Dissociation of Serum and Liver Hepatitis C Virus RNA Levels in Patients Coinfected with Human Immunodeficiency Virus and Treated with Antiretroviral Drugs

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We examined hepatitis C virus (HCV) RNA levels in serum, peripheral blood mononuclear cells (PBMC), and the liver for 135 patients with chronic HCV infections, 44 of whom were human immunodeficiency virus (HIV) positive and treated with highly active antiretroviral therapy (group A), 66 of whom were HIV negative (group B), with abnormal serum alanine aminotransferase (ALT) values, and 25 of whom were HIV negative, with ALT values of ≤1.5 times the normal value (group C). Patients had not been treated with interferon, with or without ribavirin, at the time of the study. A statistically significant correlation between HCV RNA levels in the liver and serum was reproducibly documented, whereas this was inconsistent for serum and PBMC. A comparative evaluation of HCV RNA levels in the liver and PBMC showed significantly lower values for group A than for groups B and C (P < 0.01 and P < 0.0001, respectively). In contrast, HCV RNA levels in serum were significantly higher for group A than for group B (P < 0.001). A dissociation between HCV RNA levels in serum and the liver was found for patients with HIV-HCV coinfections. Although the relative contribution of extrahepatic reservoirs, including lymphoid cells, to HCV RNA levels in serum is unclear, it may be speculated that a low intrahepatic HCV burden is caused by restored immunocompetence after successful antiretroviral therapy in coinfected patients.

Although the introduction of highly active antiretroviral therapy (HAART) has significantly improved the life expectancies of patients with human immunodeficiency virus (HIV) infection, morbidity and mortality rates from liver disease caused by hepatitis C virus (HCV) still represent an overwhelming clinical problem for HIV-infected individuals (26). These patients usually respond poorly to treatment with alpha interferon, with or without ribavirin, which may also occasionally cause severe hepatotoxicity (5, 23). Moreover, there is evidence indicating that coinfecion may accelerate the progression to severe forms of liver disease, such as fibrosing cholestatic hepatitis (29) and cirrhosis (12, 27). Impaired cell-mediated immune responses to HCV are currently thought to be an important cofactor in the failure to control virus-induced intrahepatic necroinflammatory processes (18). It has been suggested that a severe course of liver disease may also result from the restored immunocompetence induced by HAART (15).

Several lines of experimental evidence suggest that higher levels of HCV RNA in the serum are usually found in patients with HCV-HIV coinfections than in patients with HCV infections only (8, 31). Although the pathogenetic basis for this observation has never been fully elucidated, HIV-induced immune system deficiency is generally considered a potentially important factor (9). However, reproducible correlations between HCV RNA levels in serum and CD4+ T-cell counts have not been confirmed (6). For HIV-positive patients treated with HAART, controversial data showing no significant changes (35) or increased (30) or decreased (24) HCV RNA levels in serum have generally been reported. Although there is no convincing evidence in favor of a direct relationship between HCV RNA levels in the serum and the severity of liver lesions for a single HCV infection (14, 22), recent studies suggest an association between HCV RNA levels and the progression to full-blown AIDS (7), indicating that there is a synergism between the two viruses.

Intrahepatic HCV RNA levels in patients with chronic liver disease or in patients who have undergone transplants have been investigated in some studies (11, 22); however, only limited data are currently available for HIV-HCV-coinfected patients (20, 33). The aim of this study was to compare HCV viral load levels in HCV-infected versus HCV-HIV-coinfected persons and to assess quantitative differences in HCV viral burdens among the peripheral blood mononuclear cell (PBMC), liver, and serum compartments for these patient categories. HIV-coinfected patients had already been treated with HAART at the time of the study, thus ensuring adequate immune competence.

MATERIALS AND METHODS

Patients. The following three groups of patients with chronic HCV infections were enrolled in this study: (i) 44 HAART-treated HIV-positive patients (group A), (ii) 66 HIV-negative patients with persistently high alanine aminotransferase (ALT) levels in the serum (group B), and (iii) 25 HIV-negative patients with ALT levels in serum of ≤1.5 times the upper limit of what is considered normal who were part of a long-term prospective survey (4) (group C). Patients in groups B and C were enrolled between February 1998 and December 1999, whereas patients in group A were enrolled between March 1999 and July 2000. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the a priori approval by the Institutional Review Board of IRCCS.
Policlinico San Matteo. All patients gave their informed consent to donate part of the blood and liver tissue taken for diagnostic purposes to the research project. HIV-positive patients were referred to our center from ad hoc outpatient clinics for intravenous drug users where antiretroviral therapy was already instituted. The median estimated duration of HIV infection as documented by the concentration of HIV RNA copies per milliliter of serum in plasma was 12 years (range, 2 to 16 years). For all patients, the inclusion criteria were the presence of circulating anti-HCV antibodies, a positive result for HCV RNA in serum by nested reverse transcription-PCR (RT-PCR), the availability of a diagnostic liver biopsy, and no previous treatment with interferon, with or without ribavirin. The Knodell score was employed to evaluate the histological grading score (histologic activity index [HAI]) and the fibrosis stage (16). Additional requirements for HIV-infected patients were that they were between the ages of 18 and 60 years, consistently had HIV RNA levels in plasma of <12,000 copies/ml, and had ≥200 CD4+ T cells/μl in the 16 weeks preceding the biopsy procedure. All group A patients were treated with different combinations of antiretroviral drugs, including nucleoside reverse transcriptase inhibitors (zidovudine, didanosine, lamivudine, and stavudine), nonnucleoside reverse transcriptase inhibitors (efavirenz, nevirapine, and delavirdine), and protease inhibitors (PIs; saquinavir, indinavir, ritonavir, and nelfinavir), for a median time of 21 months (range, 2 to 51 months). Specifically, 30 of the 44 patients received a therapeutic regimen including PIs, whereas the remainder were treated with nucleoside reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors. Additional data, such as the estimated duration of HIV infection as well as the HIV viral load and CD4+ T-cell count before the initiation of therapy, were available for 38 of the 44 patients. The median CD4+ T-cell count before the first line of antiretroviral therapy was 118 cells/μl of blood (range, 0 to 772 cells/μl), and the median HIV RNA level in plasma was 11,500 copies/ml (range, 83 to 1,000,000 copies/ml), whereas these values were 226 cells/μl (range, 8 to 812 cells/μl) and 5,257 copies/ml (range, 118 to 1,000,000 copies/ml), respectively, prior to the initiation of HAART.

RESULTS

Demographics, biochemical and histologic disease indexes, HCV genotypes, and HIV parameters. As shown in Table 1, the median age of group A patients was significantly lower than those of group B (P < 0.001) and C (P < 0.001) patients. The median age for group C was also higher than that for group B (P < 0.05). The median ALT values as well as the degree of liver inflammation and fibrosis were similar for groups A and B (P > 0.05). Most patients in group C had normal ALT levels. There was a higher prevalence of genotypes 1 and 4 and a lower prevalence of genotypes 2 and 3 in group A (P = 0.011). HIV infections in group A patients were well controlled by HAART, as documented by the very low median HIV RNA level in plasma (25 HIV RNA copies/ml; range, 25 to 10,939) and the high median CD4+ T-cell count (470 CD4+ T cells/μl of blood; range, 196 to 1,198) at the time of liver biopsy.

Quantification of HCV RNA in liver, PBMC, and serum. Liver and serum samples were available from all patients, whereas PBMC samples were obtained from 40 of 44 (90.9%) patients in group A, 51 of 66 (77.2%) patients in group B, and all 25 (100%) patients in group C.

Upon stratification of each of the three groups of patients by demographic characteristic (age, >40 years or ≤40 years), HCV genotype (types 1 and 4 or types 2 and 3), histological activity grade (HAI score of 0 to 3, 4 to 9, or >9), and degree of fibrosis (0 to 1, 2 to 3, or 4), no significant difference between strata was detected for HCV RNA levels in the liver, PBMC, or serum. For group A, there were no significant differences in HCV RNA levels in the liver, PBMC, and serum between patients with or without detectable HIV RNA levels and high (>350) or low (≤350) CD4+ T-cell counts (data not shown). In addition, for group A, no statistically significant differences in HCV RNA levels in the liver, PBMC, and serum were detected between patients with an estimated duration of HIV infection of 10 years or less and those infected more than 10 years earlier, between patients treated for 12 months or less and those treated for more than 12 months, and between patients treated with therapeutic regimens which did or did not include PIs. A comparison of HCV RNA levels in the liver, PBMC, and serum among patients from groups A, B, and C showed sta-
Assuming a natural reading, the text states that statistically significant differences were observed in the median hepatic HCV RNA level between group A (16,146 HCV RNA copies/μg of total liver RNA; range, 469 to 131,578) and groups B and C (68,750 HCV RNA copies/μg of total liver RNA; range, 250 to 1,250,000, and 250,000 HCV RNA copies/μg of total liver RNA; range, 30,000 to 750,000, respectively). Similarly, the median HCV RNA level in PBMC was significantly lower for group A (937 HCV RNA copies/μg of total PBMC RNA; range, 0 to 8,333) than for groups B (4,166 HCV RNA copies/μg of total PBMC RNA; range, 0 to 62,500) and C (6,250 HCV RNA copies/μg of total PBMC RNA; range, 0 to 35,714). Preliminary experiments on specimens obtained from four patients indicated that the virus was predominantly localized in the B-cell subset (data not shown).

In contrast, HCV RNA levels in the serum showed an opposite trend, being significantly higher in patients from group A (14,810,000 HCV RNA copies/ml; range, 230,000 to 63,470,000) than in patients from group B (4,729,500 HCV RNA copies/ml; range, 199,000 to 74,620,000), although they were not significantly different from those in patients from group C (11,530,000 HCV RNA copies/ml; range, 252,000 to 118,900,000).

For all three groups of patients, a statistically significant correlation was found between HCV RNA levels in the liver and the serum, although the correlation was less consistent between HCV RNA levels in the serum or liver and those in PBMC.

### DISCUSSION

In this study, we have reported a systematic quantitative analysis of HCV RNA in different body compartments (serum, liver, and PBMC) for patients with HCV infection only or with HCV-HIV coinfection. Statistically significant differences emerged from the evaluation of a substantial number of patients in each category. However, even though differences between the groups were statistically significant, a considerable overlap was consistently documented among patient categories.

We were able to confirm previous surveys that reported a significant correlation between HCV RNA levels in the liver and the serum for HCV-infected patients (11, 22) and for a
small group of HIV-HCV-coinfected patients (33). However, for the present study, coinfected patients were referred to us while they were already on antiretroviral treatment, thus significantly reducing the effect of HIV-induced immune system suppression on HCV replication, which may be a profound influence (3, 30). Because of this, it is important to emphasize that >50% of the patients had virtually undetectable HIV levels and only six patients had HIV titers exceeding 1,000 copies/ml.

The discrepancy between low hepatic HCV RNA concentrations and high HCV RNA concentrations in the serum for HIV-HCV-coinfected patients may have several explanations. Increased HCV RNA levels in the serum suggest that there is an enhanced release of HCV virions into the circulation or enhanced viral replication in extrahepatic sites. However, the former hypothesis is probably untenable, since at steady state there should be equilibrium between virus production and clearance. On the other hand, recent findings suggested that the suppression of HIV-1 RNA and increases in CD4+ cell counts induced by HAART are indeed associated with significant increases in HCV RNA that are sustained over time (3). An alternative, and probably more plausible, explanation is that since HCV appears to replicate in certain subsets of PBMC (19), under basal conditions of co-infection, HIV and HCV would compete for this reservoir. With the successful clearance of HIV-infected lymphocytes, HCV RNA levels in serum would increase as more susceptible target cells become available.

Low hepatic HCV RNA levels may instead result from successful therapeutic responses to antiretroviral drugs. Indeed, it has been shown that the CD4+ T-cell reconstitution induced by HAART is responsible for better control of hepatic HCV replication, and vigorous CD4+ T-cell responses are associated with slowly progressive or asymptomatic chronic liver disease (2). It is well known that therapeutic regimens which include PIs significantly improve T-cell responses in HIV-infected subjects by enhancing CD4+ and CD8+ T-cell counts. Indeed, the sustained disappearance of HCV RNA from patients responding to HAART, most likely through the restoration of cytolytic T-cell responses, has been repeatedly reported (10, 24, 34).

The correlation of HCV RNA levels in PBMC with those in the liver and serum for the three groups of patients was less consistent. Controversial data on the actual ability of HCV to replicate in PBMC have been reported (17, 21), suggesting a limited contribution of PBMC to the total HCV RNA burden. In addition, no clear-cut differences between the three groups of patients examined were observed. However, it was not possible to distinguish between active viral replication and the mere presence of HCV RNA in lymphoid cells, as we did not specifically investigate the presence of the HCV RNA minus strand, which some investigators believe is indicative of viral replication in this cellular compartment (22). Preliminary evidence suggests that the HCV E2 envelope protein directly interacts with CD81, a putative HCV receptor (25), on B-cell membranes by inducing cross-linking of the B-cell coreceptor complex with a membrane immunoglobulin (28). This may significantly lower the B-cell activation threshold in the ab-

![FIG. 1. HCV RNA levels in liver (a), PBMC (b), and serum (c) from patients with HIV-HCV coinfections (group A), HCV infections only, with abnormal ALT levels (group B), and HCV infections only, with ALT values of ≤1.5 times the upper limit of normal (group C). Horizontal lines represent medians.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum vs liver</th>
<th>Serum vs PBMC</th>
<th>Liver vs PBMC</th>
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<td></td>
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<tr>
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<tr>
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<td>0.3700</td>
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*HCV RNA loads were calculated in copies per milliliter for serum samples and in copies per microgram of RNA for liver and PBMC samples.*
ence of viral replication. In this regard, note that, in agreement with these findings, our study showed that HCV RNA was predominantly localized in the B-cell subset and recent data have reproducibly shown productive HCV infections of B-cell lines isolated from patients with chronic HCV infection (32). Further studies are needed to fully elucidate the mechanisms of HCV pathogenesis and to clarify the role of virus replication in different compartments in both HIV-seronegative and HIV-seropositive individuals.

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