Longitudinal Analysis of Hepatitis C Virus Replication and Liver Fibrosis Progression in Renal Transplant Recipients

Jacques Izopet,1 Lionel Rostaing,2 Karine Sandres,1 Jean-Marc Cisterne,2 Christophe Pasquier,1 Jean-Louis Rumeau,3 Michel Duffaut,4 Dominique Durand,2 and Jacqueline Puel1

1Laboratoire de Virologie, Hôpital Purpan, Centre Hospitalier Universitaire (CHU) Toulouse; 2Unité de Transplantation d’Organes, Service de Néphrologie, and 3Laboratoire d’Anatomie et Cytologie Pathologiques, CHU Rangueil; 4Service de Médecine Interne, CHU Purpan, Toulouse, France

The pathogenesis of hepatitis C virus (HCV) infection was investigated by analysis of changes in viral and histologic parameters in 36 renal transplant recipients who were infected with HCV before transplantation. Each patient was classified according to development of liver fibrosis as assessed by 2 liver biopsies done 45 and 81 months after transplantation: 13 had progressing liver fibrosis (fibrosers) and 23 did not (nonfibrosers). All developed high-titer posttransplant viremia with a significant increase of 1.2 log RNA copies/mL. There were no significant differences in the increases in serum HCV RNA or genotype distributions in fibrosers and nonfibrosers. The hypervariable region (HVR)–1 of the HCV genome was analyzed by cloning and sequencing 20 clones per sample from 5 fibrosers and 5 nonfibrosers. Comparison of samples revealed that liver fibrosis progression was significantly associated with slower HVR-1 quasispecies diversification, suggesting the selection of more aggressive variants in fibrosers.

Hepatitis C virus (HCV) infection is the major cause of chronic liver disease following renal transplantation [1, 2], but it is not clear whether chronic HCV infection is detrimental to patient survival [3, 4]. HCV-positive patients have a better chance of survival if they are given a kidney transplant than do matched HCV-positive patients who remain on chronic dialysis [5], even though chronic HCV infection predisposes patients to posttransplant infections such as sepsis [6, 7]. Legendre et al. [8] reported that long-term survival of HCV-positive renal transplant patients was affected and that increased mortality was primarily caused by liver disease and sepsis.

HCV-related liver damage is driven by host cellular immune responses, including those mediated by HCV-specific T lymphocytes in immunocompetent patients [9–11]. The cellular basis of hepatic fibrosis, with excessive deposition of extracellular matrix, involves the interplay of many factors, such as T helper-derived cytokines [12] and transforming growth factor–β1 [13]. Anticalcineurin agents, such as cyclosporin A, have been used in allograft transplantation since 1982 to interfere with the host immune response. These agents may not be as detrimental for liver histology as anticipated. However, the use of antilymphocyte agents immediately after renal transplantation is associated with a significant increase in viremia in HCV-positive patients [14]. To the best of our knowledge, no study has yet evaluated the long-term effect of this sometimes dramatic increase on liver parameters. The HCV genome is highly variable due to the error-prone nature of the viral RNA-directed RNA polymerase and is most accurately described in infected persons as a population of closely related viral variants [15, 16]. HCV quasispecies all have extensive mutations in the hypervariable region (HVR)–1 of the second envelope glycoprotein gene (E2), which are believed to be associated with viral persistence by ensuring immune escape [17–20]. The evolution of HCV quasispecies after renal transplantation and the effect of their development on liver fibrosis progression are unknown.

This study evaluated long-term posttransplant HCV viremia in renal transplant patients and compared it with pretransplant findings, correlated the changes in HCV viremia and HCV genotype with liver histology, and compared the development of HVR-1 quasispecies in patients with and without liver fibrosis progression.

Patients and Methods

Patients

Several years ago we set up a clinical program to evaluate the long-term posttransplant effect of a preexisting chronic HCV infection. Study subjects underwent liver biopsies (LBs) every 3–4 years after transplantation or earlier if clinically indicated. We excluded patients infected with both HCV and hepatitis B virus or
human immunodeficiency virus, those with autoimmune disease, and those with hemochromatosis. This study was done on 36 anti-HCV–positive HCV RNA–positive patients (21 men and 15 women; mean [SD] age at transplantation, 42 ± 10 years). Thirty-four patients received cadaveric allografts and 2 received organs from living related donors. All had induction therapy based on antilymphocyte globulins (Lymphoglobulins; Pasteur Mérieux, Lyon, France) or antithymocyte globulins (Thymoglobulins; Pasteur Mérieux, Lyon, France) or antithymocyte globulins (Thymoglobulins; Pasteur Mérieux, Lyon, France). Chronic immunosuppression was treated with cyclosporin A, azathioprine, and prednisone.

The duration of HCV infection was determined from archival sera. Since 1972, it has been the policy at our center to obtain ≥1 serum sample per year from each dialysis and renal transplant patient. The samples are stored frozen at −20°C. The anti-HCV status before renal transplantation of patients in this study was assessed retrospectively on stored sera with a 3d-generation ELISA III (Ortho Diagnostics Systems, Roissy, France) and a 3d-generation recombinant assay (RIBA III; Ortho Diagnostics Systems). The mean (SD) duration of HCV infection at the first LB was 130 ± 50 months.

Each patient underwent exhaustive clinical and laboratory examination at the time of transplantation and at each LB. This included clinical examination, assessment of liver enzyme activities (alanine and aspartate aminotransferases [ALT, AST, respectively], γ-glutamyl transpeptidase [γ-GT], and alkaline phosphatase [AP] levels), and renal function tests (serum creatinine and proteinuria levels). Serum samples were kept frozen at −80°C for virologic testing. The daily dose of each immunosuppressive drug given during the posttransplant period was recorded. No patients were alcoholics: All stated they did not drink alcohol, one of us (L.R.) informed them that alcohol consumption might accelerate HCV-related liver disease, and all patients but one (i.e., 1 with cirrhosis) had AST values lower than ALT values.

Histologic Evaluation

All LBs were examined by the same pathologist (J.L.R.), who was unaware of their chronologic order. The LBs were scored according to the total Knodell score [21] with independent assessment of the grade (activity scores) and the stage (Knodell fibrosis score).

Serum HCV RNA Concentration

The HCV RNA concentration in pretreatment sera was measured by the standardized quantitative reverse-transcription polymerase chain reaction (RT-PCR) assay (AmpliC HCV Monitor; Roche Molecular Systems, Branchburg NJ) according to the manufacturer’s instructions.

HCV Genotype

HCV genotype was determined by the Inno-LiPA II HCV method (Innogenetics, Ghent, Belgium). The products of RT-PCR amplification were hybridized to immobilized probes specific for the different genotypes and subtypes.

HCV Variability

PCR amplification. After RNA extraction by the guanidinium thiocyanate–phenol-chloroform method and RT with the outer antisense primer and murine Moloney leukemia virus reverse transcriptase, nested PCR was performed using standard conditions, that is, 5 min denaturation at 95°C followed by 35 cycles (95°C for 30 s, 55°C–60°C for 30 s, and 72°C for 90 s) and a final extension at 72°C for 10 min. Outer primers were sense primer 5′-CA-GACTGCAATGGCTCAATCAT-3′ (position 1245–1267 of HCV-J) and antisense primer 5′-TTGCAGTTAAGGCAGTCC-3′ (position 1612–1630). Inner primers were sense primer 5′-CACTGGGGAGTCTGGCGGG-3′ (position 1395–1414) and antisense primer 5′-ATGTGCCAGCTGCACATTGT-3′ (position 1587–1606).

Plasmid cloning. PCR products were purified with QIAamp columns (Qiagen, Courtaboeuf, France) as specified by the manufacturer. Purified products were quantified by spectrophotometry: 10 ng of products was directly ligated into 50 ng of PCR II vector (original TA cloning kit; Invitrogen, Leek, The Netherlands) at 14°C overnight. Recombinant plasmids were used to transform Escherichia coli–competent cells according to the manufacturer’s protocol, and transformants were grown on ampicillin plates.

Nucleotide sequencing. Twenty cDNA clones from PCR products were selected. Plasmid DNAs containing HVR-1 inserts were prepared and sequenced on both strands by the dyeoxy chain termination method (ABI PRISM Ready Reaction AmpliTag Fs; dye deoxy primers; Applied Biosystems, Paris) on an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA). Electrophoresis data were analyzed by the Sequence Navigator program (Perkin-Elmer, Foster City, CA).

Computed calculation of genetic diversity and types of mutational changes. Nucleotide sequences were aligned with the CLUSTAL W program (version 1.7; see [22]). We quantified diversity as the average genetic distance calculated for all pairs of nucleotide sequences by using the DNADIST module in the PHYLIP package, version 3.572 (J. Felsenstein, Department of Genetics, University of Washington, Seattle, 1995). The calculation was based on a Kimura 2-parameter distance matrix with a transition-to-transversion ratio of 2.0. The numbers of synonymous and nonsynonymous substitutions per synonymous and nonsynonymous site, respectively, were calculated with the Jukes-Cantor correction for multiple substitutions by use of the MEGA program [23, 24].

Statistical Analysis

The serum HCV RNA concentrations were converted to log (base 10) values before analysis. All HCV RNA concentrations are expressed as the log of the number of copies per milliliter. Proportions were compared by use of the χ² test or Fisher’s exact test for expected values <5. Quantitative variables were compared with Student’s t test. P < .05 was considered significant.

Nucleotide Sequence Accession Numbers

The nucleotide sequences presented have been submitted to GenBank (accession nos. AF207080–AF207519).
Results

**Patient characteristics and evolution of liver parameters.** The types of immunosuppression given to the patients at the first (LB1) and second (LB2) biopsies are indicated in table 1. The first was taken within the 4th posttransplant year (45 ± 34 months) and the second 36 months later. There were no significant changes in ALT and AST activities after transplantation (ALT, 60 ± 44 IU/mL; AST, 43 ± 22 IU/mL at LB1; ALT, 64 ± 51 IU/mL; AST, 42 ± 29 IU/mL at LB2) from the pretransplantation values (ALT, 56 ± 60 IU/mL; AST, 37 ± 29 IU/mL) despite chronic immunosuppression. The activities of γ-GT and AP were also not significantly changed between LB1 and LB2. The total Knodell scores were low at LB1 (4.8 ± 0.5) and LB2 (4.4 ± 0.5). There was also no significant difference between fibrosis scores at LB1 (0.9 ± 0.2) and LB2 (1.2 ± 0.2). Nevertheless, 2 groups of patients were identified from liver fibrosis progression (fibrosers), estimated as the difference between the 2 consecutive LBs: there were 23 patients without liver fibrosis progression (nonfibrosers) whose liver fibrosis progression was <1 Knodell fibrosis unit and 13 fibrosers whose liver fibrosis progression was ≥1 Knodell fibrosis unit (1 U, 8 patients; 2 U, 3 patients; and 3 U, 2 patients).

**Effect of clinicobiologic characteristics, HCV genotype, and serum HCV RNA concentration on liver histology.** The sex, age, duration of HCV infection, degree of immunosuppression after transplantation, and aminotransferase activities at the time of transplantation of the 13 fibrosers and 23 nonfibrosers were similar (table 2). The distributions of HCV genotype in the fibrosers (9 [69%] of 13, genotype 1b) and nonfibrosers (11 [48%] of 23, genotype 1b) were also similar. The mean (SD) of HCV RNA concentration at the time of transplantation was 4.5 ± 1 log copies/mL. There was a significant increase after transplantation to 5.7 ± 0.5 log copies/mL at the time of LB1 (about year 4; P < .001). The virus load was stable thereafter; the figures for LB2 (~7 years after transplant) were very similar at 5.7 ± 0.6 log copies/mL. The significant increase in serum HCV RNA concentrations after transplantation had no detrimental effect on liver histology within a follow-up period of up to 81 ± 36 months after transplantation. The increases in serum HCV RNA after transplantation in the 13 fibrosers and 23 nonfibrosers were not significantly different (table 2). There was also no significant difference in pretransplant serum HCV RNA concentration between the fibrosers and nonfibrosers (table 2). Half the patients who had a pretransplant serum HCV RNA concentration ≥4.5 log copies/mL had liver fibrosis progression versus 22% for those with serum HCV RNA concentration <4.5 log copies/mL, but the difference was not significant.

**Relationship between liver fibrosis progression and HCV quasispecies development.** We analyzed quasispecies by cloning and sequencing the HVR-1 region at 2 times (LB1 and LB2) in the 5 patients whose differences between fibrosis scores on the 2 consecutive LBs were the greatest (2–3 U) to assess the influence of mutations in the HVR-1 region of the HCV genome on disease severity after transplantation. These 5 fibrosers were infected with genotype 1b. Five nonfibrosers randomly selected from the HCV-1b-infected patients whose liver fibrosis progression was <1 U served as controls. We generated 20 HVR-1 clones per HCV RNA-positive sample time and sequenced and analyzed 400 HVR-1 clones from these 10 patients. Amino acid sequences were deduced from the nucleotide sequences.

Table 3 shows the heterogeneity parameters calculated for each of the 10 patients at LB1 and LB2. The mean (±SE) pairwise within-sample genetic distances in the 10 patients were low and were similar in fibrosers (0.025 ± 0.009 at LB1, 0.017 ± 0.008 at LB2) and nonfibrosers (0.017 ± 0.007 at LB1, 0.010 ± 0.003 at LB2). The numbers of nonsynonymous substitutions per nonsynonymous site and synonymous substitutions per synonymous site were not significantly different in most patients, suggesting that both selection pressures and the accumulation of random mutations due to rapid replication contributed to the heterogeneity of the HVR-1 quasispecies. The proportions of nonsynonymous mutations per nonsynonymous site (Ka) in fibrosers (0.029 ± 0.010 at LB1; 0.020 ± 0.011 at LB2) and nonfibrosers (0.022 ± 0.08 at LB1; 0.007 ± 0.003 at LB2) were similar. The proportions of synonymous mutations per synonymous site (Ks) were also similar in fibrosers (0.020 ± 0.004 at LB1; 0.016 ± 0.005 at LB2) and nonfibrosers (0.027 ± 0.011 at LB1; 0.021 ± 0.008 at LB2). Within-sample analysis therefore indicated a largely homogeneous virus population at each time, with no significant differences in heterogeneity between fibrosers and nonfibrosers.

Pairwise comparisons of the sequences obtained at the 2 LBs (table 3) showed that the average between-samples genetic distances were significantly greater in the 5 fibrosers (0.115 ± 0.011) than in the 5 fibrosers (0.045 ± 0.006; P < .01). This suggests that nonfibrosers had more genetic changes. The proportion of nonsynonymous mutations per nonsynonymous site was also significantly greater in the 5 nonfibrosers compared to the 5 fibrosers, indicating selection of a quasispecies with different properties during development.
Table 2. Clinical, laboratory, and virologic features of 36 hepatitis C virus (HCV)-infected renal transplant recipients according to the progression of liver fibrosis on 2 consecutive liver biopsies (LBs).

<table>
<thead>
<tr>
<th>Clinical, laboratory, or virologic feature</th>
<th>Fibrosers (n = 13)</th>
<th>Nonfibrosers (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men/women)</td>
<td>8/5</td>
<td>13/10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age at infection (years)</td>
<td>36 ± 10</td>
<td>34 ± 10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Duration of HCV infection at LB1 (months)</td>
<td>139 ± 49</td>
<td>125 ± 63</td>
<td>&gt;0.05</td>
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<tr>
<td>Immunosuppression at LB1 (mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>2.9 ± 0.7</td>
<td>3.2 ± 0.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Prednisone</td>
<td>0.1 ± 0.03</td>
<td>0.1 ± 0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Alanine aminotransferase at RT (IU/L)</td>
<td>69 ± 83</td>
<td>48 ± 40</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Aspartate aminotransferase at RT (IU/L)</td>
<td>42 ± 31</td>
<td>35 ± 28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a/1b</td>
<td>0/9</td>
<td>5/11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>2/3/4</td>
<td>2/1/1</td>
<td>6/1/0</td>
<td></td>
</tr>
<tr>
<td>Serum HCV RNA concentration (log copies/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA at RT</td>
<td>4.9 ± 0.9</td>
<td>4.3 ± 1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ΔRNA at LB1</td>
<td>+1 ± 0.9</td>
<td>+1.3 ± 1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ΔRNA at LB2</td>
<td>+0.9 ± 0.8</td>
<td>+1.4 ± 1.2</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

NOTE. RT, renal transplantation.

Table 3. Characteristics of hypervariable region 1 quasispecies in 10 hepatitis C virus (HCV)-1b-infected renal transplant recipients with or without progressing liver fibrosis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at infection, years</th>
<th>Fibrosis Knodell score</th>
<th>Intersample variability, fibrrosers vs. nonfibrosers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LB1</td>
<td>LB2</td>
</tr>
<tr>
<td>Fibrosers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>28</td>
<td>1 3</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>32</td>
<td>0 3</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>18</td>
<td>1 3</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>40</td>
<td>1 4</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>33</td>
<td>1 3</td>
</tr>
<tr>
<td>Nonfibrosers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>47</td>
<td>0 0</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>24</td>
<td>0 0</td>
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<tr>
<td>8</td>
<td>F</td>
<td>35</td>
<td>0 0</td>
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<tr>
<td>9</td>
<td>M</td>
<td>39</td>
<td>1 1</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>46</td>
<td>0 0</td>
</tr>
</tbody>
</table>

NOTE. LB, liver biopsy; M, male; F, female; gd, mean pairwise genetic distance; Ka or rKa, average no. of nonsynonymous mutations per nonsynonymous site; Ks or rKs, average no. of synonymous mutations per synonymous site.

* Fibrosers vs. nonfibrosers, P < 0.01.
Figure 1. Consensus amino acid sequences of hypervariable region (HVR-1) in samples obtained during 2 biopsies 36 months apart from 10 hepatitis C virus-1b-infected renal transplant recipients, 5 with progressing liver fibrosis (fibrosers) and 5 without (nonfibrosers). Consensus amino acid sequences of HVR-1 in samples obtained before renal transplants (RTs) are shown for 1 fibroser (patient 5) and 1 nonfibroser (patient 6). LB, liver biopsy.

plant recipients who had 2 consecutive LBs 36 months apart after transplantation. Although the HCV genotype and virus kinetics made no contribution to the natural history of the HCV infection in our patients, detailed analysis of the HVR-1 region of the HCV genome by molecular cloning and the sequencing of multiple clones indicated that the progression of liver fibrosis was associated with slower diversification of HVR-1 quasispecies.

The main features of this study are, first, the accurate determination of the duration of infection, which was assessed from archival sera, in contrast to the protocol in most studies, in which the duration of infection was estimated from a history of intravenous drug use, transfusion, or surgery; second, the lack of alcohol intake by our patients, as this is a known confounding variable that influences the natural history of liver fibrosis [25]; and third, the availability of paired LB taken 4 and 7 years after transplantation, which allowed us to classify patients as fibrosers or nonfibrosers by use of a standardized scoring system. In contrast to the findings for immunocompetent patients [26], male sex, age at infection >40 years, and the duration of HCV infection were not associated with the progression of liver fibrosis. The lack of association could be due to the long period that our patients were on maintenance hemodialysis (~10 years), which could be when chronic hepatitis C develops slowly. Another explanation could be the small sample size in our study compared with that with immunocompetent patients [26].

The long-term follow-up of our patients after transplantation showed a 1.2 log increase in HCV RNA concentration. This stable high-titer viremia was similar to that observed in the short term in renal or liver transplant patients [14, 27, 28]. This increase in HCV RNA after transplantation probably reflects the loss of immune control due to immunosuppressive drugs and the establishment of a new equilibrium between virion production and clearance. We found no correlation between serum HCV RNA increase and liver fibrosis progression. The lack of a link between serum HCV RNA concentrations and disease severity or disease progression is also the rule in immunocompetent patients. In contrast to the data of Charlton et al. [29] for liver transplant recipients, the pretransplant virus load did not predict outcome. This difference could be related to the lack of de novo infection of the liver in our patients.

The influence of viral genotype on the pathogenesis of liver disease is highly controversial. Several case-control studies [30–32] and 1 prospective cohort study [33] on immunocompetent patients found a relationship between genotype 1b and the development of hepatocellular carcinoma in cirrhotic patients. However, 2 other prospective cohort studies did not confirm those findings [34, 35]. Some reports indicate that liver transplantation patients infected with genotype 1b are more likely to develop acute hepatitis and chronic active hepatitis than those infected with other genotypes [36–39], but other investigators have detected no such relationship [40, 41]. The genotype distributions in fibrosing and nonfibrosing renal transplant patients did not differ, even though the liver fibrosis developed more rapidly in patients infected with the 1b subtype. Our results suggest that parameters of viral heterogeneity other than those specifying genotype are involved in the natural history of HCV infection, because genotype-specific differences should be more apparent in immunosuppressed patients, in whom HCV replication is higher than in immunocompetent patients.

We performed a longitudinal analysis of HVR-1, the most genetically diverse region of the HCV genome, to evaluate the possibility that distinct viral quasispecies play a role in the pathogenesis of progressive HCV infection. We examined 10 HCV-1b-infected patients, 5 of whom had developing liver fibrosis and 5 of whom had stable fibrosis score, as assessed by 2 consecutive LBs 3 years apart. Although fibrosers and nonfibrosers had similar within-sample variability at each time, reflecting homogeneous virus populations in both groups, the between-sample analysis revealed less genetic diversification of HVR-1 in fibrosers than in nonfibrosers. The smaller proportion of nonsynonymous mutations per nonsynonymous site in fibrosers than in nonfibrosers indicates different selection pressures in these patients and suggests the selection of particularly fit variants in fibrosers. Slower HCV quasispecies diversification
measured by a heteroduplex mobility assay in liver transplant recipients is also associated with the development of bridging fibrosis or severe bridging necrosis in the allograft within the first 18 months after transplantation [42]. The slow change in genetic diversity in patients with severe liver disease in that study concerned not only HVR-1 but also other subgenomic regions of HCV, such as E1 and nonstructural genes. These results may, therefore, suggest that more cytopathic variants are propagated in patients with developing liver fibrosis progression, as was suggested by isolated case reports of rapidly progressing hepatitis C infections in immunosuppressed patients [43-45]. The molecular interactions between viral and host proteins could account for both virus selection and alteration of the host signaling pathways resulting in fibrogenesis.

It was recently shown that HCV E2 protein can modulate the activity of the double-stranded RNA-dependent protein kinase, which is a mediator of apoptotic responses and a tumor suppressor, at least in part by phosphorylating eIF-2α and limiting mRNA translation [46]. In addition, there may be movement of quasispecies variants in fibrosers from hepatic to non-hepatic compartments, because HVR-1 is close to regions of E2 involved in the interaction with CD81, a putative cellular receptor of HCV [47, 48]. Our results may also reflect differences in the strength and quality of the residual immune response after immunosuppressive therapy, resulting in the selection of different virus quasispecies in fibrosers and nonfibrosers. Analysis of the hepatic concentrations of mRNA for key regulatory cytokines such as interleukin-4, -10, and -2, interferon-γ, tumor necrosis factor-α, and transforming growth factor-β will be useful for identifying a relationship between the cytokine profile and the development of liver fibrosis.

Our study has some limitations. First, the liver HCV quasispecies were not characterized. Second, the association between low virus diversification and severe liver disease could reflect an important mechanism in liver fibrosis progression; however, it could also simply be a phenomenon unrelated to the pathogenesis. Thus, this longitudinal study of renal transplant recipients who acquired HCV before transplantation demonstrates there is no correlation between the increase in serum HCV RNA after transplantation and liver fibrosis progression. The HCV genotype also has no influence on the progression of the disease, but analysis of HVR-1 quasispecies indicates that slower quasispecies diversification is associated with progression of liver fibrosis, suggesting there is selection of more aggressive HCV variants. Whether these variants are directly pathogenic or are more aggressive because of host-specific immune-mediated mechanisms requires further investigation.

Acknowledgments

We thank Martine Dubois and Michèle Cazabat for excellent technical assistance and Eliane Coutanceau for secretarial assistance.

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