Hepatitis C Virus Persistence after Spontaneous or Treatment-Induced Resolution of Hepatitis C

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It is presumed that resolution of hepatitis C, as evidenced by normalization of liver function tests and disappearance of hepatitis C virus (HCV) RNA from serum, as determined by conventional laboratory assays, reflects virus eradication. In this study, we examined the expression of the HCV genome in the sera, peripheral blood mononuclear cells (PBMC), and, on some occasions, monocyte-derived dendritic cells (DC) long after resolution of hepatitis C by using a highly sensitive reverse transcription (RT)-PCR–nucleic acid hybridization (RT-PCR–NAH) assay. The samples obtained from 16 randomly selected patients (5 with spontaneous and 11 with treatment-induced resolution), monitored for up to 5 years, were studied by qualitative and semiquantitative RT-PCR–NAH and by real-time RT-PCR to detect the HCV RNA positive strand. The replicative HCV RNA negative strand was examined in PBMC after culture with a T-cell proliferation stimulating mitogen. The findings show that HCV RNA was carried in the convalescent-phase sera and/or PBMC in all 16 individuals investigated. Also, DC from six of seven patients were reactive for the HCV genome. Importantly, traces of the HCV RNA negative strand, suggesting progressing virus replication, were detected in the majority of mitogen-stimulated PBMC, including four samples collected 5 years after recovery. Sequencing of the HCV 5’ untranslated region fragment revealed genotype 1b in four of nine individuals examined and genotypes 1a and 2a in three and two patients, respectively. These results imply that HCV RNA can persist at very low levels in the serum and peripheral lymphoid cells and that an intermediate replicative form of the HCV genome can persist in PBMC for many years after apparently complete spontaneous or antiviral therapy-induced resolution of chronic hepatitis C.

Hepatitis C virus (HCV) is a small positive-strand RNA virus of approximately 9,400 nucleotides that chronically infects an estimated 170 to 350 million people worldwide. Of those acutely afflicted, only 15% recover, while the remaining 85% succumb to chronic hepatitis (4). Furthermore, up to one-fifth of the individuals with chronic hepatitis C progress to cirrhosis, and these patients are at a greater risk of developing hepatocellular carcinoma (9).

It is generally accepted that HCV replicates by making a cRNA strand known as the negative or replicative strand. Although the liver is the main site of virus replication, there is an increasing body of evidence for virus propagation in extrahepatic locations, including cells of the lymphatic and the central nervous systems (17, 28). In regard to infection of lymphoid cells, HCV positive and negative strands were detected in the peripheral blood mononuclear cells (PBMC) and the bone marrow from chronically infected patients (26, 29, 37). It was also shown that HCV can propagate in lymphoid cell cultures and that the virus derived is infectious (33, 34). The notion of natural HCV tropism for lymphoid cells is supported by a significant overrepresentation of certain lymphoproliferative disorders in the HCV-infected population. For instance, type II mixed cryoglobulinemia occurs 11 times more frequently in patients with HCV than in those without (6). Also, non-Hodgkin lymphoma appears to be, albeit less strongly, associated with HCV infection (24).

The current RT-PCR-based assay approved for clinical diagnostics, i.e., the Amplicor HCV v2.0 assay (Roche Molecular Diagnostics, Pleasanton, Calif.), detects HCV RNA with a sensitivity of 1,000 virus genomic equivalents (vge) per ml (or 500 IU/ml). Other assays can identify HCV RNA at 52 vge/ml (or 10 IU/ml) (i.e., the Versant HCV RNA qualitative assay; Bayer Corp., Tarrytown, N.J.). This implies that small quantities of HCV occurring either in serum or within cells may escape detection. Therefore, considering the natural history of HCV infection, there exists a possibility that the virus may not be completely eradicated at the time of clinical and serological resolution of hepatitis. This situation may occur following spontaneous recovery or antiviral therapy.

Lymphotropism is a characteristic of many DNA and RNA viruses capable of inducing persistent infection (8, 27). A number of studies, including those with highly hepatotropic hepatitis B virus (19, 30) and woodchuck hepatitis virus (3, 21, 22), have demonstrated that pathogenic virions can persist at low levels in cells of the lymphatic system years after resolution of liver disease, if not for life. In the present study, considering the fact that a symptomatic long-term hepatitis C is a frequent consequence of HCV infection, we asked whether individuals convalescent from hepatitis C may also persistently carry the virus. In addition, although HCV infection of PBMC has been documented in patients with chronic hepatitis C (17, 26, 28, 31,
TABLE 1. Clinical characteristics and detection of HCV RNA in sera and lymphoid cells from patients with serological resolution of hepatitis C

<table>
<thead>
<tr>
<th>Recovery type and patient</th>
<th>Age/sex</th>
<th>Route of infection</th>
<th>Estimated duration of infection (yr)</th>
<th>Antiviral treatment type and duration (wk)</th>
<th>Follow-up after SVR to treatment (mo)</th>
<th>HCV RNA in PBMC DC:</th>
<th>HCV RNA genotype</th>
<th>Over all positivea</th>
<th>HCV RNA positive/total tested (%)</th>
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<td>+</td>
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<td>IFN/R (48)</td>
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<td>IVDU</td>
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<td>IFN/R (24)</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>NT</td>
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</tbody>
</table>

**Notes:**
- a: M, male; F, female; Blood tx, blood transfusion; NA, not available or not applicable; ND, not determined; NT, not tested; IFN, alpha interferon; R, ribavirin; 12a and 12b, samples from the same patient collected at 30 (a) and 15 (b) months after clinical recovery.
- b: HCV RNA measured by the Roche Amplicor HCV v2.0 assay (sensitivity, 1,000 vge/ml).
- c: HCV RNA measured by nested RT-PCR–NAH with UTR and E2 region-specific primers (sensitivity, ≤10 vge/ml).
- d: HCV RNA measured by nested RT-PCR–NAH with UTR-specific primers (sensitivity, >10 vge/ml).
- e: HCV genotype determined in recovered serum and/or PBMC samples as described in Materials and Methods.
- f: Overall positivity defined as HCV RNA reactivity in either serum, PBMC, or a combination thereof.
- g: Patients with SVR after a 24- to 48-week therapy with IFN or combination treatment with IFN/R.

To assess whether HCV may persist after resolution of hepatitis C, we examined sera and PBMC from individuals with either spontaneous or therapeutically induced resolution of chronic hepatitis C, as evidenced by sustained normalization of biochemical indicators of liver function tests and clearance of HCV RNA from serum, as determined by conventional laboratory assays. In this study, the samples were analyzed using reverse transcription (RT) and nested PCRs followed by Southern blot hybridization of the amplified products to virus-specific probes (RT-PCR–NAH assay; sensitivity, ≤10 vge/ml). Applying this enhanced detection method, we found HCV genomes in sera and/or PBMC in all recovered individuals investigated and observed that the genome can persist for at least 60 months after apparently complete sustained clearance of HCV RNA, as assessed by the current RT-PCR assays. In addition, traces of the virus RNA negative strand were identified in mitogen-stimulated PBMC collected at the end of the observation period, suggesting that virus replication persisted in lymphoid cells long after clinical resolution of hepatitis C.

**MATERIALS AND METHODS**

**Patients and samples.** Parallel serum and PBMC samples from 16 randomly selected patients (12 men and 4 women; ages 32 to 57), who had recovered from HCV infection based on clinical, biochemical, and virological criteria, were investigated in this study (Table 1). Five persons (patients 1 to 5) had evidence of past HCV infection; i.e., they were seropositive for anti-HCV (EIA; Abbott Diagnostics, Maidenhead, United Kingdom) and had undetectable levels of HCV RNA in serum (Roche Amplicor HCV v2.0) and normal levels of alanine aminotransferase in serum. Four of these five individuals had risk factors for exposure to HCV, i.e., multiple transfusions of factor VIII since childhood because of hemophilia (n = 1) or long-term intravenous drug use (IVDU) (n = 3) (Table 1). Prior to inclusion in this study, these five subjects had been monitored regularly for a period of between 12 and 36 months. The blood results showed normal liver function and undetectable HCV RNA in the serum or three or more occasions, as determined by the Roche Amplicor HCV v2.0 assay using total RNA extracted with guanidine thiocyanate, as recommended by the manufacturer (sensitivity, 1,000 copies/ml).

Eleven other patients were known to have chronic hepatitis C, as proven by biopsy and serology; i.e., they were seropositive for anti-HCV and HCV RNA and had received antiviral treatment for 24 or 48 weeks (Table 1). Ten of these eleven patients received combination treatment with alpha interferon 2b (Intron-A) at 3 million U three times per week and ribavirin at 1,000 or 1,200 mg daily (Schering-Plough, Kenilworth, N.J.), while one patient received alpha interferon monotherapy with Roferon-A (Roche, Welwyn Garden City, United Kingdom) at 4.5 million U three times a week. Although some of these patients acquired HCV infection due to IVDU in the past (Table 1), none of these patients showed sustained virological response (SVR) to antiviral treatment; i.e., HCV RNA was undetectable in the serum 6 months after the end of treatment, and every 6 to 12 months liver function tests and HCV RNA were checked. During

37), a similar investigation after resolution of hepatitis C has not been conducted. This line of research is important, since silent persistence of small quantities of HCV might have potentially epidemiologic implications and could be responsible for reactivation of the disease after termination of antiviral therapy or in immunocompromised patients.
the follow-up, HCV RNA was not detectable in these individuals by the Roche Amplicor HCV v2.0 assay (patients 6 to 16; Table 1). In addition, serum samples from the five patients with the 5-year follow-up (patients 6 to 10; Table 1) were also screened for the presence of HCV RNA by the NID assay (National Institute of Diabetes and Digestive and Kidney Diseases Core Laboratory at Caltech) and a real-time PCR assay. The liver function tests were repeatedly normal in all 11 cases during follow-up. The samples (serum and PBMC) used in this study were obtained between 12 and 60 months after these 11 patients showed greater expression of both HCV positive and replicative RNA strands relative to that of untreated cells (T. N. Q. Pham and T. I. Michalak, unpublished observations). Therefore, this method was adopted as a routine step in this study.

This approach would enhance identification and expression of its genome, which otherwise were undetectable by the conventional assay. A serum sample and a cDNA-derived sample from patient 16 were analyzed for 50 cycles and the validity of controls (the test samples. The specificity of amplification was confirmed by agarose gel electrophoresis and by sequencing the amplicons and the validity of controls (the test samples. The specificity of amplification was confirmed by agarose gel electrophoresis and by sequencing the amplicons and the validity of controls (the test samples. The specificity of amplification was confirmed by agarose gel electrophoresis and by sequencing the amplicons and the validity of controls (the test samples. The specificity of amplification was confirmed by agarose gel electrophoresis and by sequencing the amplicons and the validity of controls (the test samples. 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The sensitivity and specificity of the detection was established using synthetic HCV RNA positive and negative strand fragments, which were generated by the HCV UTR-E2 fragment clones, as templates. For this purpose, randomly chosen clones were sequenced using amini T7 DNA cycle sequencing system (Promega) to select those carrying a positive or a negative strand. After plasmid linearization, the strands were transcribed with T7 RNA polymerase from a Megascript T7 high-yield transcription kit (Ambion, Austin, Tex.). For negative-strand detection, 5 µg of test RNA in 10 µl of RNA-free TE buffer was denatured for 1 min at 95°C, and then the temperature was lowered to 70°C. All subsequent steps were carried out at this temperature. The RNA sample was supplemented with 10 µl of preheated 2× RT buffer (20 mM Tris-HCl, pH 8.3, with 180 mM KCl, 2 mM MnCl2, 1.6 mM deoxyxynucleoside triphosphate mix, 15 pM UTR1 primer, and 5 µl of tRNA DNA polymerase) and cycled for 2 min at 60°C. cDNA extension was continued for an additional 15 min. Then, 80 µl of preheated PCR mixture containing a chelating buffer (10 mM Tris-HCl buffer, pH 8.3, with 100 mM KCl, 750 µM dGTA, 0.05% Tween 20, and 5% glycerol) with 2.5 mM MgCl2 and 15 pM antisense UTR1 primer was added, and amplification was carried out for 45 cycles. Finally, 10 to 20 µl of the direct PCR product was used as the template for the second round of amplification, which was done under the same conditions as those described for standard RT-PCR. The remaining PCR products were analyzed by agarose gel electrophoresis. The sensitivity of this assay was described above. The strand-specific PCR combined with hybridization analysis was capable of detecting ~5000 to the correct (negative) strand, while unspec-
RESULTS

HCV RNA in sera after resolution of hepatitis C. Sera were collected from individuals monitored for 12 to 60 months after spontaneous or antiviral treatment-induced recovery from chronic hepatitis C. These persons were repeatedly nonreactive for HCV RNA when tested under standard clinical laboratory RNA isolation and RT-PCR amplification conditions. However, the same sera subjected to RNA extraction with Trizol and analyzed by nested RT-PCR–NAH with UTR and/or E2 region-specific primers (sensitivity, ≤10 vge/ml) demonstrated persistent presence of HCV genomes (Table 1). Thus, four of the five individuals with apparently complete virological recovery from self-limiting hepatitis C continued to carry circulating HCV RNA when tested by the in-house assay. Among the persons who resolved hepatitis C due to antiviral therapy, the HCV genome was identified in sera of all of the 11 patients examined. However, two serum samples collected from one individual (patient 12) at 15 and 30 months after recovery gave opposing results. Thus, while the former sample was HCV RNA nonreactive, the latter was evidently positive when tested by nested RT-PCR–NAH. Overall, HCV RNA was detected in 15 (88%) out of 17 serum samples analyzed. The quantities of HCV RNA carried in the late-convalescent-phase sera usually varied between 10 and 10^2 vge per ml, as estimated by densitometric analysis of the RT-PCR–NAH signals. The rigorous precautions undertaken, which included contamination and negative controls at each step of RNA and cDNA preparation and PCR amplification as well as validation of the positive signals and controls by NAH, unequivocally ensured the authenticity of HCV RNA detection.

Quantification of HCV RNA by real-time RT-PCR. Serum samples from 10 patients, 4 with self-limiting and 6 with antiviral treatment-induced resolution of hepatitis C, which were HCV positive by the nested RT-PCR–NAH, were examined for viral RNA load by our in-house-developed real-time RT-PCR (sensitivity, ≤10^2 vge/ml). Of the 10 samples, 4 (cases 1, 3, 6, and 15) showed quantifiable amounts of HCV genome by a Light Cycler (Roche), while the remaining 6 were entirely nonreactive (data not shown). The HCV RNA loads measured in the positive samples were around 5 × 10^2 vge/ml. The results indicated that, while HCV RNA could be detected and quantified in some convalescent-phase sera by real-time RT-PCR, overall this technique provides only a moderate advantage over standard laboratory RT-PCR assays and was not superior to the RT-PCR–NAH protocol normally used for detection of the silent presence of HCV sequences.

Upregulation of HCV RNA expression in mitogen-stimulated PBMC. Taking advantage of previous observations that mitogen stimulation of lymphoid cells may enhance replication of the residing virus and allow a more ready detection of its genome (3, 13), we included cultures of PBMC from the recovered individuals with IL-2 and PHA as a routine step prior to RNA extraction. As illustrated in Fig. 1, this measure allowed for detection of HCV RNA in apparently virus-nonreactive PBMC or augmented HCV RNA expression in cells which, prior to the treatment, displayed weak HCV RNA signals. Using this approach, the HCV RNA positive strand was detected in 13 (81%) out of the 16 cases investigated (Table 1). In contrast, when unstimulated cells were examined, three of the nine tested were found reactive (data not shown).

Detection of HCV RNA in parallel sera and PBMC. Examination of paired sera and IL-2 and PHA-treated PBMC using the RT-PCR–NAH assay with UTR and E2 region-specific primers revealed that in fact all individuals investigated remained HCV RNA reactive (Table 1). Figure 2a illustrates the detection of HCV RNA in three cases after spontaneous recovery from chronic hepatitis C. Of the five individuals examined in this group, the viral genome was identified in three (60%) in both serum and PBMC. The other two (40%) were positive in either serum or PBMC (Table 1). Figure 2b depicts the identification of HCV RNA in parallel serum and PBMC samples collected from three individuals 5 years after appa-
HCV RNA was negative in serum by amplification of the UTR and E2 regions. Interestingly, in case 13, although HCV RNA signals were detected in serum and PBMC of case 2 were reactive for both genomic sequences in cases 2 and 13, but also E2 region-specific primers were used, the same sample was reactive with the E2 primers. PBMC from this patient showed HCV reactivity detectable by ultracentrifugation were HCV RNA negative, except in a single specimen (case 11). This indicated that a potential contamination of extracellular HCV RNA to positive signals detected in DC was unlikely.

**HCV RNA sequence analysis.** Direct sequencing of the nested 5′-UTR amplicons derived from sera and, on some occasions, from in vitro stimulated PBMC allowed for determination of the HCV genotype in 2 of 5 individuals with spontaneous recovery and in 6 of 11 patients with SVR due to antiviral therapy (Table 1). Genotype 1b was identified in four cases, and genotype 1a was identified in three individuals, whereas genotype 2a was prominent in two patients. In two instances (cases 12 and 16), samples obtained at two different time points of follow-up were sequenced. Thus, in patient 12, the sequences from the 15- and 30-month points after resolution of hepatitis were compared. In case 16, the first sample was obtained during symptomatic chronic hepatitis and the second 12-month sample after clinical recovery. As documented in Fig. 4, the sequences detected were identical in both samples from each of the patients, and they were of genotype 1b (10), albeit there were minor differences between these sequences and the prototype sequence of HCV genotype 1b.

**HCV RNA in DC.** DC derived from the monocyte population of PBMC were available for examination from seven convalescent cases (Table 1). When comparable amounts of total RNA extracted from these DC were transcribed and cDNA was analyzed by the nested RT-PCR–NAH, six cell preparations were found to be HCV RNA reactive (Fig. 5). Interestingly, DC from one individual (case 12, sample collected 30 months after SVR) showed HCV RNA despite the fact that the total PBMC population was HCV nonreactive by RT-PCR–NAH (Table 1). DC culture supernatants concentrated by ultracentrifugation were HCV RNA negative, except in a single specimen (case 11). This indicated that a potential contribution of extracellular HCV RNA to positive signals detected in DC was unlikely.

**HCV RNA negative strand in PBMC.** As indicated, 81% of the PBMC samples examined after culture in the presence of IL-2 and PHA displayed the HCV RNA positive strand (Table 1). To determine whether this expression was accompanied by the presence of the replicating virus genome, the HCV RNA was analyzed by the nested RT-PCR–NAH using both UTR and E2 region-specific primers. Total RNA was extracted from sera (S) and PBMC (P) from case 2, with self-limited acute infection, and from case 13, with therapeutically induced resolution of hepatitis C, 10 (91%) were found positive for HCV RNA in serum, and 9 (90%) out of 10 whose IL-2- and PHA-treated PBMC were tested also carried viral sequences. Although, one should be cautioned to note that detection of the HCV RNA positive-strand sequences by RT-PCR does not necessarily reflect the existence of the entire virus genome.

**FIG. 2.** Detection of HCV RNA in parallel serum and PBMC samples from individuals convalescent from chronic hepatitis C. Sera (S) and IL-2- and PHA-stimulated PBMC (P) from individuals who (a) resolved hepatitis spontaneously and were monitored for at least 12 months thereafter or (b) resolved hepatitis due to antiviral therapy and were monitored for 60 months after the apparently complete virological recovery were tested for HCV RNA by nested RT-PCR–NAH assay. Serum and PBMC from a healthy individual (Healthy) and water (DW and NW) and cDNA-free mock (M) samples were used as negative controls. Positive samples showed the expected 244-bp band.

**FIG. 3.** Identification of HCV RNA in parallel samples of serum and PBMC by RT-PCR–NAH using both UTR and E2 region-specific primers. Total RNA was extracted from sera (S) and PBMC (P) from case 2, with self-limited acute infection, and from case 13, with therapeutically induced recovery from chronic hepatitis. Serum and PBMC from a healthy individual and a patient with clinically evident chronic hepatitis C (CH) served as negative and positive controls, respectively. Water (DW and NW) and cDNA-free mock (M) samples were included as contamination controls. Positive samples showed the expected 244-bp band for UTR and the 442-bp fragment for the E2 region by Southern blot hybridization analysis.

**TABLE 1.** Identification of HCV RNA in parallel samples of serum and PBMC by RT-PCR–NAH using both UTR and E2 region-specific primers. Total RNA was extracted from sera (S) and PBMC (P) from case 2, with self-limited acute infection, and from case 13, with therapeutically induced recovery from chronic hepatitis. Serum and PBMC from a healthy individual and a patient with clinically evident chronic hepatitis C (CH) served as negative and positive controls, respectively. Water (DW and NW) and cDNA-free mock (M) samples were included as contamination controls. Positive samples showed the expected 244-bp band for UTR and the 442-bp fragment for the E2 region by Southern blot hybridization analysis.
negative strand, which is considered to be a reliable indicator of actually progressing replication, was quantified. Since this replication intermediate normally occurs at frequencies lower than those of the positive strand, the replicative strand was examined only in PBMC which were found reactive for the positive strand. Figure 6 illustrates the detection of the HCV RNA negative strand in IL-2- and PHA-treated PBMC isolated from patient 4 with a 12-month follow-up after spontaneous recovery and from four individuals with follow-up between 12 and 60 months after apparently complete SVR due to antiviral treatment (Table 1). Overall, this form of RNA was detected in 9 (75%) out of 12 PBMC samples investigated. The expression of the replicative strand varied between 30 and 3 × 10^4 vge/μg of total cellular RNA, as estimated by quantification of hybridization signals. It is of note that culture of PBMC with IL-2 and PHA enhanced expression of the HCV RNA negative strand by approximately twofold (Fig. 6).

FIG. 4. Nucleotide sequence alignment of the 5′-UTR fragments of HCV RNA amplified from serum or PBMC of individuals with apparently complete serological resolution of chronic hepatitis C following antiviral therapy. Samples from case 12 were obtained 15 (PBMC) or 30 months (serum) after sustained response to treatment. In case 16, the first sample was taken during symptomatic chronic hepatitis (Chronic) and the second 12 months after evidence of sustained clinical resolution of hepatitis C. The sequences obtained were aligned with the prototype HCV genotype 1b (10). Nucleotide sequences from the patients’ samples identical to those in the HCV genotype reference (top line) are shown as dashes, and differences are identified by letters.

FIG. 5. Expression of the HCV RNA positive strand in monocyte-derived DC from individuals with long-term recovery from chronic hepatitis C. Cultured DC (C) and concentrates of their culture supernatant (S) were tested for HCV RNA by nested RT-PCR–NAH. Three micrograms of DC RNA and all RNA extracted from the pellet obtained after ultracentrifugation of 10 ml of DC culture supernatant were used for analysis. Water (DW and NW) instead of cDNA and mock (M) sample treated as a test RNA sample served as negative controls, while DC and their culture supernatant derived from a patient with chronic hepatitis C (CH) were used as positive controls. Hybridization signals showed 244-bp bands.

FIG. 6. Detection of the HCV RNA negative strand in PBMC from individuals with clinical and apparently complete virological resolution of hepatitis C. Total RNA was extracted from IL-2- and PHA-stimulated PBMC isolated from individuals with self-limited (case 4) or therapeutically induced (cases 7, 11, 12, and 16) recovery from hepatitis C and from control untreated (UT) and IL-2- and PHA-treated (T) PBMC obtained from a healthy donor and a patient with actively progressing chronic hepatitis C (CH). RNA was reverse transcribed and cDNA was amplified by PCR with HCV RNA negative strand-specific 5′-UTR primers. Serial dilutions of a synthetic HCV RNA negative strand (sHCV RNA neg) amplified in parallel were used as semiquantitative standards. Water (DW and NW) and mock (M) samples were used as negative and contamination controls. The positive signals (244-bp fragments) were visualized by hybridization to the recombinant HCV UTR-E2 probe. Values under the panel represent relative densitometric units (DU) given by positive hybridization signals.
investigation. This finding is not surprising, considering that several laboratories have shown the presence of the HCV RNA replicative strand in peripheral and organ lymphoid cells of patients with chronic hepatitis C (7, 17, 23, 37), as we also found in this study (Fig. 6). The present data clearly indicate that carriage of HCV in peripheral lymphoid cells is not terminated at the time of clinical resolution of chronic hepatitis C, but rather it subsides to a level that is not readily detectable by the currently used laboratory assays, and the virus genome persists at these low levels for a long time after apparently complete recovery.

In the present study, culture of lymphoid cells with IL-2 and PHA markedly enhanced detection of both the HCV RNA positive and replicative strands. PHA is a potent nonspecific inducer of T-cell proliferation, whereas IL-2 is a cytokine important for T-cell growth and in vitro survival (12). In this context, it has been shown that T-cell (11, 25, 33) as well as B-cell (23) lines can be infected in culture by HCV, although the infection was usually inefficient and transient. Furthermore, the most recent and unambiguous study documented that HCV infects B cells and that these cells are capable of producing infectious HCV virions (35). Although the molecular mechanism of mitogen-induced upregulation of HCV replication and genome expression in lymphoid cells is unknown and will require separate studies, this finding is not unique. Similar observations have been made in infections with other viruses which display a tropism for lymphoid cells, including serologically occult infection caused by woodchuck hepatitis virus that invariably involves the host’s lymphatic system (3, 20). The strategy of enhancing HCV genome detection in PBMC established in this study provides a valuable aid to the diagnosis of serologically silent HCV persistence. Subsequent work should include a definition of culture conditions under which an increased HCV genome expression can be achieved in both T and B cells. This might further enhance the identification of HCV genomes in situations where low virus loads are expected and where sera and resting lymphoid cells remain seemingly virus nonreactive.

In light of recent findings demonstrating that monocyte-derived DC from patients with chronic hepatitis C might be a reservoir of replicating HCV (2, 7), we assessed whether DC obtained from the recovered individuals by a similar procedure also carry the virus. Here, we provide molecular evidence for the presence of HCV RNA in DC from six of the seven cases examined (Table 1 and Fig. 3). Although the amount of the genomic material was not sufficient to determine the presence of the HCV replicative strand, the absence of a viral RNA signal in the concentrated DC culture supernatant, except for that of a single specimen (case 11), strongly suggests that the HCV RNA detected originated from the cells but not from a culture aliquot. The allostimulatory function and maturation of DC in individuals with resolved hepatitis C do not seem to be noticeably affected (1, 2). However, the persistence of trace amounts of the virus in DC, which are essential for T-cell activation, may play an important role by providing a sustained stimulus to HCV-specific T cells. Consistent with this interpretation is the existence of strong HCV-specific cytotoxic T- and Th-lymphocyte responses for many years after resolution of hepatitis C (5, 14, 36).

The present study shows that HCV RNA can persist, albeit at very low levels, in the serum and circulating lymphoid cells for years after apparently complete clinical and virological resolution of chronic hepatitis C. If the present findings reflect the existence of the biologically competent, infectious virus, this silent persistence may have important epidemiological and pathogenic implications. Among other factors, these trace amounts of the virus may lead to reactivation of hepatitis C after termination of antiviral therapy or due to severe immunosuppression and may support perpetuation on the subclinical level of liver disease which becomes symptomatic years after exposure to the virus. They may also constitute a source from which HCV may spread through blood and organ donations to susceptible individuals.

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