Frequent Compartmentalization of Hepatitis C Virus with Leukocyte-Related Amino Acids in the Setting of Liver Transplantation

Frédéric Schramm,1,2,3 Eric Soulier,1,2 Cathy Royer,1,2 Thierry Weitten,1,2 Samira Fafi-Kremer,1,2 Nicolas Brignon,1 Nicolas Meyer,4 Bernard Ellero,5 Marie-Lorraine Woehl-Jaegle,5 Carole Meyer,5 Philippe Wolf,6 Michel Doffoël,6 Thomas F. Baumert,1,2,6 Françoise Stoll-Keller,1,2,3 and Evelyne Schvoerer1,2,3

1Institut National de la Santé et de la Recherche Médicale, Unité 748, 2Faculté de Médecine, Université Louis Pasteur, and 3Laboratoire de Virologie, 4Département d’Information Médicale, 5Service de Chirurgie Générale et Transplantations Multiorganes, and 6Service d’Hépato-gastroentérologie, Centre Hospitalier Régional Universitaire de Strasbourg, Strasbourg, France

Background. Nonrandom distribution of hepatitis C virus (HCV) quasispecies (compartmentalization between blood plasma and leukocytes) suggests the presence of HCV leukotropic variants. HCV compartmentalization in the setting of liver transplantation (LT) is poorly understood. To study HCV leukotropic variants, we investigated the evolution of HCV compartmentalization after immunosuppression in liver transplant recipients.

Methods. Plasma and peripheral blood mononuclear cell (PBMC) samples were collected from 5 liver transplant recipients before and after LT. We used clone sequencing to analyze the hypervariable region 1 (HVR1)–E2 384–419 region, which plays a key role in HCV entry and the induction of neutralizing responses, and assessed compartmentalization through phylogenetic analyses and Mantel’s test.

Results. Compartmentalization was frequent in the LT setting. HCV quasispecies were more homogeneous after LT in both the plasma and PBMC compartments, with a significant decrease in quasispecies complexity (P = .003) and genetic distances (P = .004) after transplantation. Our analysis identified 8 PBMC-related amino acid residues in HVR1.

Conclusions. HCV compartmentalization between plasma and PBMCs and the emergence of leukotropic variants could be potentiated by immunosuppression in liver transplant recipients. The identification of defined leukotropic variants may contribute to the understanding of virus-host interactions after transplantation.

End-stage cirrhosis related to chronic hepatitis C virus (HCV) infection has become the main indication for liver transplantation (LT). Virological recurrence is almost inevitable, occurring during the first hours after LT [1]. HCV reinfection generally runs an accelerated course in liver transplant recipients [1], and its pathophysiological mechanisms are still poorly understood. HCV circulates in infected patients as a complex mixture of closely related but genetically distinct viral variants called “HCV quasispecies” [2]. Selection of HCV variants escaping antibody-mediated neutralization may play an important role in liver reinfection during the early posttransplantation period [3]. Immunosuppression has also been defined as a prognostic factor in the outcome of recurrent hepatitis [4].

Low-level viral replication in different extrahepatic tissues and cell types, including peripheral blood mononuclear cells (PBMCs), has been suggested to be involved in the pathogenesis of HCV recurrence after LT [5–10]. Nonrandom distribution of HCV quasispecies (HCV compartmentalization between plasma and PBMCs or among the different PBMC subsets) suggests the presence of HCV leukotropic variants [8, 9]. By in-
fecting several peripheral blood cell types, such as B and T lymphocytes, monocytes, macrophages, and dendritic cells, HCV could interfere with antiviral cellular and humoral immune responses [10, 11].

Analysis of the molecular characteristics of leukotropic viral strains will play an important role in advancing our knowledge of interactions between HCV and leukocytes. LT is a compelling model of HCV infection in naive liver, with a well-defined initiation of infection and the ability to monitor virus evolution at specific time points [4]. HCV quasispecies compartmentalization has been reported in liver transplant recipients [11]. However, little is known about the evolution of HCV compartmentalization and possible amino acid signatures of leukotropic HCV strains in liver transplant recipients.

A key role of viral tropism is viral entry mediated by the viral envelope glycoproteins E1 and E2. The HCV hypervariable region 1 (HVR1) and its adjacent C-terminal stretch, comprising amino acids 384–419 of envelope glycoprotein E2, has been shown to play a crucial role in viral entry by interacting with hepatocyte coentry factors, such as scavenger receptor BI and highly sulfated heparan sulfate [12, 13]. Furthermore, this region has been shown to represent a major target of neutralizing antibodies [14, 15] containing several B cell epitopes [16].

Here, by performing sequence analysis of E2 HVR1 and the coding region of its adjacent C-terminal stretch, we have demonstrated that HCV compartmentalization occurs frequently in patients who have undergone LT. Furthermore, we have identified a cluster of leukocyte-related amino acid residues in HVR1.

**METHODS**

**Patients.** A prospective study of HCV infection had been conducted in 17 patients undergoing LT for HCV-related end-stage cirrhosis at the Transplantation Center of University Hospital in Strasbourg, France [3]. Five patients were chosen because they were infected with the most prevalent viral genotypes in the original cohort: 1b (n = 3; patients A, C, and D) and 3a (n = 2; patients B and E). These patients also exhibited differences in histological progression (Metavir scores): patients A, D, and E did not have histological evidence of recurrent hepatitis until 34, 12, and 21 months after LT, respectively, whereas patients B and C had early recurrence of histological liver disease (5 and 4 months after LT). All patients received immunosuppressive treatment to avoid liver-graft rejection; 3 patients (patients B, C, D, and E) received antiviral treatment after liver transplantation.

![Patient A](image1)

![Patient B](image2)

**Table 1. Patient characteristics.**

<table>
<thead>
<tr>
<th>Patient (sex)</th>
<th>Genotype*</th>
<th>HCCb</th>
<th>Viral load, 1 × 10^6 IU/mLc</th>
<th>Immunosuppressive therapyd</th>
<th>Recurrence (Metavir score)e</th>
<th>Antiviral treatmentf</th>
<th>Route of infection (duration of infection BT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (male)</td>
<td>1b</td>
<td>Yes</td>
<td>Positive Positive 2:1</td>
<td>TAC/RAP/COR</td>
<td>Recurrence at M34 (A1F0)</td>
<td>. . .</td>
<td>Transfusion (33 years)</td>
</tr>
<tr>
<td>B (female)</td>
<td>3a</td>
<td>Yes</td>
<td>184 309 4645</td>
<td>TAC/MMF/COR</td>
<td>Recurrence at M5 (A1F0)</td>
<td>M5 ND (ND)</td>
<td></td>
</tr>
<tr>
<td>C (female)</td>
<td>1b</td>
<td>Yes</td>
<td>2620 87 ND</td>
<td>TAC/RAP/COR</td>
<td>Recurrence at M4 (A1F1)</td>
<td>M6 ND (ND)</td>
<td></td>
</tr>
<tr>
<td>D (female)</td>
<td>1b</td>
<td>Yes</td>
<td>907 1556 &gt;7892</td>
<td>TAC/RAP/COR</td>
<td>Recurrence at M12 (A1F1)</td>
<td>M12 ND (ND)</td>
<td></td>
</tr>
<tr>
<td>E (male)</td>
<td>3a</td>
<td>No</td>
<td>1268 13 2279</td>
<td>TAC/COR</td>
<td>Satisfactory at M21 (A0F0)</td>
<td>. . .</td>
<td>ND (ND)</td>
</tr>
</tbody>
</table>

**NOTE.** BT, before liver transplantation; COR, corticoid; D, day; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, month; MMF, mycophenolate; ND, not determined; RAP, rapamycin; TAC, tacrolimus.

* HCV genotyping (LiPA HCV II; Siemens Medical Solutions Diagnostics).
* HCV-related HCC observed before transplantation.
* Viral load (Versant HCV RNA 3.0 Assay [bDNA]; Siemens Medical Solutions Diagnostics; sensitivity, 615 IU/mL). The positive samples below the bDNA assay threshold were tested with a more sensitive technique (Amplicor; Roche; sensitivity, 50 IU/mL).
* Treatment takes into account the entire posttransplantation follow-up.
* Metavir score, established by histological examination of the liver, is given for the date of the first observation of recurrent hepatitis. Necroinflammatory activity (A) is graded from A0 to A3, and fibrosis (F) is graded from F0 to F4 (cirrhosis).
* When antiviral treatment (pegylated interferon/ribavirin) was given after liver transplantation, the date of treatment initiation is indicated.

![Figure 1. Unrooted neighbor-joining tree of the 534 hypervariable region 1–E2 384 – 419 nucleotide sequences. Sequences were determined from plasma and peripheral blood mononuclear cell samples from all patients. Reference sequences belonging to genotype 1b and 3a were included (GenBank accession nos. AF356827 and AF046866, respectively). Bootstrap values between patient samples are indicated; values always exceeded 65%. Horizontal bars indicate the no. of nucleotide substitutions per site. HCV, hepatitis C virus.](image3)
and D) received antiviral treatment (pegylated interferon/ribavi

and D) received antiviral treatment (pegylated interferon/ribavirin) after LT (at months 5, 6, and 12, respectively) (table 1). No patient coinfected with hepatitis B virus or HIV was retained for the study. Approval for this work was obtained in 2002 from the institutional authorities for human studies in Alsace, France, in accordance with the Declaration of Helsinki.

**Sampling, RNA extraction, reverse transcription, and polymerase chain reaction (PCR) amplification.** HCV quasispecies were analyzed in plasma and PBMC samples collected before LT and at 7 days, 1 month, and ~1 year after LT. PBMC samples were washed with PBS and treated with trypsin and EDTA before nucleic acid extraction to eliminate possible contamination of PBMCs by HCV present in plasma or adsorbed to the PBMC membranes. Briefly, the cells were washed in PBS and then incubated with trypsin (0.05%; Gibco) for 5 min at 37°C, followed by 2 washing steps in PBS and fetal calf serum (30%). RNA was extracted from 2 × 10⁶ PBMCs with the NucliSENS Isolation Kit (bioMérieux), and from plasma (140 µL) with the QIAamp Viral RNA Mini Kit (Qiagen). RNA (~250–300 ng per reaction in plasma and 700 HCV copies per reaction in PBMCs) was subjected to PCR and nested PCR using high-fidelity ProofStart DNA polymerase (Qiagen) and primers that have been published elsewhere [17]. Negative controls consisting of RNase/DNase-free water were included in all experiments.

**Cloning and sequence analysis of viral isolates.** Fragments obtained by PCR were gel purified and cloned into *Escherichia coli*, as described elsewhere [3]. A mean of 14 viral clones per sample were sequenced bidirectionally using the OpenGene DNA Sequencing System (Siemens Medical Solutions Diagnostics). Electrophoregrams were visually inspected, and corrected sequences were analyzed with Molecular Evolutionary Genetics Analysis (MEGA) software (version 4; http://www.megasoftware.net) [18]. We performed alignments using the ClustalW algorithm. A final nucleotide sequence of 109 bp, including HVR1, was used for the quasispecies analyses (HVR1-E2 384–419). Quasispecies complexity was expressed by normalized Shannon entropy (Sn): 

\[ Sn = -\sum i (pi \ln pi)/ln N, \]

where pi is the frequency of each amino acid sequence and N is the total number of sequences analyzed in each compartment, ranging from 0 (no diversity) to 1 (maximum diversity). Pairwise nucleotide distances were calculated according to the Kimura 2-parameter method. Synonymous distances (dn) and nonsynonymous distances (ds) were estimated using the Nei-Gojobori method with the Jukes-Cantor correction. We calculated \( dN - dS \) values as an index of selective pressure operating on the viral quasispecies. Phylogenetic trees were constructed by the neighbor-joining method, using the Kimura 2-parameter model (1000 bootstrap resampling replications with MEGA). To determine whether sequences within each compartment (plasma or PBMCs) were more similar to each other than to sequences from other compartments, we performed phenetic analysis using Mantel’s test, a generalized regression permutation method comparing 2 distance matrices, with values ≤.05 corresponding to a compart-

![Figure 2](https://academic.oup.com/jid/article-abstract/198/11/1656/961362)
mental structure of the quasispecies [9, 19]. We also looked for temporal phenetic structure in each compartment, using Mantel’s test (Permute! software, version 3.4; http://www.bio.umontreal.ca/casgrain/en/labo/permute/index.html). To explore amino acid signature patterns specific to PBMC or plasma variants, we used the VESPA (Viral Epidemiology Signature Pattern Analysis) program (http://hcv.lanl.gov/content/sequence/VESPA/vespa.html). We assessed the relevance of each signature individually, taking into account the frequency and relative proportions of 1 residue at each position for PBMCs compared with plasma. Amino acid alignments for each patient and all clones show the detailed distribution of amino acids. GenBank accession numbers of clones are EU679662–EU680195.

**Statistical analysis.** Data were analyzed using linear mixed models, which are an extension of analysis of variance that allow random effects to be specified separately from the standard fixed effects. Interaction over time was estimated for every model. Analyses were run with R software (nlme package). Time was considered a continuous fixed effect factor. Differences were considered significant at $P \leq .05$.

**RESULTS**

**Evolution of viral variants.** Plasma and PBMC compartmentalization was analyzed in HVR1-E2\(_{384-419}\) fragments, which were amplified by PCR in all samples except for the PBMCs of patient C at 6 months. A total of 534 clones (289 from plasma and 245 from PBMCs) were sequenced (patient A, $n = 108$; patient B, $n = 116$; patient C, $n = 78$; patient D, $n = 112$; patient E, $n = 120$). Alignment of HVR1-E2\(_{384-419}\) segments showed a distinct patient-specific sequence pattern, ruling out the possibility of interpatient sample contamination (figure 1).

Amino acid sequence analysis demonstrated that most of the PBMC and plasma samples displayed a quasispecies pattern, with the presence of a predominant variant (arbitrarily defined as a variant representing $>30\%$ of the quasispecies) and a varying number of minor variants (figure 2A), except in 5 post-LT samples showing 1 unique variant with no minor variants:
Figure 4. Phylogenetic trees showing the longitudinal distribution of all of the hepatitis C virus (HCV) variants, which were previously separated according to time (see figure 3). Viral variants are represented with the same symbols as in figure 3: peripheral blood mononuclear cell (PBMC) (black squares) and plasma (white squares) variants before liver transplantation (LT), PBMC (black triangles) and plasma (white triangles) variants at day 7 after LT, PBMC (black diamonds) and plasma (white diamonds) variants at 1 month after LT, and PBMC (black circles) and plasma (white circles) variants at the latest time point after LT. Bootstrap values ≥65% (based on 1000 replicates) are indicated. HCV variants that have been shown to constitute compartment-specific clusters in time-related subtrees are depicted by additional symbols: 2 stars for plasma variants and 1 star for PBMC variants. Horizontal bars indicate the no. of nucleotide substitutions per site.
plasma from patient A at day 7, the 3 post-LT PBMC samples from patient B, and PBMCs from patient E at 16 months.

Genetic diversity and selective pressure. The quasispecies diversity assessed by genetic distances and genetic complexity and the selective pressure evaluated by \( d_N - d_S \) values were established in each compartment and at each time point (figure 2B and 2C). Complexity and genetic distances showed homogenization of HCV quasispecies after LT compared with before LT, in both plasma and PBMCs (figure 2B). Indeed, the average intrapatient genetic distance decreased after LT, in both

![Figure 5. Amino acid pattern of hepatitis C virus (HCV) quasispecies studied in hypervariable region 1 (HVR1)-E2_384-419. A, Sequence logo representations based on amino acid alignments of all plasma (289 clones) and all peripheral blood mononuclear cell (PBMC) (245 clones) sequences, obtained by use of the WebLogo program developed by Schneider and Stephens [21] and upgraded by Crooks et al. [20] (http://weblogo.berkeley.edu/). Consensus amino acids appear on the top of the graph, and other amino acids appear according to decreasing frequency from top to bottom; the height of each letter indicates the relative frequency of each residue at each position; and the overall height of the stack indicates the sequence conservation, in bits. Basic residues (histidine [H], lysine [K], and arginine [R]) are highlighted by boldface type. A classic representation of the residues found at each position is also shown under the corresponding sequence logos, allowing identification of less represented amino acids. B, Alignment of plasma and PBMC consensus sequences obtained from patients infected with HCV genotype 1b (patients A, C, and D) or 3a (patients B and E). Genotypes are indicated in parentheses. Amino acids identical to those observed in the consensus sequence obtained from genotype 1b plasma variants are represented by dots.](https://academic.oup.com/jid/article-abstract/198/11/1656/961362)
plasma (from a mean ± SD of 0.064 ± 0.015 before LT to 0.030 ± 0.008 after LT) and PBMCs (from 0.046 ± 0.011 before LT to 0.019 ± 0.007 after LT); the average genetic complexity evolved in the same way in plasma (from 0.54 before LT to 0.39 after LT) and PBMCs (from 0.38 before LT to 0.34 after LT). Thus, complexity and genetic distances globally decreased (homogenization) according to the time after LT (P = .003 and P = .004, respectively), with no difference between the plasma and PBMC compartments (P > .05). Concerning specific patients, homogenization of HCV quasispecies from before LT to the late time point was observed in both plasma and PBMCs in 3 (patients B, D, and E) of the 4 patients (patients A, B, D, and E) with available late data (month 12 or 16). They all showed decreased complexity and genetic distances, and patient D demonstrated a constant low genetic distance for PBMCs (figure 2B).

The dN - dS values revealed positive immune selective pressure for positive values and, conversely, conservative constraints for negative values. No tendency was observed in this group of 5 patients. Indeed, dN - dS values appeared to be highly variable, with no significant variations over time (P > .05) or according to the compartmental origin of viral clones (P > .05) (figure 2C). We found no link between this index at a given time point and subsequent changes in quasispecies.

**Phylogenetic and phenetic analyses.** To analyze HCV compartmentalization after LT, bootstrapped phylogenetic trees were constructed from nucleotide sequences for each subject,

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**Figure 6.** Signature sequence analysis. A, Consensus sequences are presented for the 3 patients (patients A, D, and E) in whom peripheral blood mononuclear cell (PBMC)-related amino acid residues were found by the Viral Epidemiology Sequence Pattern Analysis (VESPA) algorithm. PBMC-related residues are indicated by boldface type. Relative frequencies of corresponding amino acids in the query sequence set (PBMCs) and the background sequence set (plasma) are shown, and relevant amino acids are highlighted by gray squares. B, Amino acid alignments of hypervariable region 1–E1 (58) for the 5 patients (patients A–E). Left-hand columns show the compartment origin and the time of sampling; variants are identified by lowercase letters, and values in parentheses indicate the nos. of identical clones. Consensus sequences were generated for each patient and are displayed at the top of each alignment; amino acids identical to those of the derived consensus sequences are represented by dots, and gaps are represented by dashes. BT, before liver transplantation; D, day; M, month.
with an independent analysis of each time point (figure 3). A clustering of viral variants according to compartment origin was identified in all 5 patients (100%), with compartmentalized structures revealed at 15 (79%) of 19 time points. Distinct clusters of PBMC variants (supported by bootstrap values ≥65%) were present before LT in 1 of 5 patients (patient A) and after LT in 4 of 5 patients (8 of 14 post-LT time points). Distinct clusters of plasma variants were present before LT and 1 month after LT in patients A and B; in the pre-LT sample of patient C; before LT, at day 7, and at 1 month for patient D; and at all post-LT time points for patients E.
points for patient E. In total, 8 (42%) of 19 time points indicated both phenetic structure (significant Mantel’s test results) and phylogenetic clustering of variants according to compartmental origin (figure 3)—that is, compartmentalization before LT in 2 of 5 patients and after LT in 4 of 5. The only patient (patient A) displaying HCV compartmentalization at all time points had a low HCV RNA load at the 1-year follow-up time point and histological recurrence of hepatitis only at month 34. Among the plasma and PBMC samples, phenetic temporal structure was found only in PBMCs from patients B, C, and E (not patients A and D), indicating sequential replacement over time of the quasispecies present at a given time point in those PBMCs (data not shown). Phylogenetic trees including all available time points were also constructed for each patient (figure 4). In these trees, plasma clusters with significant bootstrap values were observed in all patients, including either pre- or post-LT sequences. For post-LT variants, PBMC-specific clusters with significant bootstrap values were observed in 3 of 5 patients (patients A, C, and D). Additional symbols (1 star for PBMC clusters and 2 stars for plasma clusters) allow us to identify HCV clusters previously shown in time-related subtrees. In all patients, plasma variants observed at day 7 were either identical to or clustered with variants present before LT in both plasma and PBMCs.

**Amino acid sequence analysis.** To identify leukocyte-specific amino acid signatures, we analyzed amino acid sequences of isolated variants. First, despite high global variability, several positions within HVR1 were highly conserved. Furthermore, positions found elsewhere to be nearly invariant in HVR1 of plasma variants (385, 389, 393, 403, 406, and 409) [3] were largely conserved in both plasma and PBMC variants in our study (logo representations [20, 21] (figure 5A). In the area of the E2384–419 region after HVR1, known to be highly conserved [14], positions 412, 413, and 415–419 were nearly invariant in plasma and PBMC samples. In HVR1-E2384–419, only positions 403, 413, and 415 were entirely conserved in both plasma and PBMC samples. Basic residues were found in consensus sequences in plasma and PBMCs at positions 384, 386, 394, and 410 in HVR1. As expected, the consensus sequences of HCV variants appeared significantly different between the 2 genotypes (1b and 3a), whereas they were more similar within the same genotype, whatever the compartment (figure 5B).

Second, using the VESPA algorithm and taking into account the frequency of each atypical residue in the PBMC and plasma sequence set, 8 PBMC-related amino acids were retained at 5 (26%) of 19 time points, in patients A (2 residues present before LT, 3 at 1 month, and 1 at 12 months), D (1 residue present before LT), and E (1 residue present at day 7) (figure 6A). The pre-LT and 1-month PBMC-related amino acids of patient A included a basic residue (histidine at position 405 of HVR1) that was found at a frequency of 100% in PBMCs and was present in pre-LT and 1-month plasma sequences at frequencies of 31% and 0%, respectively. However, by adding amino acid alignments for each patient and from all available clones (figure 6B), we did not detect hot spots for mutations that could systematically distinguish plasma from PBMC variants, except for an aspartic acid at position 404 in HVR1, which was present in all plasma variants of patient A at 1 month but which was never found in PBMC variants. This is why we preferred to consider leukocyte-related residues retained by VESPA rather than “signatures.” The 3 patients with PBMC-related residues (patients A, D, and E) did not show histological HCV recurrence within the first year after LT. The 2 patients without PBMC-related residues (patients B and C) showed early histological HCV recurrence (5 and 4 months after LT). No link was observed between the presence of PBMC-related amino acids or compartmentalization and response to antiviral treatment after LT, but there were only 3 treated patients, preventing any statistical analysis or conclusion (data not shown).

**DISCUSSION**

In this study, we have demonstrated frequent PBMC and plasma compartmentalization of HCV in the LT setting and homogenization of HCV quasispecies after LT. Furthermore, we have identified 8 PBMC-related amino acid residues in HVR1 of E2. Several authors have observed low-level viral replication in extrahepatic cell types, including PBMCs [5–10]. Compartmentalization thought to reflect autonomous extrahepatic replication in PBMCs could be enhanced by immunosuppression and was recently observed in HIV/HCV-coinfected patients [22]. In vivo studies have demonstrated evidence of low HCV replication in lymphoid cells from HIV-infected patients [6, 7], and HCV has been recently shown to infect T cells and to affect interferon-γ signaling [23]. The existence of leukotropic-fitted variants has indeed been suggested previously; minor quasispecies variants were selected both in vitro in human T and B cell lines and in vivo in PBMCs from infected chimpanzees [23–26]. Knowledge about HCV compartmentalization and the clinical course of HCV-related disease in the LT setting is limited. Patients without transplants and with HCV compartmentalization have been shown to have high rates of sustained virological response to pegylated interferon/ribavirin therapy [27]. In our study, the only patient with HCV compartmentalization at all time points (patient A) presented with low HCV RNA load at the 1-year follow-up time point, and the 3 patients with HCV with PBMC-related amino acid residues did not demonstrate histological HCV recurrence within the first year after LT. However, the number of patients included in this study limits the statistical analysis of potential links between PBMC-specific compartmentalization or residues and HCV load, histological parameters, or clinical parameters.

Findings from other studies have suggested that the bottleneck effect generated by new liver grafts results in the selection of viral variants in the quasispecies [4, 28, 29]. Homogenization of HCV quasispecies, corresponding to a decrease in quasispecies complexity and genetic distances in plasma after LT, has been
Our results suggest a significant evolution to the homogenization of HCV quasispecies in both the plasma and the PBMC compartment after LT. Concerning the source of HCV recurrence, the extrahepatic contribution to HCV plasma viremia was assumed to be very low [31, 32]. Moreover, in a study of 6 patients, liver grafts were infected mainly by liver-derived viruses present in plasma, despite some PBMC strains being detected in serum soon after LT [33]. However, HCV viremia decreases after liver removal [1], and immune cells massively infiltrate the liver graft soon after LT [34]. Thus, extrahepatic reservoirs could contribute to liver-graft reinfection. In our study, plasma variants emerging after LT, reflecting hepatic replication in the liver graft, were related to variants present in both the plasma and the PBMC compartment before LT. Therefore viral strains adapted to both environments (plasma [i.e., the liver] and mononuclear cells) that remain in the circulation during liver removal could be the main source of graft reinfection by HCV, meriting further investigation.

Similar to findings in HIV infection, in which cell tropism may be determined on the basis of amino acid substitutions in the envelope V3 loop [35], a specific amino acid pattern involved in cell entry could play a key role in HCV cell tropism. Blackard et al. [22] observed HCV PBMC-related amino acid signatures within HVR1 in HIV/HCV-coinfected patients. Using the same tool (VESPA [36]), we identified 8 PBMC-related residues in HVR1, of which 7 had similarities to those observed by Blackard et al. [22]. Threonine at position 395 and basic amino acids at position 405 (2 histidines in our study and 1 lysine in the study of Blackard et al. [22]) were observed in both studies. Moreover, 2 serines and 2 asparagines at positions 397 and 404, respectively, were observed in our study; Blackard et al. observed PBMC signatures at the same 2 latter positions, albeit with different amino acids. Similarly, Ducoulombier et al. [8] identified positions 397 and 404 as potential mutation sites that could distinguish plasma from naïve (IgD+) B cell variants, with amino acids different from ours in B cells.

In addition, the presence of PBMC-related amino acids, combined with the absence of major changes in sequential quasispecies in PBMCs from patients A and D, could suggest that PBMC strains share signatures. However, because of signature variations (as defined by VESPA) observed in amino acid alignments by time point and patient, with the corresponding residues possibly present in plasma in our study or in a previous study [8], we preferred to consider those corresponding amino acids to be leukocyte-related residues rather than signatures.

HVR1 was chosen for the identification of leukotropic amino acid patterns, because this globally basic region [37] has been shown to play a crucial role in the viral entry process [15, 38] and, therefore, in cell tropism [39–41]. HVR1 is required for binding to highly sulfated heparan sulfate [13, 41] and scavenger receptor BI [12], 2 key molecules in viral attachment and entry [39, 40, 42, 43]. Moreover, the HVR1-E2_384–419 region contains neutralizing epitopes that are potentially important in the anti-HCV immune response [39, 44]. Our interpatient analysis found strong similarity in the conservation pattern between plasma and PBMC variants in the HVR1-E2_384–419 region, supporting the notion that specific amino acid residues are vital for viral entry and subsequent fitness.

Finally, even though HCV replication in leukocytes was not demonstrated by negative strand–specific reverse-transcription PCR in our study, our data confirm the frequency of HCV compartmentalization between PBMCs and plasma in LT. We observed this by analyzing HVR1 in unsorted PBMCs; such analyses are nevertheless believed to be less sensitive than exploration in sorted cells (especially B lymphocytes) [8]. Frequent PBMC compartmentalization of HCV in the LT setting suggests that immunosuppression could favor the emergence of leukotropic variants [7, 11]. Higher levels of HCV replication were observed in immunosuppressed subjects, such as HIV/HCV-coinfected patients and the transplant recipients included in our study (table 1) [7, 45]. Furthermore, immunosuppression, by increasing viral production, could modulate the expression of receptors or cofactors for HCV entry and replication in nonhepatic cells, as shown for HIV [46].

In conclusion, our study has demonstrated frequent HCV compartmentalization between plasma and PBMCs in LT and leukotropic variants with PBMC-related amino acid residues. Further studies using recent models of HCV infection, HCV pseudotyped particles, and cell culture–derived infectious HCV [40] will allow investigation of the functional relevance of the identified amino acids to cell tropism, HCV-host interactions, and the pathogenesis of HCV infection.

References


