Selective Transmission of Hepatitis C Virus Quasi Species through a Needlestick Accident in Acute Resolving Hepatitis

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Background. Little is known about the transmission of variant hepatitis C virus (HCV) genome through needlestick injuries.

Methods. To demonstrate how HCV quasi species are transmitted and adapt to the new host in acute resolving infection, we analyzed the nucleotide and deduced amino acid sequences of the hypervariable region 1 (HVR-1) in the E2 domain of HCV in both the source of the virus (“donor”) and the person who received the virus through a needlestick accident (“recipient”). In addition, we also performed phylogenetic analysis of HCV quasi species in these patients to document the viral transmission.

Results. We obtained a total of 33 clones at different time points by using polymerase chain reaction amplification and cloning and sequencing of HVR-1. A predominant HVR-1 variant (in 4 of 10 isolates) in the donor was not present in the recipient 6 and 14 weeks after the accident. In contrast, a minor variant (in 1 of 10 isolates) in the donor became the predominant strain in the recipient 6 weeks (in 10 of 12 isolates) and 14 weeks (in 6 of 11 isolates) after the accident. Additional phylogenetic analysis showed high homology of nucleotide sequences between isolates obtained from the donor and isolates obtained from the recipient. In addition, the variants in the recipient’s virus showed substantial genetic preservation in the course of acute resolving hepatitis.

Conclusions. These data suggested that a minor HCV variant from a donor was transmitted to the recipient through a needlestick injury and that it prevailed as the dominant species. The preserved genetic homogeneity of the transmitted viral variants in patients with acute HCV infection may account for their clinical outcomes of resolving hepatitis.
the presence of antibody to HCV (anti-HCV). To improve our understanding of the transmission of HCV quasi species through a needlestick accident and the molecular evolution in acute resolving hepatitis, we analyzed the nucleotide and amino acid sequences of the HVR-1 region of HCV genomes recovered from the donor and the recipient.

MATERIALS AND METHODS

Patients. We observed patient C (the donor), a 30-year-old man, and patient W (the recipient), a 25-year-old woman. The donor, who had chronic myeloid leukemia, had developed chronic hepatitis C through blood transfusion. Before the needlestick accident, the recipient, a health care worker, had undergone blood testing, which revealed normal liver function test results and negative results for anti-HCV. Both of the patients tested negative for hepatitis B surface antigen (HBsAg; Abbott Laboratory) and for anti-HIV (Abbott Laboratory), as determined using commercial kits. The donor had already tested positive for anti-HCV before the event. Neither the donor nor the recipient had a history of heavy alcohol use, injection drug use, or serological markers suggestive of autoimmune hepatitis. Metabolic liver diseases, such as Wilson disease, hereditary hemochromatosis, or α-1 antitrypsin deficiency, were excluded on the basis of clinical and laboratory data. Serum alanine aminotransferase activity, anti-HCV, and HCV quasi species data for the donor were determined at the time of the needlestick accident. Moreover, we observed the recipient for clinical manifestations, presence of anti-HCV, and presence of HCV quasi species at the time of the needlestick accident and weekly to monthly after the event (total duration of follow-up, 36 months). Serial serum samples were obtained from the recipient and were stored at −20°C until use.

Serological assays. Serum samples were examined for anti-HCV using a second-generation EIA (Abbott Laboratory).

Detection of HCV RNA, HCV genotyping, and quantitation of HCV cDNA level. Qualitative detection of serum HCV RNA was done by using the 5′ untranslated region RT-PCR amplification assay [11]. HCV genotypes were identified using

Figure 1. Phylogenetic analysis of the hypervariable region 1 (HVR-1) sequences of hepatitis C virus in both the donor and the recipient performed using the 6-parameter method. The phylogenetic tree is constructed by using a computer program of the neighbor-joining method. Bootstrap values are shown along each main branch (>20% are shown). The GenBank accession numbers of reported sequences are indicated in the phylogram. The bar indicates evolutionary distance. A, Phylogenetic tree of HVR-1, by nucleotide sequences. B, Phylogenetic tree of HVR-1, by deduced amino acid sequences.
Figure 2. Clinical course of patient W (“recipient”), who had acute resolving hepatitis C virus (HCV) infection. The dotted line indicates the upper limit of normal serum alanine aminotransferase (ALT) activity. Results of tests for antibody to HCV (anti-HCV) are presented with the following cut-off index values: 2+, >2.5; 1+, 1.5–2.5; and −, <1.5.

type-specific primers [12]. Serum HCV cDNA levels were quantified using a competitive PCR assay [13].

Amplification, cloning, and sequencing of HVR-1. HVR-1 was detected by RT-PCR, with oligonucleotide primers designated for HVR-1 amplification (outer sense, 904 5′-CAGGACTGCAATTGCTCAATCTA-3′; outer antisense, 481 5′-TTGCAGTTTAAGGCCAGTCC-3′; inner sense, 1054 5′-CAGCTGGGATGCTCGTG- CGGG-3′; and inner antisense, 483 5′-ATGTGGCGAGCTGCGATTG-3′), as previously described [14]. The amplified products (212 bases; nucleotides 1054–1265) were ligated to pCR-Script SK (+) vectors and then transformed to Escherichia coli XL 1-Blue MRF competent cells (Stratagene). Plasmid DNAs were extracted from white colonies using the Winard minipress DNA purification system (Promega). The presence of inserted DNA was confirmed by SalI and BamHI digestion and electrophoresis. The sequences of inserted DNA were determined using fluorescence-labeled primers with a sequencer (model 373A; Applied Biosystems). Sequencing conditions were specified in the protocol for the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). Paired primers 1054 and 483 were used as sequencing primers for the detection of HVR-1.

Genetic diversity and phylogenetic tree analysis. The genetic diversity and the evolutionary relationship of HCV HVR-1 sequences (nucleotides 1150–1230) in the donor and the recipient were determined using the 6-parameter method [15]. Nucleotide sequences of the 3 genotypes (1a, 1b, and 2a) were obtained from the GenBank, European Molecular Biology Laboratory, and DNA Data Bank of Japan databases. The accession numbers of 11 sequences are indicated in figure 1. A phylogenetic tree was constructed by neighbor-joining methods using the MEGA 3 program by Kumar et al. [16] on the basis of nucleotide sequences of the amplified HVR-1 sequence of the HCV genome. The data set was bootstrap-resampled 1000 times to ascertain support for major branches of the tree.

RESULTS

As shown in figure 2, the recipient was asymptomatic until 6 weeks after the needlestick accident, when nausea and vomiting developed and the patient’s urine became tea colored. The serum alanine aminotransferase level of the recipient was 1425 IU/L (normal level, <40 IU/L), and seroconversion of anti-HCV was documented 6 weeks after the event.

Genotype analysis of HCV in both patients revealed that the genotype was 1b. The HCV cDNA level of the donor was 2 × 10^5 copies/mL at the time of the event. For the recipient, the HCV cDNA level could not be detected until 6 weeks after
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Figure 3. Nucleotide sequences of the cloned cDNA products in hepatitis C virus (HCV) hypervariable region 1 (HVR-1; nucleotides 1150–1230) recovered from the donor (D0-01 to D0-10) at the time of the accident, the recipient (R1-01 to R1-12) at 6 weeks after the accident, and the recipient (R2-01 to R2-11) at 14 weeks after the accident. The consensus sequence is determined by the total of 33 clones from both the donor and the recipient. The asterisk indicates the master sequence in the 3 samples. The nucleotide sequences of D0-01 and D0-02; R1-01 to R1-08 and R2-01 to R2-05; R1-09, R1-10, and R2-06; and R1-11 and R2-07 to R2-10 are identical.

The event, when it was \(5 \times 10^6\) copies/mL. The detection of the HCV cDNA coincided with the first peak in alanine aminotransferase activity (alanine aminotransferase level, 1425 IU/L). HCV cDNA was transiently undetectable 10 weeks after the event, when alanine aminotransferase activity regressed (alanine aminotransferase level, 64 IU/L). The HCV cDNA level was again detectable (\(5 \times 10^3\) copies/mL) 14 weeks after the event, when the second peak in alanine aminotransferase activity occurred (alanine aminotransferase level, 316 IU/L). After the second attack, a third peak in alanine aminotransferase activity (alanine aminotransferase level, 99 IU/L) occurred 5 months after the event. However, HCV cDNA was undetectable after the second peak in alanine aminotransferase activity. From months 2 to 25, high cut-off indices of anti-HCV (>2.5 or 2+) were observed. The indices (1.5–2.5 or 1+) decreased from months 25 to 35 and were less than the cut-off level (<1.5 or −) 36 months after the event.

We further analyzed the nucleotide sequences of HVR-1 in serum samples obtained from the donor and the recipient. Ten clones (D0) were found in the donor’s samples. Twelve clones (R1) and 11 clones (R2) were found in the recipient’s samples 6 weeks and 14 weeks after the needlestick accident, respectively (figure 3). Two of the 10 donor’s clones were the same (D0-01 and D0-02). In R1 serum, 8 and 2 of the 12 recipient’s clones with the same nucleotide sequences constituted the major variants (R1-01 to R1-08) and the minor variants (R1-09 and R1-10), respectively. In R2 serum, 5 and 4 of the 11 recipient’s clones with the same nucleotide sequences constituted the major variants (R2-01 to R2-05) and the minor variants (R2-07 and R2-10), respectively. There were 9 different but genetically closely related clones in D0, 4 in R1, and 4 in R2. The deduced amino acid sequences in HVR-1 of all clones are shown in figure 4. Synonymous substitutions (i.e., changes in the nucleotides without changes in the amino acids) were found between D0-01 and D0-03, D0-01 and D0-04, R1-01 and R1-09, and R2-01 and R2-06. The genetic variation of the HCV clones in samples from the donor was greater than that in samples from the recipient. Moreover, our data did not show identical nucleotide or amino acid sequences of HCV HVR-1 between the donor and the recipient. Therefore, we performed phylogenetic tree analysis of HVR-1 (figure 1A and 1B) to unravel the genetic relationship among these HCV quasi species. The analysis revealed that the HCV strains from both patients originated from the same cluster. We found 95.1% and
Figure 4. Deduced amino acid sequences of the corresponding nucleotide sequences in hepatitis C virus (HCV) hypervariable region 1 (HVR-1; amino acids 384 to 410) recovered from the donor (D0-01 to D0-10) at the time of the accident, the recipient (R1-01 to R1-12) at 6 weeks after the accident, and the recipient (R2-01 to R2-11) at 14 weeks after the accident. The consensus sequence is determined by the total 33 clones from both the donor and the recipient. The asterisk indicates the master sequence in the 3 samples. The deduced amino acid sequences of D01-01 to D01-04; R1-01 to R1-10 and R2-01 to R2-06; and R1-11 and R2-07 to R2-10 are identical.

88.9% similarity in sequences of nucleotides and deduced amino acids of HVR-1 between D0-05 and R1-01, respectively. In addition, 3 of the 4 HCV variants that were determined to be different by nucleotide sequence analysis of HVR-1 were well preserved between 6 weeks and 14 weeks after the needlestick accident, during which period repeated flares of hepatitis activity occurred. Eighty-five percent of the HVR-1 amino acid sequence (23 of 27 amino acids) in the recipient’s serum sample (R1 and R2) was preserved.

DISCUSSION

Despite the discovery of HCV by molecular biological methods [3] and the sequencing of the entire genome, our knowledge about this virus and the nature of the protective immune responses remains limited [17]. Some investigators have argued that poor host immune response to HCV is the main determinant in the development of viral persistence, whereas others have pointed to viral factors [18].

By using PCR amplification and cloning and sequencing of HVR-1, we found that the minor variant of HCV quasi species in the donor was transmitted to the recipient and became the major variant after transmission. After experiencing repeated flares of hepatitis, the recipient eventually cleared the virus. This study has raised important issues of why the minor variant in the donor predominated in the recipient and why viral clearance occurred in the recipient. Studies of the transmission of the infectious HCV clones were mainly performed using chimpanzee models, with contradictory results. One study has shown, by comparison of amino acid sequences in HVR-1 of HCV clones, that the major quasi species of the human inocula was identical to that of infected chimpanzee serum [19]. Another report showed that the minor variant, rather than the major variant, became the major variant in infected chimpanzee or human lymphocytic cell lines after transmission [20]. Selective transmission of HCV quasi species in humans has been found after receipt of blood transfusion [21], transmission of infection from the mother to the infant [22, 23], and after a needlestick injury [24] (as was also confirmed in our study).

We propose 3 possible mechanisms to explain the phenomenon: (1) selection of the variants at the time of entry, (2) selective amplification of the variants in the new host, and (3) immune elimination in the recipient of the donor’s major variant. First, it is possible that one variant has a selective advantage over other variants during viral entry. In HIV-1 infection, the different macrophage-tropic abilities of the virus may determine the efficiency of replication in the submucosal space [25]. We believe that there may be different tissue tropism between the variants, although this remains to be proven. Second, it is possible that multiple variants enter the new host but that only 1 of these variants, because of its biological advantage, is selectively amplified to become the dominant strain [26]. This is supported by the sequence homogeneity in patients with hemophilia who have seroconversion and who were presumably inoculated with multiple HIV-1 variants parenterally [27]. However, it is difficult to evaluate the relative amplification advantage because of the lack of an in vitro HCV model. Third, it is possible that, after the transmission, the major variant from the donor mounts a stronger immune pressure than the minor variants through different replication and mutation rates, resulting in earlier viral clearance [17]. However, we could not detect HCV viremia in the recipient’s serum sample using a sensitive PCR assay until 6 weeks after the event. Moreover, neither hepatitis nor seroconversion was noted during the first 6 weeks after the event, making this proposed mechanism unlikely. Therefore, our data favor the selective transmission and amplification of HCV quasi species in the recipient.

Unlike the patient who developed chronic hepatitis in the study by Saito et al. [24], our recipient developed acute resolving hepatitis C after the needlestick accident. The HCV quasi species in our study were much less genetically diverse and complex than the quasi species in theirs. Of particular note, most of the HCV quasi species recovered in R1 and R2 serum samples had homogeneous nucleotide and amino acid sequences. It is noteworthy that the evolution of HCV HVR-1 plays a role in viral clearance, as supported by Farci et al. [28]
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...and Laskus et al. [29], who showed that the relative evolutionary stasis of HVR-1 in HCV quasi species is associated with acute resolving hepatitis, whereas the evolutionary complexity is associated with persistent infection.

In conclusion, the significance of the report is 3-fold. First, our data demonstrate that a minor HCV quasi species in the donor may be successfully transmitted to a recipient and then preponderate in the new host. Second, molecular evolutionary analysis of HCV can help document viral transmission and could potentially predict outcome. Finally, the evolutionary stasis of HVR-1 in HCV quasi species may be associated with the resolution of acute HCV infection.

Acknowledgments

Potential conflicts of interest. All authors: no conflicts.

References


