were first described in solid organ transplant recipients in 2008 [4, 5] and have been subsequently reported by several European teams [6–8]. This emerging disease is defined as a persistent viral infection, with hepatitis lasting for >6 months. The incidence of HEV infection after kidney transplantation has been estimated to be 2.7 cases/100 person-years [9], and half of the kidney transplant recipients infected with HEV develop chronic HEV infection (CHE) [10]. Rapid progression to cirrhosis, liver transplantation, and death has also been reported [5, 6, 11]. No curative treatment for HEV hepatitis is currently recommended. Both pegylated interferon
and ribavirin were effective in treating CHE in few patients, but interferon is contraindicated in kidney transplant recipients [12, 13].

The evolution of HEV infection toward CHE seems to be dependent on the immunological status of the patient. Indeed, CHE has been described only in severely immunocompromised patients, namely solid organ transplant recipients, human immunodeficiency virus–infected patients [14, 15], and individuals with hematological diseases [12]. In solid organ transplant recipients, CHE development has been found to be related to the dose or the type of immunosuppressive drugs taken [10, 16], and one-third of CHE patients clear HEV after reduction of the dose of immunosuppressive drugs [10]. The mechanisms leading to CHE are poorly understood. CHE has been recently associated with impaired HEV-specific T-cell responses in organ transplant recipients [17]. On the other hand, a microarray analysis performed on the livers of chimpanzees that were experimentally infected with HEV showed enhanced expression of interferon-stimulated genes (ISGs), which suggests activation of the interferon response by HEV [18].

In the prospective study presented here, we compared the transcriptional profiles of whole blood from kidney transplant recipients with CHE and matched kidney transplant recipients without viral infection. Microarray studies demonstrated that patients with CHE exhibited a specific peripheral signature including 30 upregulated genes, 25 of which are annotated ISGs. The upregulation of 5 type I ISGs selected from the CHE signature was confirmed by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Interestingly, the expression level of these genes was associated with the persistence of HEV infection. These results suggest that the persistence of HEV infection is related to increased expression of ISGs and that studying the peripheral transcriptional signature may be useful in understanding the chronic evolution of HEV infection in kidney transplant recipients.

**MATERIALS AND METHODS**

**Patients**

The registry number of the clinical trial is NCT01090232. The study protocol was approved by the Comité de Protection des Personnes Sud Méditerranée I, the ethical committee of the Assistance Publique-Hôpitaux de Marseille. Patients signed an informed consent form to participate in the study.

Sixteen kidney transplant recipients monitored at the Marseille University Hospital were enrolled in the study from May to November 2010. Eight control patients and 8 patients with CHE (CHE patients) were matched for sex, age, time since kidney transplantation, and immunosuppressive treatment (presence of a calcineurin inhibitor). CHE was defined by the persistence of HEV RNA in the blood for >6 months. The date of HEV infection was considered to be the first date on which elevated liver enzyme levels were observed. Consumption of uncooked pig liver sausage, which has been identified as a risk factor for HEV transmission to humans [19], was reported in 5 patients. The mode of HEV transmission for the 3 remaining patients was not known. At inclusion, no CHE patient had received antiviral treatment. Control patients were free of acute or chronic viral infection at the time of inclusion. Serological determination of hepatitis B virus surface antigen, human immunodeficiency virus, and hepatitis C virus (HCV) was negative for all patients. Their clinical characteristics are presented in Table 1. Briefly, the median age of patients was 55 years (range, 44–77 years). The median time since kidney transplantation at inclusion was 87 months (range, 43–182 months). The median time since HEV infection was 44 months (range, 12–75 months) in CHE patients. Results of blood liver tests were normal for all of the control patients. The numbers of peripheral leukocytes were similar in CHE and control patients.

The virological status of CHE patients was determined as follows. Serial dilutions of a plasmid containing a specific HEV region were used for HEV RNA quantification. Serum HEV RNA was detected using an in-house qRT-PCR assay and sequencing of the partial open reading frame 2 of the viral genome, as described previously [19]. Phylogenetic analysis revealed that all of the viral strains belonged to genotype 3. Four patients were infected with subtype 3f, 3 were infected with subtype 3c and 1 was infected with subtype 3e.

**Microarray Analysis**

Whole blood (2.5 mL) was collected from patients by use of PAXgene blood RNA tubes (Qiagen), and samples were stored at –80°C before RNA extraction. Microarrays were performed as described [20]. In brief, the RNA was extracted using the RNeasy Mini Kit (Qiagen) after a DNase I step to eliminate DNA contaminants. The quantity and the quality of RNA were assessed using a Nanodrop (Thermo Science) and a 2100 Bioanalyzer (Agilent Technologies), respectively. The microarray study was performed using Agilent 4X44 K Whole Human Genome microarrays, which include 45 000 probes. The RNA was labeled with cyanine-3 CTP by using an Agilent Low RNA Input Fluorescent Amplification kit. The samples were deposited on microarray slides, and the hybridization was performed using the QIamp labeling kit. The slides were scanned with a G2505B DNA microarray scanner (Agilent Technologies) at a resolution of 5 μm. The images were analyzed with Feature Extraction Software 10.5.1.

The microarray data analysis was performed, and the figures were created using R (v.2.13) and the Bioconductor software suite. The raw data were filtered and normalized using the Agi4 44PreProcess library. Unsupervised and supervised analyses were carried out using hierarchical clustering, principal component analysis (made4 library) [21] and...
the significance analysis of microarray (SAM) algorithm (siggenes library) [22]. Genes were considered to be differentially expressed when the false-discovery rate [23] was <10%, and the absolute fold-change (FC) was >2.0. Functional enrichment analysis was performed on selected genes with the DAVID bioinformatics tool [24], using the Gene Ontology (GO) [25], INTERPRO [26], and KEGG pathways [27, 28]. Key words were selected when the corrected P value (Benjamini-Hochberg) for enrichment was <.01. The data were generated in compliance with the Minimum Information About a Microarray Experiment (MIAME) guidelines and were deposited in the National Center for Biotechnology Information’s Gene Expression Omnibus (accession number GSE36539).

qRT-PCR
qRT-PCR was carried out as described elsewhere [20]. Reverse transcription of 100 ng of RNA was performed with the MMLV-RT kit (Invitrogen). Complementary DNA was obtained using oligo(dT) primers and MMLV reverse transcriptase (Invitrogen), and qPCR experiments were performed using SYBR Green Fast Master Mix (Roche Diagnostics) and a SmartCycler (Cepheid). The primers were designed using Primer3 [29], and their sequences are provided in Supplementary Table 1. The results were normalized using the housekeeping gene β-actin and are expressed as 

\[ FC = 2^{-\Delta\Delta Ct} \]

where 

\[ \Delta\Delta Ct = (Ct_{\text{Target}} - Ct_{\text{Actin}})_{\text{CHE}} - (Ct_{\text{Target}} - Ct_{\text{Actin}})_{\text{control}} \],

as described elsewhere [30].

Statistical Analyses
Qualitative parameters were expressed as the median and min-max range. Quantitative parameters were expressed as absolute counts and percentages. Differences between groups were assessed for statistical significance with the nonparametric Mann–Whitney U test, with a P value cutoff of .05. The experimental data were presented as medians and interquartile ranges.

### Table 1. Characteristics of Patients With Chronic Hepatitis E Virus Infection (CHE) and Control Patients at Inclusion

<table>
<thead>
<tr>
<th>Patient, by Group</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Kidney Transplantation</th>
<th>Time Since Event (mo)</th>
<th>HEV Load (log₁₀ copies/mL)ᵃ</th>
<th>ALT Level (IU/L)ᵇ</th>
<th>AST Level (IU/L)ᶜ</th>
<th>GGT Level (IU/L)ᵈ</th>
<th>TB Level (µmol/L)ᵉ</th>
<th>Immunosuppressive Treatment†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHE A</td>
<td>60</td>
<td>M</td>
<td>152</td>
<td>75</td>
<td>6.7ᵃ</td>
<td>80</td>
<td>65</td>
<td>119</td>
<td>17</td>
<td>Tac, Pr</td>
</tr>
<tr>
<td>CHE B</td>
<td>52</td>
<td>F</td>
<td>43</td>
<td>30</td>
<td>7.6</td>
<td>39</td>
<td>25</td>
<td>18</td>
<td>NA</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>CHE C</td>
<td>46</td>
<td>M</td>
<td>72</td>
<td>45</td>
<td>7.9ᵇ</td>
<td>9</td>
<td>21</td>
<td>50</td>
<td>13</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>CHE D</td>
<td>56</td>
<td>M</td>
<td>62</td>
<td>47</td>
<td>7.2</td>
<td>16</td>
<td>16</td>
<td>28</td>
<td>11</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>CHE E</td>
<td>68</td>
<td>M</td>
<td>182</td>
<td>48</td>
<td>6.0</td>
<td>72</td>
<td>56</td>
<td>35</td>
<td>13</td>
<td>CsA, Pr</td>
</tr>
<tr>
<td>CHE F</td>
<td>47</td>
<td>M</td>
<td>75</td>
<td>13</td>
<td>NA</td>
<td>43</td>
<td>36</td>
<td>60</td>
<td>NA</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>CHE G</td>
<td>47</td>
<td>M</td>
<td>85</td>
<td>21</td>
<td>3.8</td>
<td>24</td>
<td>28</td>
<td>19</td>
<td>24</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>CHE H</td>
<td>76</td>
<td>M</td>
<td>160</td>
<td>43</td>
<td>5.9</td>
<td>169</td>
<td>114</td>
<td>NA</td>
<td>NA</td>
<td>Tac, Pr</td>
</tr>
<tr>
<td>Control A</td>
<td>60</td>
<td>M</td>
<td>167</td>
<td>...</td>
<td>...</td>
<td>11</td>
<td>16</td>
<td>NA</td>
<td>NA</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>Control B</td>
<td>60</td>
<td>F</td>
<td>43</td>
<td>...</td>
<td>22</td>
<td>27</td>
<td>39</td>
<td>NA</td>
<td>NA</td>
<td>Tac, MPA, Pr</td>
</tr>
<tr>
<td>Control C</td>
<td>47</td>
<td>M</td>
<td>71</td>
<td>...</td>
<td>28</td>
<td>20</td>
<td>37</td>
<td>NA</td>
<td>CsA, MPA, Pr</td>
<td></td>
</tr>
<tr>
<td>Control D</td>
<td>58</td>
<td>M</td>
<td>62</td>
<td>...</td>
<td>19</td>
<td>26</td>
<td>26</td>
<td>20</td>
<td>CsA, Aza, Pr</td>
<td></td>
</tr>
<tr>
<td>Control E</td>
<td>44</td>
<td>M</td>
<td>94</td>
<td>...</td>
<td>44</td>
<td>25</td>
<td>NA</td>
<td>NA</td>
<td>Tac, Pr</td>
<td></td>
</tr>
<tr>
<td>Control F</td>
<td>46</td>
<td>M</td>
<td>88</td>
<td>...</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>CsA, Aza, Pr</td>
<td></td>
</tr>
<tr>
<td>Control G</td>
<td>68</td>
<td>M</td>
<td>176</td>
<td>...</td>
<td>21</td>
<td>23</td>
<td>53</td>
<td>NA</td>
<td>CsA</td>
<td></td>
</tr>
<tr>
<td>Control H</td>
<td>74</td>
<td>M</td>
<td>133</td>
<td>...</td>
<td>18</td>
<td>13</td>
<td>33</td>
<td>NA</td>
<td>CsA, Pr</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Aza: azathioprine, CsA, cyclosporin; GGT, gamma-glutamyltranspeptidase; MPA, mycophenolic acid; NA, not available; Tac, tacrolimus; TB, total bilirubin; Pr, prednisone.

ᵃ At inclusion, except for CHE patients A and C.
b Reference, 3–41 IU/L.
c Reference, 0–38 IU/L.
d Reference, 8–60 IU/L.
ᵉ Reference, <26 µmol/L.
ᶠ Received at the time of HEV diagnosis.
ᵍ Measured 32 days before inclusion.
h Measured 10 days before inclusion.
RESULTS

Transcriptional Signature of CHE Patients

A whole-genome microarray approach was used to define the peripheral transcriptional signature of CHE. The principal component analysis showed that the overall gene expression of CHE patients and control patients was organized in 2 different groups (Figure 1A). Supervised analysis using the SAM algorithm identified 41 probes, corresponding to 30 genes that were upregulated in CHE patients, compared with control patients (Figure 1B). In contrast, no genes were downregulated. The ratio of gene expression ranged from 2.5- to 15.2-fold between CHE patients and matched control patients. Of note, 1 CHE patient (DP) showed an atypical pattern of gene expression, regardless of the gene studied.

When we performed functional enrichment analysis of the 30 genes that were upregulated in CHE patients, we found that 20 genes were related to the innate immune response. When we compared the CHE transcriptional signature with the recently established Interferome database, which contains 1996 ISGs [31], we found that 25 of the 30 genes that were upregulated in CHE patients (83% of the total number of modulated genes) were annotated ISGs. The DAVID tool isolated 2 particular groups of genes associated with the interferon pathway. The first group was composed of 4 genes that are known to encode interferon-induced proteins with tetratricopeptide repeats (IFIT1, IFIT2, IFIT3, and IFIT5). The second group was composed of 3 genes that share 2′-5′-oligoadenylate synthetase-related domains (OAS2, OAS3, and OASL). The other upregulated genes of the interferon pathway included the genes that encode CXCL10, a ubiquitin-like modifier (ISG15), HERC5, interferon-induced protein 44-like (IFI44L), MX1, radical S-adenosyl methionine domain containing 2 (RSAD2), epithelial stromal interaction 1 (EPSTI1), and XAF1. The expression levels of 5 genes involved in the type I interferon response (ISG15, IFIT1, IFIT44L, RSAD2, and EPSTI1) that were upregulated in the microarray were also determined by qRT-PCR. The microarray and qRT-PCR results were strongly correlated ($R^2 = 0.91$, $P = .0008$). We found that expression of the ISG15, IFIT1, IFIT44L, RSAD2, and EPSTI1 was upregulated in 7 CHE patients as compared to 7 matched control patients (Figure 2). Of note, both techniques indicated a higher interindividual variability of expression in CHE patients, compared with controls. Taken together, these results showed that the CHE patients exhibited a specific transcriptional program in which interferon-stimulated effectors were prominent.

CHE Signature and HEV Persistence

One can hypothesize that the interindividual variability between CHE patients that was found in the microarray and

---

Figure 1. Chronic hepatitis E virus infection (CHE)–associated signature of gene expression. A, The graphical representation of principal component analysis shows a clear separation between CHE patients and matched controls. B, The gene expression signature associated with CHE is represented on a heat map. The CHE patients and control patients are represented in columns. The expression levels of the genes are color coded from blue to red. Dendrograms on the top (samples) and the left (genes) show the results of the hierarchical clustering that was computed with Pearson correlation distance and average linkage.
qRT-PCR may be related to the course of HEV infection. To address this issue, we sorted the CHE patients into 3 groups: the first one included patients who experienced early HEV clearance within 6 months after inclusion in the study (median time, 4 months; range, 1.3–4.6 months), the second group included patients who experienced HEV clearance with a delay of >6 months after inclusion (median time, 11.5 months; range, 8.9–17.4 months), and the last group included patients whose HEV infection had not resolved at the time of data analysis (>17.4 months after inclusion). The patterns of gene expression observed by microarray differed considerably between the 3 groups of CHE patients. The expression of ISG15, IFIT1, IFI44L, RSAD2, and EPSTI1 was significantly (P ≤ .01) higher in patients who had not cleared the HEV infection than in patients who cleared the HEV infection early on (Figure 3). The ratios of gene expression ranged from 6.6-fold, for EPSTI1, to 25.6-fold, for RSAD2. The expression of ISG15, IFIT1, IFI44L, RSAD2, and EPSTI1 was intermediate in patients who cleared HEV infection in its late stages. Similarly, the determination of gene expression levels by qRT-PCR showed that these genes were more highly expressed in patients who did not clear the HEV infection than in patients who had cleared the HEV infection in its early phases.

Finally, we attempted to analyze the interindividual variability between CHE patients and the viral load. The HEV RNA level was measured in 7 CHE patients. The HEV load was the lowest in patients who experienced early clearance of HEV infection, intermediate in patients who cleared HEV infection in its late stages, and highest in patients who had not cleared HEV infection (Figure 3). Taken together, these results suggest that upregulated expression of EPSTI1, ISG15, IFIT1, IFI44L, and RSAD2 is associated with persistent HEV infection.

**DISCUSSION**

CHE has been increasingly described in kidney transplant recipients since 2008 [4, 5], but the underlying mechanisms of this new disease remain obscure. In this study, we compared the transcriptional profiles of whole blood from kidney transplant recipients with CHE and matched kidney transplant recipients without HEV infection. We found that CHE patients were characterized by a specific transcriptional signature consisting of 30 upregulated genes. Of note, 1 CHE patient showed an atypical gene expression pattern, compared with the other patients, but we were unable to explain this apparent discrepancy on the basis of clinical and biological data. Our results were close to those obtained in a microarray study conducted on liver biopsy specimens from chimpanzees. In that study, Yu et al found that 58 genes were upregulated and no genes were downmodulated in chimpanzees with acute HEV infection [18]. Interestingly, 20 genes that were upregulated in our study were also upregulated in chimpanzees, suggesting that the peripheral signature that we found in humans characterizes HEV infection.

The specific signature of CHE included 25 genes annotated as ISGs, which are known to be involved in antiviral activity. In our study, we found the genes encoding CXCL10 (also known as IP-10) and ISG15 to be upregulated. CXCL10 is a...
A chemokine that attracts T lymphocytes, natural killer cells, and monocytes [32]. CXCL10 has been reported to be essential in the development of a protective response against viral infection [33]. ISG15, a ubiquitin homolog, has numerous target proteins that are important in the interferon response. These targets include transcription factors, such as Janus kinase 1 and signal transducer and activator of transcription 1 (STAT1); pattern-recognition receptors, such as retinoic acid-inducible gene I; and antiviral effector proteins, such as MX1, PKR, and RNaseL [34]. It has been demonstrated that increased levels of ISG15 lead to an increase in the antiviral activity of interferon [35], suggesting that the upregulated expression of ISG15 in CHE patients is related to high antiviral activity. Other ISGs, such as OAS2, OAS3, OASL, and MX1, which were found to be upregulated in CHE patients, encode proteins known to be antiviral effectors [34]. In addition to these genes, other ISGs were upregulated. These included the genes that encode HERC5, a ligase that conjugates ISG15 to protein substrates; RSAD2 (viperin), one of the most highly induced interferon effector proteins; the IFIT complex (IFIT1, IFIT2, IFIT3, and IFIT5 genes), which antagonizes viruses by sequestering specific viral nucleic acids [36]; and IFI44L, a paralog of IFI44, a 44-kD protein originally identified as the HCV-associated microtubular aggregate in the hepatocytes of chimpanzees infected with HCV [37]. Of note, it has been recently demonstrated that IFI44L has high anti-HCV antiviral activity [38]. Other genes that were upregulated in CHE patients encode proteins such as XAF1 and BATF2 (SARI), which have been reported to mediate apoptosis [39, 40] and may constitute a line of defense of immune cells against viruses [41]. To our knowledge, no antiviral function has been described for EPSTI1, another ISG expressed in tissues characterized by extensive epithelial-stromal interaction [42, 43]. Our results were consistent with the activation of the interferon system reported after acute HEV infection in chimpanzees [18]. In contrast, during persistent HEV infection of an epithelial cell line, it has been found that HEV inhibits type I interferon signaling through STAT1 phosphorylation. Interestingly, the expression of MX1 and OAS genes is similar in infected and noninfected cells [44], whereas these genes were upregulated in CHE patients. It is likely that this in vitro model of HEV infection is not convenient for studying patients with CHE. We can hypothesize that the upregulated expression of ISGs in CHE patients is related to activation of the interferon pathway, as previously described for human viral infections, such as HCV infection [45–47].
We found that the ISG response of patients who did not clear their HEV infection was largely higher than the ISG response of patients who cleared HEV infection quickly. These results showed that kidney transplant recipients did not spontaneously resolve CHE despite activation of the interferon system. It is likely that the increased expression of ISGs in CHE patients favors viral persistence through induction of a refractory state of the interferon signaling pathway. This hypothesis is supported by earlier observations demonstrating that strong induction of ISGs in the liver does not systematically lead to the resolution of chronic HCV infection; in fact, this induction impedes the response to interferon therapy [45–47]. It has been suggested that patients with chronic HCV infection exhibit defects in the downstream steps of ISG transcription, rendering them resistant to both endogenous interferon and interferon therapy [45]. In CHE, we presume that the absence of the few critical ISGs directly involved in antiviral activity prevents the elimination of HEV or that posttranscriptional modifications of critical ISG proteins alter their antiviral activity. Ribavirin, which was found effective to treat CHE in few kidney transplant recipients, has been reported to enhance the antiviral effects of interferon in chronic HCV infection through ISGs [48]. Nonetheless, it is difficult to speculate on the antiviral mechanism of ribavirin in HEV infection. Indeed, HEV clearance is rapidly achieved after ribavirin monotherapy in kidney transplant recipients [12] whereas ribavirin monotherapy is not efficient against HCV [48].

Finally, we found that the HEV loads were higher in patients who did not clear their HEV infection than in patients who cleared HEV infection, suggesting that timing of HEV clearance was associated with HEV loads. As the level of expression of selected ISGs was associated to the timing of HEV clearance, ISG expression may be a marker of ongoing viremia or an independent prognostic marker of HEV clearance. The absence of sequential assessment of ISGs did not allow the discrimination between these hypotheses. Further studies are needed to elucidate the role of ISGs in CHE.

In conclusion, the blood of kidney transplant recipients with CHE exhibited a specific transcriptional signature associated with a dysregulated interferon response that is correlated with a persistent HEV infection. Our results opened new ways to explore the mechanisms of chronic HEV infection in kidney transplant recipients and to improve the monitoring of these patients.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We are very grateful to Dr Christian Capo for advice and suggestions in the writing of the manuscript.

Financial support. This work was supported by funds from the Assistance Publique-Hôpitaux de Marseille, Marseille, France (contract 2009-A00945-52). V. M. was supported by the Assistance Publique-Hôpitaux de Marseille.

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


