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Evidence of Distinct Populations of Hepatitis C Virus in the Liver and Plasma of Patients Co-Infected With HIV and HCV

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Abstract

Viral diversity is an important predictor of hepatitis C virus (HCV) treatment response and may influence viral pathogenesis. HIV influences HCV variability in the plasma; however, limited data on viral variability are available from distinct tissue/cell compartments in patients co-infected with HIV and HCV. Thus, this exploratory study evaluated diversity of the hypervariable region 1 (HVR1) of HCV in the plasma and liver for 14 patients co-infected with HIV and HCV. Median intra-patient genetic distances and entropy values were similar in the plasma and liver compartments. Positive immune selection pressure was observed in the plasma for five individuals and in the liver for three individuals. Statistical evidence supporting viral compartmentalization was found in five individuals. Linear regression identified ALT ($P = 0.0104$) and AST ($P = 0.0130$) as predictors of viral compartmentalization. A total of 12 signature amino acids that distinguish liver from plasma E1/HVR1 were identified. One signature amino acid was shared by at least two individuals. These findings suggest that HCV compartmentalization is relatively common among patients co-infected with HIV and HCV. These data also imply that evaluating viral diversity, including drug resistance patterns, in the serum/plasma only may not adequately represent viruses replicating within the liver and, thus, deserves careful consideration in future studies.

Keywords

HIV; HCV; co-infection; diversity; quasispecies; compartmentalization

INTRODUCTION

Similar to other RNA viruses, variability is a hallmark of HCV infection. Within an infected individual, HCV exists as a population of distinct but closely related viral variants, termed the *viral quasispecies*. Quasispecies diversity has important biologic implications for viral persistence, host cell tropism, antiviral drug resistance, and the development of an effective HCV vaccine (reviewed in [Farci, 2011]). The associations between HCV heterogeneity and

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liver transplantation outcome, disease progression, and chronicity of infection have been explored previously [Martell et al., 1994; Gretch et al., 1996; Brambilla et al., 1998; Manzin et al., 1998; Sullivan et al., 1998; Ray et al., 1999; Farci et al., 2000; Hayashi et al., 2000; Curran et al., 2002]. An association between HCV variability and treatment outcome has been reported as well [Pawlotsky, 2000; Layden-Almer et al., 2005; Sherman et al., 2010]. To date, most studies of HCV diversity have focused on the hypervariable region 1 (HVR1). HVR1 is subject to selective immune pressure [Weiner et al., 1992], and subsequent studies have demonstrated that HVR1 diversity may correlate with viral clearance [Chen and Wang, 2005; Liu et al., 2012].

Due to shared routes of transmission, co-infection with HCV is increasingly recognized as a major cause of morbidity and mortality among HIV-positive persons [Bica et al., 2001; Tedaldi et al., 2003; Salmon-Ceron et al., 2000]. In the US, 150,000–300,000 persons are infected with HIV and HCV, representing 15–30% of all HIV-infected persons and 5–10% of all HCV-infected persons [Alter et al., 1999; Sherman et al., 2002]. It is well established that HIV negatively impacts the clinical course of HCV disease. While the precise mechanism(s) remain unclear, HCV RNA levels are significantly elevated during HIV/HCV co-infection as compared to HCV mono-infection [Yokozaki et al., 2000; Tedaldi et al., 2003]. Furthermore, HIV seroconversion and antiretroviral therapy (ART) initiation [Beld et al., 1998; John et al., 1998; Rutschmann et al., 1998; Ragni and Bontempo, 1999; Chung et al., 2002; Cooper and Cameron, 2002; Pett et al., 2002] are both associated with sustained increases in HCV RNA. The clinical course of HCV infection is also accelerated in patients co-infected with HIV and HCV, as they experience more advanced liver fibrosis, cirrhosis, and death than patients with HCV mono-infected [Soto et al., 1997; Lesens et al., 1999; Yee et al., 2000; Bica et al., 2001; Puoti et al., 2001; Ragni and Belle, 2001; Martinez-Sierra et al., 2003; Mohsen et al., 2003; Poynard et al., 2003; Tedaldi et al., 2003]. Further compounding the clinical management of HCV, HIV co-infection results in decreased response rates to HCV treatment compared to HCV mono-infection [DiMartino et al., 2001; Perez-Olmeda et al., 2003; Chung et al., 2004]. Previous studies have focused largely on HCV mono-infected individuals and reported a non-random distribution of viral variants in various cell types and tissues (reviewed in [Farci, 2011]). This implies that the requirements for HCV replication are cell type-dependent and/or that there are distinct selection pressures acting upon viral variants depending upon their major site(s) of replication.

Relatively, few studies have assessed HCV diversity during HIV/HCV co-infection. Two cross-sectional studies found increased diversity in HVR1 in patients co-infected with HIV and HCV compared to patients with HCV mono-infection [Sherman et al., 1996] or to HIV/HCV co-infected subjects with higher CD4 cell counts [Toyoda et al., 1997]. Others have demonstrated that HCV was more diverse and under less selection pressure in patients co-infected with HIV and HCV compared to the patients with HCV mono-infection [Tanaka et al., 2007]; however, this was not observed in all studies [Mao et al., 2001; Shuhart et al., 2006]. Genomic regions other than the viral envelope proteins also exhibit intra-patient diversity, but the selection pressures on these regions may be distinct. Higher quasispecies diversity was observed in HVR1 compared to core or the 5' untranslated region from the same patients co-infected with HIV and HCV [Blackard et al., 2004]. Similarly, higher E1/HVR1 genetic distances were observed compared to NS5B sequences from the same

patients [Blackard et al., 2012]. This is particularly interesting given that HCV titers are elevated during HIV co-infection [Beld et al., 1998; Yokozaki et al., 2000; Tedaldi et al., 2003] and that co-infection is associated with lower HCV treatment response [DiMartino et al., 2001; Perez-Olmeda et al., 2003; Chung et al., 2004]. However, direct comparisons of HCV diversity in non-structural genes between mono-infected and patients co-infected with HIV and HCV are rare. Similarly, others have shown that p7 diversity was higher during HCV mono-infection compared to HIV/HCV co-infection, and liver fibrosis was inversely correlated with p7 synonymous substitutions [Li et al., 2012]. Therefore, viral diversity and compartmentalization was characterized in a well-characterized cohort of patients co-infected with HIV and HCV.

METHODS

Study Patients

For this pilot study, informed consent was obtained from 14 patients co-infected with HIV and HCV genotype 1 enrolled at the University of Cincinnati. Liver biopsy and plasma samples were obtained prior to the initiation of antiretroviral therapy (ART).

PCR Amplifications

RNA was extracted from 140 μ l of plasma using the QIAamp Viral RNA Kit or from homogenized liver biopsies (typically 1–2 mm in length) using the RNeasy Mini kit. cDNA corresponding to E1/HVR1 was generated using the antisense primer 5'-AGG CTT TCA TTG CAG TTC AAG GCC TTG CTA TTG ATG TGC C-3' (nucleotides 1639–1600) as described elsewhere [Blackard et al., 2004, 2007]. First round PCR amplification using the sense primer 5'-GCG TCC GGG TTC TGG AAG ACG GCG TGA ACT ATG CAA CAG G-3' (nucleotides 802–841) was performed as follows: 94°C for 2 min, followed by 35 cycles at 94°C for 1 min, 65°C for 2 min, and 72°C for 2 min, with a final elongation step at 72°C for 8 min. Second round PCR conditions were the same as the first round and utilized the antisense primer 5'-AGT TCA AGG CCG TGC TAT TGA TGT GCC AAC TGC CGT TGG T-3' (nucleotides 1626–1587) and the sense primer 5'-GGC ATG GGA TAT GAT GAT GAA CTG GTC CCC TAC-3' (nucleotides 1295–1327). These primers have been utilized extensively and amplify HCV genotypes 1–4. All RT-PCR amplifications included one reaction containing no reverse transcriptase and another containing no template as negative controls. PCR products were gel purified and ligated into the pGEM-T Easy Vector. An average of 11 plasmids per sample type per individual were sequenced using dye terminator chemistry and edited using CodonCode Aligner. Previous reports have shown that a similar approach accurately and reproducibly represents the overall HCV quasispecies diversity within an individual and that multiple amplifications of the same template yielded similar estimates of quasispecies diversity [Torres-Puente et al., 2003; Blackard et al., 2004].

Phylogenetic Analysis and Quantification of Diversity

All alignments were performed using the neighbor-joining method implemented in Clustal X [Thompson et al., 1997]. GenBank references used to confirm genotype included D10749 (1a), M62321 (1a), AF511950 (1a), AF177040 (H77; 1a), D90208 (1b), AY460204 (1b), D14853 (1c), AY051292 (1c), AF238481 (2a), AB047639 (2a), AB030907 (2b), AY232746

(2b), D50409 (2c), AF046866 (3a), D17763 (3a), D28917 (3a), D49374 (3b), Y11604 (4a), DQ516084 (4a), FJ025854 (4b), Y13184 (5a), AF064490 (5a), Y12083 (6a), and D84262 (6b). The statistical robustness and reliability of the branching order within each phylogenetic tree were confirmed by bootstrap analysis using 1,000 replicates [Felsenstein, 1985]. Phylogenetic inference was performed using a Bayesian Markov chain Monte Carlo (MCMC) approach as implemented in the BEAST v1.7.2 program [Drummond et al., 2012] under an uncorrelated log-normal relaxed molecular clock and the general time-reversible model with nucleotide site heterogeneity estimated using a gamma distribution. The MCMC analysis was run for a chain length of 50,000,000 with sampling every 5,000th generation. Results were visualized in Tracer v1.5 to confirm chain convergence, and the effective sample size (ESS) was calculated for each parameter. All ESS values were >500 indicating sufficient sampling. The maximum clade credibility tree was selected from the posterior tree distribution after a 10% burn-in using TreeAnnotator v1.7.2. Clonal sequences have been submitted to GenBank and given the accession numbers KC192048–KC192373.

Intra-patient genetic distances were calculated by pairwise comparison of nucleotide sequences using the Kimura method of MegAlign (DNASTAR; Madison, WI). Shannon entropy $\left(S_n = - \sum (p_i \ln p_i) / \ln N\right)$, where p_i is the frequency of each distinct amino acid sequence, and N is the total number of sequences analyzed, was also calculated. Non-synonymous (dN)-synonymous (dS) values were calculated via the Nei-Gojobori method in MEGA v5.1 [Kumar et al., 2008].

Evaluation of Compartmentalization

Compartmentalization of HCV variants was assessed using Mantel's test as described previously using PASSaGE version 2 with 10,000 permutations and the Slatkin-Maddison (S-M) test for population gene flow as implemented in the HyPhy program using 10,000 permutations [Slatkin and Maddison, 1989; Afonso et al., 1999; Ducoulombier et al., 2004; Pond et al., 2005; Roque-Afonso et al., 2005; Zehender et al., 2005; Sobesky et al., 2007; Schramm et al., 2008; Ramirez et al., 2009; Rosenberg and Anderson, 2011]. However, because there is no agreed upon gold standard for evaluating viral compartmentalization, multiple methods were utilized. P values <0.05 were considered statistically significant evidence that viral sequences from the liver were compartmentalized as compared to those from the corresponding plasma. Signature amino acid patterns that distinguish liver viral variants from those in the corresponding plasma were identified using the Viral Epidemiology Signature Pattern Analysis program with a threshold of 70% [Korber and Myers, 1992].

Statistical Analyses

Paired-sample t tests were used to compare intra-patient variability in the plasma versus liver. All analyses were performed using SAS software. Best subset regression models were used to determine which predictor variables—alanine aminotransferase (ALT), aspartate aminotransferase (AST), age, CD4 cell count, $\log_{10}$ HCV viral load, $\log_{10}$ HIV viral load, race, and gender—to include in a multiple regression model. Least squares linear regression was then used to model the relationship between these clinical and/or demographic

predictors (with P values > 0.10) and the various methods utilized to assess compartmentalization.

RESULTS

Patient Characteristics

The mean age for study participants was 50.5 years and included 11 males and 3 females (Table I). Twelve participants were African American. The median CD4 cell count was 342 cells/mm³. Median ALT and AST levels were 56.0 and 85.5 IU/ml, respectively. Median baseline HIV and HCV RNA levels were 4.6 log₁₀ copies/ml and 6.3 log₁₀ IU/ml, respectively. All participants were ART-naïve and were not receiving interferon or direct-acting HCV agents at the time of sample collection.

Intra-patient Variability

All consensus E1/HVR1 nucleotide sequences belonged to HCV genotype 1, and plasma and liver sequences clustered by patient in all instances (Fig. 1). Intra-patient variability was similar between plasma and liver samples with respect to median genetic distance (0.98% in plasma versus 0.78% in liver; $P = 0.65$), entropy (0.89 vs. 0.80; $P = 0.21$), and dN-dS (−0.0004 vs. −0.0011; $P = 30$). Positive dN-dS values—indicating positive immune selection pressure—were observed in the plasma and liver for six and six individuals, respectively.

Analysis of HCV Compartmentalization

By phylogenetic comparison of patient-specific viral variants from the plasma and liver, three subjects—Cin03, Cin05, and VCU01—had plasma-specific E1/HVR1 variants (Supplementary Fig. S1). HCV compartmentalization was explored further using the Slatkin-Maddison (S-M) test and Mantel's test, which do not rely on interpretation of a phylogenetic tree (Table II). When comparing patient-specific plasma- and liver-derived E1/HVR1 variants, the S-M test demonstrated statistically significant quasispecies compartmentalization for Cin05 ($P = 0.002$) and VCU05 ($P = 0.0004$) and trends towards compartmentalization for Cin03 ($P = 0.088$) and Cin10 ($P = 0.071$). Similarly, Mantel's test demonstrated statistically significant evidence of compartmentalization for Cin05 ($P = 0.001$) and Cin10 ($P = 0.017$). Thus, five patients showed evidence of viral compartmentalization using at least one measurement.

Next, potential predictors of viral compartmentalization were evaluated (Table III). Using the phylo-genetic test, best subset regression identified CD4 cell count and gender as factors possibly associated with HCV compartmentalization (adjusted R^2 for the model = 0.2162). With Mantel's test, ALT, AST, log₁₀ HCV, log₁₀ HIV, race, and gender were identified as factors possibly associated with compartmentalization (adjusted $R^2 = 0.5273$). Using the S-M test, ALT, AST, and age were identified as factors possibly associated with compartmentalization (adjusted $R^2 = 0.1536$). Using a combination of phylogenetic, Mantel's, and/or SM tests, age, race, and gender were identified as factors possibly associated with compartmentalization (adjusted $R^2 = 0.2668$). The Mantel's test model had the highest R^2 value and was deemed the best predictive model overall. Linear regression

identified ALT ($P = 0.0104$) and AST ($P = 0.0130$) as predictors of viral compartmentalization (R^2 value = 0.5365 and P value for the model = 0.0453).

Signature Sequence Analysis of Compartment-Specific Amino Acids

To identify specific amino acids associated with compartmentalization in patients co-infected with HIV and HCV, signature sequence analysis was performed as shown in Figure 2. A total of 12 (range: 1–7) signature amino acids were detected in E1/HVR1 for Cin05, Cin07, and Cin10. One signature amino acid—position 401—was shared by two individuals.

CONCLUSIONS

During HIV infection, viral compartmentalization has been documented in the brain, cerebrospinal fluid, and genital tract, and there are emerging data for HIV compartmentalization within the gut, lung, liver, kidney, and breast milk (reviewed in [Blackard, 2012]). HIV alters HCV diversity [Sherman et al., 1996; Toyoda et al., 1997; Mao et al., 2001; Shuhart et al., 2006; Tanaka et al., 2007; Li et al., 2012]. Extrahepatic complications of HCV infection include glomerulonephritis, mixed cryoglobulinemia, porphyria cutanea tarda, arthralgias, diabetes, autoimmune disorders, and thyroid disease. Extrahepatic replication of HCV has been reported in peripheral blood mononuclear cells (PBMCs)—including granulocytes, monocytes/macrophages, dendritic cells, and B lymphocytes—as well as extrahepatic tissues [Laskus et al., 1997, 1998a,b, 2000; Maggi et al., 1997, 1999; Shimizu et al., 1997; Lerat et al., 1998; Navas et al., 1998; Okuda et al., 1999; Roque-Afonso et al., 1999; Caussin-Schwemling et al., 2001; Radkowski et al., 2002; Goutagny et al., 2003; Radkowski et al., 2004; Bartolomé et al., 2008]. Extrahepatic replication of HCV has important implications for transmission, disease progression, and treatment [Bare, 2009]. For instance, several studies detected HCV RNA in PBMCs without detection in the corresponding serum or liver [Saleh et al., 1994; Taliani et al., 1995; Mazur et al., 2001]. In addition, patients with negative-strand HCV RNA in PBMCs had lower IFN responses compared to those with no negative-strand RNA in PBMCs [Gong et al., 2003]. Studying HCV diversity provides further evidence of extrahepatic replication by demonstrating a non-random distribution of viral variants in hepatic and/or extrahepatic compartments (reviewed in [Farci, 2011]). By definition, viral compartments are characterized by a restriction of viral gene flow between cells or tissues [Nickle et al., 2003] and occur due to physical isolation of a particular tissue/cell-type, selective migration of susceptible cells, different determinants of virulence or tropism depending upon the cell-type (i.e., distinct levels of entry factors or co-factors required for replication), and/or localized selection pressures. Significant diversity exists among hypervariable region 1 (HVR1) variants in the serum/plasma, liver, and/or PBMC variants [Maggi et al., 1997, 1999; Shimizu et al., 1997; Navas et al., 1998; Okuda et al., 1999; Zehender et al., 2005; Ramirez et al., 2009] due to variation in the viral envelope determinants of HCV entry and/or the varied capacity of the host immune response to interact with and remove certain viral variants. Diversity in the 5' untranslated region (UTR) and/or internal ribosome entry site (IRES) also exists in PBMCs [Di Liberto et al., 2006; Vera-Otarola et al., 2009]. The precise mechanisms leading to the development of compartmentalization have not been adequately examined but likely include (i) differential immune selection pressures, (ii) cell-

type specific differences in viral replication, (iii) local concentrations of antiviral drugs and/or drug resistance, and (iv) co-infections that alter the cellular microenvironment. Functional studies further demonstrate that HCV variation also affects translational efficiency and implies that HCV adapts for efficient replication in a cell type-dependent manner [Laporte et al., 2000, 2003; Lerat et al., 2000; Forton et al., 2004].

In the current analysis, both phylogenetic and statistical evidence of viral compartmentalization were observed between the liver and the plasma for a subset of patients co-infected with HIV and HCV. These findings are supported by other studies demonstrating that the circulating quasispecies population does not always reflect the composition of the intrahepatic quasispecies population. For example, when examining quasispecies diversity in the brain, lymph node, liver, and serum, 50% of E2/HVR1 variants in the liver were not observed in the serum [Forton et al., 2004]. When evaluating UTR diversity in the serum and liver by a single-strand conformational polymorphism assay, 4 of 6 liver samples contained variants not present in the corresponding sera [Jang et al., 1999]. Others reported that E2/NS1 variants present in the liver and/or PBMCs were not detected in the corresponding plasma, while other variants were present only in the plasma [Maggi et al., 1997]. Similarly, at least one HVR1 sequence unique to the serum was detected in 12 of 13 patients analyzed [Okuda et al., 1999]. Thus, HCV compartmentalization is relatively frequent in vivo, although only a minority of studies have included patients co-infected with HIV and HCV or evaluated the clinical factors associated with compartmentalization.

To our knowledge, only one other study has evaluated factors associated with HCV compartmentalization. Using a single-strand conformational polymorphism assay, others reported that compartmentalization was more frequent in former injection drug users and less frequent with genotype 1 [Di Liberto et al., 2006]. In this study, ALT and AST were identified as variables significantly associated with viral compartmentalization, suggesting that higher transaminase levels may be associated with higher rates of hepatocyte cell death and turnover. However, the current study only included individuals with HCV genotype 1; therefore, the role of HCV genotype could not be examined further. Others have examined the association between hepatic injury—as measured by ALT/AST levels—and viral diversity [Nagayama et al., 1999; Bozdayi et al., 2000; Farci et al., 2006; Hanada et al., 2007]. In a pilot study of five patients, reduced transaminase levels were associated with decreased immune pressure targeting the E2 region, suggesting that the vigor of the immune response may drive both ALT elevations and viral diversity [Hanada et al., 2007]. HVR1 diversity also correlates with ALT levels in perinatally infected children [Farci et al., 2006]. Unfortunately, these studies did not evaluate compartmentalization or patients co-infected with HIV and HCV as included in the current study. In theory, the development of viral compartmentalization may serve as a defense mechanism by which the virus hides for certain immunologic responses and/or impacts the degree of immune-mediated liver injury, although this hypothesis has not been adequately explored to date.

Limitations to the current study include the modest number of study patients evaluated in this pilot study, the possible lack of generalizability across HCV genotypes, and an inability to evaluate compartment-specific HCV variants over time. As well, by sequencing a limited number of variants and a small portion of the HCV genome, the true magnitude of viral

diversity and compartmentalization was likely underestimated. As discussed elsewhere, there are several methods for evaluating viral compartmentalization; however, there is no consensus as to which are most accurate. Nonetheless, the utilization of several complementary methods—as performed here —provides the most reliable assessment of viral compartmentalization [Zárate et al., 2007].

The current study focused on the viral *envelope* as a critical determinant of HCV replication, cell tropism, and compartmentalization. However, non-structural genomic regions are also involved in viral replication, tropism, and/or compartmentalization. For instance, diversity in the 5' UTR/IRES affects translational efficiency, implying that HCV has adapted for replication in a cell type-dependent manner [Laporte et al., 2000, 2003; Lerat et al., 2000; Forton et al., 2004; Di Liberto et al., 2006; Vera-Otarola et al., 2009]. Our group also reported that UTR diversity was associated with plasma HCV RNA [Blackard et al., 2004], although this has not yet been examined in extrahepatic sites. As well, NS5A variability modifies its transcriptional activity and regulates virus-host interactions in a cell type-dependent manner [Pellerin et al., 2004]. Similarly, NS5B variability impacts HCV RNA levels and treatment outcome in vivo [Lohmann et al., 2000; Lévêque and Wang, 2002; Hamano et al., 2005; Itakura et al., 2005; Le Pogam et al., 2008; Blackard et al., 2010]. We identified previously NS5B compartmentalization in PBMCs even when E1/E2 compartmentalization was absent [Blackard et al., 2012].

Multiple explanations may support the presence of divergent viruses in the serum and liver. First, different viral variants may possess distinct replication kinetics. For variants that replicate at low levels, this could provide a means of avoiding immunologic surveillance and maintaining persistent infection. Alternatively, liver-specific variants could represent defective genomes that cannot be packaged and released into the peripheral circulation or newly emerged variants that have not yet entered the circulation. Finally, it is possible that rapid elimination of major viral variants by neutralizing antibodies leads to over-representation of minor variants over time; however, longitudinal analysis of viral variants will be necessary to explore such a possibility in the future. Importantly, the association between compartmentalization and treatment outcome has not been examined to date in the context of direct-acting antiviral agents. These findings imply that evaluating viral diversity—including drug resistance patterns—in the serum/plasma only may not adequately represent viruses replicating within the liver. This potential divergence has implications for the effective management of chronic HCV infection and deserves careful consideration in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Consensus E1/HVR1 nucleotide sequences from 14 pairs of matched plasma and liver biopsies analyzed using a Bayesian inference approach. Relevant posterior probabilities greater than 90% are shown. Relevant bootstrap values >700 out of 1,000 from a Neighbor-Joining approach are shown in parentheses. Closed circles denote HCV genotype 1 references, while open circles denote HCV genotype 2–6 references.

*

AALVVAQLLRIPQAIMDMIAGAHWGVLAGIAYFSMVGNWAKVLVLLLFAGVDAE--THVTGGNAGR^TTAGLVGLLTPGA^KQNIQLIN

Cin03 - Liver biopsy (0)
V.....--.....
 Cin03 - Plasma
A.....--.....
 Possible signature
--.....

Cin05 - Liver biopsy (4)
Q--.H.T....Q.T...A...S.....
 CIN05 - plasma
G--.T.S....Y.A...T...V.....
 Possible signature
--.....Q.T...A...S.....

Cin07 - Liver biopsy (7)
Q--...S....GH.....ST.A.....
 Cin07 - Plasma
S--...T....AS.....TV.P.....
 Possible signature
Q--...S....GH.....ST.A.....

Cin10 - Liver biopsy (1)
--.....A.....
 Cin10 - Plasma
--.....V.....
 Possible signature
--.....A.....

VCU04 - Liver biopsy (0)
V...L.....
 VCU04 - Plasma
A...F.....
 Possible signature

Fig. 2.

Signature sequence analysis showing amino acid positions at which the distributions of liver- and plasma-specific E1/HVR1 variants are significantly different. Shown are the consensus amino acids at each position relative to the reference strain H77. Numbers in parentheses indicate the number of amino acid signatures between the plasma-liver sequences. The underlined amino acids represent signatures, while the signature amino acid denoted by an asterisk (*) is shared by two individuals.

TABLE I

Demographic and Clinical Characteristics of Patients Co-infected With HIV and HCV

Characteristic	
Age at enrollment (years) $\pm$ SD	50.5 $\pm$ 9.2
Race	
African American	12 (85.7%)
Caucasian	2 (14.3%)
Gender (% male)	11 (78.6%)
Log ₁₀ HIV viral load (copies/ml) $\pm$ SD	4.6 $\pm$ 0.6
Log ₁₀ HCV viral load (IU/ml) $\pm$ SD	6.3 $\pm$ 0.6
Median CD4 cell count (cells/mm ³) $\pm$ SD	342.0 $\pm$ 119.5
AST (IU/ml) $\pm$ SD	85.5 $\pm$ 89.2
ALT (IU/ml) $\pm$ SD	56.0 $\pm$ 80.6

IU, international units; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, standard deviation.

TABLE II

Compartmentalization of HCV E1/HVR1 Sequences in the Liver Versus the Plasma

Patient ID	Phylogenetic compartmentalization ^a	Slatkin-Maddison test	Mantel's test
Cin01	No	NS	NS
Cin02	No	NS	NS
Cin03	Yes (4)	0.088	NS
Cin04	No	NS	NS
Cin05	Yes (6) *	0.002	0.001
Cin07	No	NS	NS
Cin08	No	NS	NS
Cin09	No	NS	NS
Cin10	No	0.071	0.017
VCU01	Yes (2)	NS	NS
VCU02	No	NS	NS
VCU03	No	NS	NS
VCU04	No	NS	NS
VCU05	No	0.0004	NS

NS, not significant (P -value > 0.10).

^aPhylogenetic compartmentalization indicates the presence of clustered sequences from the same location separated by a posterior probability of at least 0.9. Numbers in parentheses indicate the number of clones within a cluster of sequences.

* The asterisk indicates an additional cluster of three sequences. Bold values indicate statistically significant evidence of compartmentalization.

TABLE III

Results of Best Subset Regression for Four Models of HCV Compartmentalization

Model	Adjusted R ²	Model variables
1 = Phylogeny	0.2162	CD4, gender
2 = Mantel's test ^a	0.5273	ALT, AST, log ₁₀ HCV, log ₁₀ HIV, race, gender
3 = Slatkin-Maddison test	0.1536	ALT, AST, age
4 = Models 1, 2, and/or 3	0.2668	Age, race, gender

^aBest predictive model based on highest adjusted R².