The Presence of Active Hepatitis C Virus Replication in Lymphoid Tissue in Patients Coinfected with Human Immunodeficiency Virus Type 1

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The existence of extrahepatic replication sites of hepatitis C virus (HCV) remains controversial. Highly strand-specific Tth-based reverse transcriptase–polymerase chain reaction was used to search for the presence of viral RNA negative strand in lymph nodes from 16 patients with AIDS and in peripheral blood mononuclear cells (PBMC) from 14 other human immunodeficiency virus (HIV)–positive patients. Negative-strand HCV RNA was detected in lymph node samples from 10 patients (63%) and in PBMC from 5 (36%). This suggests that, at least under circumstances of impaired immunity associated with HIV infection, HCV is lymphotropic in vivo. However, the clinical implications of these findings need to be further investigated.

Hepatitis C virus (HCV) has been suggested to be a lymphotropic virus: Some investigators have reported the detection of HCV RNA negative strand, which is a replicative intermediate, in peripheral blood mononuclear cells (PBMC) [1], and it has been reported that human T and B cell lines are capable of supporting a productive infection [2]. However, in vitro cell culture studies do not necessary reflect actual events occurring in an infected host, and the specificity of reverse transcriptase–polymerase chain reaction (RT-PCR) for the detection of HCV RNA negative strand has been recently questioned [3]. Thus, subsequent studies using assays optimized for strand specificity failed to demonstrate the presence of viral negative strand in PBMC [3, 4].

Even less is known about HCV replication in other lymphoid tissue, as studies using strand-specific assays are few; in a single published study, Lanford et al. [3] did not detect HCV RNA negative strand in multiple organs, including lymph nodes, from a chronically infected chimpanzee. However, the results of this study should be interpreted with caution, as it was conducted on a single ape that had a low level of viral replication in the first place.

Indeed, the results of these studies should be considered inconclusive, as the replication at extrahepatic sites could be below the sensitivity limit of current strand-specific assays; in fact, these seem to be at least 1 log less sensitive than standard RT-PCR [3, 4]. Another obstacle to a study of lymphoid tissue replication is the general unavailability of biologic material other than PBMC.

To help resolve the important but controversial issue of HCV lymphotropism, we searched for the presence of HCV RNA negative strand in PBMC and lymph nodes from a group of human immunodeficiency virus (HIV)-infected subjects. We reasoned that the commonly encountered elevation in HCV replication in this group [5] would facilitate the detection of HCV RNA negative strand and that tissue samples could be obtained during autopsy of patients who died of AIDS-related complications. In addition, studying HIV-infected subjects seems important, as the presence of HCV replication in lymphoid tissue could hypothetically affect HIV, much like what is described for such common opportunistic infections as Mycobacterium tuberculosis or cytomegalovirus [6, 7]. To ascertain strand specificity of our RT-PCR assays, cDNA synthesis was done at high temperature using the thermostable enzyme Tth [3, 4].

Materials and Methods

Biologic samples. PBMC were collected from 14 HIV-1–positive drug addicts who were found to be HCV RNA–positive in serum. All were hepatitis B surface antigen–negative, and none had received any antiviral therapy before the study. PBMC were isolated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation, washed three times with PBS (pH 7.4), and stored frozen at −80°C until use. RNA was extracted by means of a modified guanidinium thiocyanate–phenol-chloroform technique using commercially available kits (Ultraspec 2 and Ultraspec 3; Biotex Laboratories, Houston). The amount of extracted RNA loaded into the RT-PCR corresponded to 2–3 × 10⁶ cells or 20 μL of serum.

Tissue samples were collected from 16 HIV-1–infected drug addicts who died from AIDS-related complications between March 1997 and February 1998. All were HCV RNA–positive in serum, and 2 were hepatitis B surface antigen–positive. Mediastinal lymph node samples were obtained from each subject during rou-
tine autopsy conducted within 48 h of death and were stored at
−80°C until analysis. As positive controls, liver tissue samples
from 8 of them were collected as well.

RNA was extracted after tissue homogenization as described
above. For each sample, two different amounts of extracted RNA
(6 μg and 1 μg, as determined by spectrophotometry) were initially
used for RT-PCR. These values were not chosen arbitrarily, since
in preliminary studies we determined that RNA concentrations >6
μg markedly inhibit reaction. Whenever the sample was positive
at a concentration of 1 μg of RNA, the RNA template was serially
10-fold diluted in water for the purpose of titer determination.

Strand-specific RT-PCR with Tth. The Tth-based RT-PCR de-
tection of the negative HCV RNA strand was done as described
elsewhere [4]; in brief, the cDNA was generated in 20 μL of
reaction mixture containing 50 μM sense primer (5’-A/GAC/TCA-
CTCCCCCTGTGAGGAAC-3’; nt 35–55), 1 × RT buffer (Perkin
Elmer, Norwalk, CT), 1 mM MnCl₂, 200 μM (each) dNTP, and 5
U of Tth (Perkin Elmer). After 20 min at 65°C, Mn²⁺ was chelated
with 8 μL of 10× EGTA chelating buffer (Perkin Elmer), 50 μM
antisense primer (5’-TGA/GTGCACGGTCTACGAGACCTC-3’;
nt 342–320) was added, the volume was adjusted to 100 μL, and
the MgCl₂ concentration was adjusted to 2.2 mM. The amplifica-
tion was done as described [4]. The final product was analyzed by
agarose gel electrophoresis and Southern hybridization with a 32P-labeled internal oligoprobe. As described previously [4], this assay
could detect ~100 genomic equivalent (Eq) molecules of the cor-
rect strand while unspecifically detecting ≥10⁴ genomic Eq of the
incorrect strand. When 1 or 6 μg of total cellular RNA extracted
from normal human livers was added, the sensitivity of the reac-
tions was lowered by no more than 1 log, while the specificity of
the assay was not affected.

RT-PCR with Moloney murine leukemia virus (MMLV) RT.
MMLV RT–based detection of HCV RNA has been described in
detail elsewhere [4]. This assay was found to be very sensitive but
totally unspecific. As previously reported [4], it was capable of
detecting 10 genomic Eq of the correct template, but at the same
time, it also unspecifically detected ≥10⁴ genomic Eq of the in-
correct strand.

Extensive measures, outlined elsewhere [4], were used to pre-
vent and detect carryover contamination. All RT-PCR runs in-
cluded positive controls consisting of end-point dilutions of respec-
tive RNA strands, and negative controls included normal livers,
lymph nodes, and PBMC.

All titers were determined by analyzing 10-fold serial dilutions
of the RNA template, since at this dilution, the results were reliably
reproducible. The titers were calculated by assuming that the end-
point dilution contains 10 genomic Eq when tested by the MMLV
RT–based assay and 10² genomic Eq when tested with the Tth-
based assay. The Tth strand-specific assay was used to determine
the titers of both positive and negative strands; however, the
MMLV-based assay was used independently to confirm the posi-
tive-strand titers. Statistical analysis was done using SPSS for
Windows (SPSS, Chicago).

Results

HCV RNA in PBMC. All 14 patients from whom PBMC
were collected were positive for the presence of HCV RNA
plus strand in serum and PBMC in titers ranging from 5 × 10¹
to 5 × 10³ genomic Eq/mL and 10² to 10⁴ genomic Eq/2–3
× 10⁶ cells, respectively (table 1). HCV RNA negative strand
was not detected in any of the serum samples; however, 5
PBMC samples were positive for the presence of HCV RNA
negative strand when tested with Tth-based assay in two inde-
pendent experiments (table 1). The mean CD4 cell count of
patients with the detectable presence of viral negative strand
in PBMC was not different from the mean CD4 cell count of
patients with undetectable HCV RNA negative strand. For each sample, two different amounts of extracted RNA
Serum (Eq/mL) and PBMC (Eq/2–3
× 10⁶ cells) were collected were positive for the presence of HCV RNA
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tive-strand titers. Statistical analysis was done using SPSS for
Windows (SPSS, Chicago).

Table 1. Detection of positive and negative strands of HCV RNA
in serum and peripheral blood mononuclear cells (PBMC) in 14 HIV-
infected drug addicts.

<table>
<thead>
<tr>
<th>No.</th>
<th>CD4 cells/mm³</th>
<th>Serum (Eq/mL)</th>
<th>PBMC (Eq/2–3 × 10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ strand</td>
<td>– strand</td>
<td>+ strand</td>
</tr>
<tr>
<td>1</td>
<td>785</td>
<td>5 × 10⁴</td>
<td>Neg</td>
</tr>
<tr>
<td>2</td>
<td>405</td>
<td>5 × 10⁴</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>5 × 10⁴</td>
<td>Neg</td>
</tr>
<tr>
<td>4</td>
<td>281</td>
<td>5 × 10⁴</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>132</td>
<td>5 × 10⁴</td>
<td>Neg</td>
</tr>
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</tr>
<tr>
<td>14</td>
<td>591</td>
<td>5 × 10⁴</td>
<td>Neg</td>
</tr>
</tbody>
</table>

NOTE. Presence and titers of positive strand were determined indepen-
dently by Moloney murine leukemia virus reverse transcriptase– and Tth-
based assays, while presence of negative strand was determined by Tth-based
assay only. Neg, negative.
In the current study, we demonstrated for the first time the presence of active HCV replication in PBMC and lymph nodes in patients infected with HIV. In our earlier study, using exactly the same techniques as in the current investigation, we failed to demonstrate the presence of HCV RNA negative strands in PBMC from HIV-negative subjects [4]. The reasons for this discrepancy are currently unclear; however, it is likely that, due to the impairment of containment effects of the immune system, extrahepatic replication in HIV-positive subjects is high enough to enable its detection even with less than perfect assays. Nevertheless, the presence of viral replication in lymphoid tissue did not correlate with the CD4 cell count, which implies that immunosuppression alone may be insufficient and additional conditions, such as the presence of adaptive mutations, may be necessary for extrahepatic replication to commence. Similarly, the presence of viral replication in lymphoid tissue did not correlate with the titers of HCV RNA positive strand in serum, suggesting that factors governing HCV replication at extrahepatic and hepatic sites may be different.

Alternatively, HCV replication could be more efficient in activated cells. It could also be speculated that weakened immune pressure against infecting HCV leads to the development or persistence of HCV variants capable of replication in PBMC, and some recent studies on chimpanzees suggest the existence of strains with particular affinity for lymphocytes [8]. Interestingly, the only chimpanzee in that study in which a lymphotropic strain persisted for many years had received immunosuppressive therapy.

The idea that immunosuppression facilitates extrahepatic viral replication is supported by the results of our recent study conducted on HCV-positive liver transplant recipients: While viral RNA negative strand was not detected in PBMC before transplantation, PBMC collected 1 month after transplantation tested positive in 3 of 9 studied patients (unpublished data).

Which population of lymphoid cells is infected by HCV is unclear. However, as lymph nodes in AIDS patients are depleted of dendritic and T cells [9], it is likely that HCV replicates in the cells of monocyte/macrophage lineage or even in B cells. The latter possibility is intriguing, as chronic HCV infection was recently associated with B cell lymphoproliferative disorders, such as mixed cryoglobulinemia and B cell non-Hodgkin’s lymphoma [10]. The concept that B lymphocytes harbor HCV infection in vivo is supported by a recently published study by Zehender et al. [11], who found that HCV RNA associated with PBMC was concentrated in the CD19 cell fraction.

It has been reported that concomitant HIV infection facilitates transmission of HCV [5] and that it accelerates the development of severe liver disease [12], and these effects could be largely attributed to the enhancement of HCV replication in the setting of immunodeficiency. However, there is currently no strong evidence for an opposite effect, and the natural history of HIV infection does not seem to be influenced by concomitant HCV infection [13]. This would be in contrast to some opportunistic infections, such as cytomegalovirus or Mycobacterium tuberculosis, which are likely to play an active role in the pathogenesis of AIDS [6, 7]. However, HCV could infect a different cell population than HIV does.

The prevailing opinion that HCV has no influence on HIV infection does not remain unopposed: One recent report suggested an association between HCV genotype and progression of HIV disease [14], and in some studies, perinatal transmission of HIV was found to be more frequent when mothers were coinfected with HCV [15].

In summary, we documented the presence of active HCV replication in PBMC and lymph nodes in a large proportion of HIV-infected patients. This suggests that, at least under circumstances of impaired immunity associated with HIV infection, HCV is lymphotropic in vivo. However, the clinical implications of these findings need to be further investigated.

References


