Experimental Transmission of Hepatitis C Virus–Associated Fulminant Hepatitis to a Chimpanzee

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Hepatitis C virus (HCV) was transmitted from a patient with fulminant hepatitis C to a chimpanzee. The patient had developed two episodes of fulminant hepatitis C, each occurring after a separate liver transplantation. Serial serum and liver samples from the patient and the chimpanzee were analyzed for HCV replication, genotype, quasispecies heterogeneity, and antibodies. In the patient, the levels of HCV replication in serum and liver correlated with the degree of hepatocellular necrosis and the clinical expression of fulminant hepatitis. The same HCV strain, genotype 1a, was recovered from both episodes of fulminant hepatitis. An unusually severe acute hepatitis was also observed in the chimpanzee. The viruses recovered from the patient and the chimpanzee were almost identical and displayed relatively little quasispecies heterogeneity. Thus, the same HCV strain induced two episodes of fulminant hepatitis in a single patient and severe hepatitis in a chimpanzee, suggesting that the pathogenicity or virulence of a specific HCV strain may be important in the pathogenesis of fulminant hepatitis C.

The role of hepatitis C virus (HCV) as a causative agent of fulminant hepatic failure is still controversial. Whereas studies conducted in Japan documented the presence of antibodies to HCV or serum HCV RNA in 40%–60% of patients [1, 2], most of the studies performed in Western countries failed to provide evidence of an HCV etiology in acute non-A, non-B liver failure [3]. Recently, we described a patient in whom the acquisition of HCV infection was temporally associated with the development of fulminant hepatic failure, thus implicating HCV in the etiology of this dramatic clinical syndrome [4]. Another recent study, conducted in the Los Angeles area, reported a high prevalence (60%) of serum HCV RNA in patients with non-A, non-B fulminant hepatitis, associated with low socioeconomic status and Hispanic ethnicity [5]. Nevertheless, the cases of fulminant hepatic failure related to HCV so far documented in Western countries are very rare.

Chimpanzees have played a critical role in the study of non-A, non-B hepatitis. However, attempts to transmit hepatitis from patients with fulminant non-A, non-B hepatitis to chimpanzees were not successful [6]. In this report, we describe the first successful experimental transmission of an HCV strain implicated in the development of two episodes of fulminant hepatic failure in a single patient. The HCV strain (TN) recovered from this patient caused an unusually severe acute hepatitis when transmitted to a chimpanzee. The clinical profile of the disease, as well as the genetic characteristics of the HCV strain, were extensively analyzed.

Materials and Methods

Source of the HCV inoculum. The source of the HCV inoculum was a 35-year-old white woman, who underwent liver transplantation for cryptogenic end-stage liver cirrhosis. The source of HCV infection was transfusion of red blood cells for chronic anemia, on two occasions, within 2 months before the first liver transplantation, prior to the introduction of universal anti-HCV screening. The explanted liver showed inactive cirrhosis (figure 1). At postoperative week 8, the patient developed fulminant hepatic failure with progressive encephalopathy and coagulopathy, for which she was placed on the list for urgent retransplantation, performed 3 weeks later (figure 1). Serial liver biopsies obtained from postoperative week 2 showed no signs of allograft rejection but rather presented a picture indicative of acute hepatitis of increasing severity, culminating in submassive hepatocellular necrosis (figure 1). Four weeks after the second liver transplantation, the patient was dis-
charged from the hospital but was readmitted at postoperative week 16 because she had again developed fulminant hepatic failure with progressive encephalopathy and severe coagulopathy. She died in hepatic coma 10 weeks later (figure 1). Serial liver biopsies obtained after the second liver transplantation again show no signs of allograft rejection but rather present a picture of acute hepatitis that rapidly progressed to severe chronic active hepatitis and eventually to cirrhosis with active necrosis and collapse (figure 1).

Serial serum samples were tested for the presence and amount of HCV RNA, the HCV genotype, quasispecies heterogeneity, and antibodies. None of the serum samples had detectable levels of IgM antibodies to hepatitis A, hepatitis B surface antigen, IgM antibodies to hepatitis B core antigen, or antibodies against cytomegalovirus, Epstein-Barr virus, or human immunodeficiency virus. All serum samples were negative for the presence of autoantibodies. To exclude the possibility of coinfection with hepatitis B virus (HBV) or hepatitis G virus (HGV), all serum samples were tested for the presence of HBV DNA and HGV RNA by the polymerase chain reaction (PCR).

**Experimental infection of a chimpanzee.** Serum (100 μL) obtained from the patient 5 days before the first liver transplantation was inoculated intravenously into a seronegative chimpanzee. The presence and the levels of HCV RNA were measured in serum at intervals of 1, 2, or 4 weeks during the 6 months after inoculation. A sample obtained 2 weeks after inoculation was amplified with a set of primers that span the E1/E2 region, and the PCR product was analyzed both by direct sequencing and by sequencing of 10 molecular clones. Antibodies to HCV were measured weekly throughout the observation period. Liver biopsy specimens were obtained before inoculation and at 1-week intervals during the experimental study. The animal was caged alone and maintained under approved-facility conditions.

**Anti-HCV testing.** All serum samples were tested for antibodies to HCV (anti-HCV) by first- and second-generation EIAs (Ortho Diagnostic Systems, Raritan, NJ).

**Detection, titration, genotyping, and sequencing of HCV RNA.** Total RNA was extracted from 100 μL of serum and amplified by PCR, as previously described [7], using two sets of nested primers.
The first set, derived from the 5′ noncoding region [8], was used to investigate the course of HCV viremia, and the second set, from the E1 and E2 genes [8], including the hypervariable region 1 (HVR1), was used to determine the HCV genotype and the degree of viral quasispecies. The level of HCV RNA in serum was measured by the branched-chain DNA (B-DNA) test with the Amplex assay (Chiron, Emeryville, CA). The PCR products from the E1/E2 region were analyzed both by direct sequencing and by sequencing of 10 molecular clones, as previously described [4].

Detection of serum HBV DNA and HGV RNA. HBV DNA was extracted from 100 μL of serum, as previously reported [4]. Serum HGV RNA was extracted [7], and PCR was performed with nested primers derived from the 5′ noncoding region of the HGV genome [9]. The outer primer pair consisted of 5′-CAGGCAG-CCGCCAAAAGGTGG-3′, starting at map position 93, and 5′-AACTCGCCGGCTACTATTGTTGG-3′, starting at map position 363. The inner primers were 5′-CCGCCAAAAGGTGAATTGG-3′, starting at map position 100, and 5′-CGGCCTACGCCCTATTGGT-CAAG-3′, starting at map position 357.

Liver histology and immunohistochemical staining of HCV antigen. A total of 16 liver specimens obtained from the patient, including 2 explanted livers, were evaluated for recurrent viral hepatitis or changes due to rejection. The extent of hepatocellular necrosis was estimated as the percentage of the evaluated tissue.

All liver tissues were stained for an HCV antigen encoded by the fourth nonstructural (NS4) gene, and the percentage of cells that were antigen-positive was determined, as previously described [4]. Weekly liver biopsies were obtained from the chimpanzee for histologic examination.

Results

Patient. The course of HCV viremia in the patient is illustrated in figure 1. Serum HCV RNA was continuously detected by PCR throughout the observation period until the patient’s death. As measured by the B-DNA assay, the levels of HCV RNA increased to >10⁸ equivalents per milliliter before the development of fulminant hepatic failure and then decreased after the second liver transplantation. The levels increased again to ~10⁶ equivalents per milliliter 2 weeks before the onset of the second episode of fulminant hepatic failure. All serial samples were negative for HBV DNA but became positive for HGV RNA sequences 5 days after the first liver transplantation. HGV RNA sequences were detected throughout the course of the disease except for 2 samples. Sixteen liver specimens were analyzed for the intrahepatic expression of the
HCV NS4 antigen. Staining for the antigen was negative in the native liver, then became positive and remained positive until the second liver transplantation (figure 1). Initially, the antigen was undetectable in the new liver, but then it reappeared and persisted until the patient’s death. The number of positive cells increased with time after each liver transplantation, in parallel with the development of severe hepatocellular necrosis (figure 1). Antibody seroconversion by second generation assay occurred 10 days after the first liver transplantation, 20 weeks earlier than with first generation assay (figure 1).

Chimpanzee. Chimpanzee 1422 was inoculated with serum obtained from the patient 5 days before the first liver transplantation and during the incubation period for fulminant hepatitis C. The serum used for inoculation was negative for antibodies to HCV (by first- and second-generation EIAs), positive for HCV RNA (by PCR), and negative for HBV DNA and HGV RNA (by PCR). The course of HCV infection in the chimpanzee is shown in figure 2. Serum HCV RNA first appeared 1 week after inoculation and persisted through week 12 of the acute phase. Antibody seroconversion occurred at week 14, and antibodies continued to be detected throughout the observation period. HCV viremia (detected by B-DNA) reached the highest level, $10^7$, on week 9, just before the alanine aminotransferase (ALT) peak; the titer then suddenly decreased below the level of sensitivity of this test, although serum HCV RNA remained detectable by PCR for 3 additional weeks. No HGV sequences were detected in any of the serial samples tested. The animal developed an acute self-limited hepatitis characterized by an unusually high level of serum ALT, which reached a peak of 744 IU/L (figure 2) and by histopathologic changes, which were compatible with a diagnosis of severe acute hepatitis, from weeks 6–12 after inoculation.

Sequence analysis of HCV recovered from the patient and the chimpanzee. Comparison of sequential nucleotide sequences, obtained by both direct sequencing and molecular cloning of part of the E1 and E2 genes, including the HVR1, demonstrated that the HCV strain was essentially the same before and after the first liver transplantation (figure 3). However, at day 4 and at week 2 after the second liver transplantation, a second virus strain with a sequence divergence of 42% in the domain of the HVR1 (figure 3) became detectable. The 2 virus strains belonged to the same genotype, 1a. The second virus strain co-existed with the first strain, which remained quantitatively predominant (9 clones to 1 at day 4 and 6 to 3 at week 2). Subsequently, at week 3, the virus population was again represented exclusively by the original virus strain throughout the follow-up period. Comparative sequence analysis of 62 clones obtained from the patient at different time points demonstrated a low degree of diversity in the viral quasispecies within the HVR1. Of 62 clones, 52 (84%) were either identical to the consensus sequence or differed by a single amino acid (data not shown).

Direct sequencing of a sample obtained 2 weeks after inoculation demonstrated that the virus strain from the chimpanzee was the same as that used for inoculation (figure 3). This was

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Figure 3. Multiple sequence alignment of 31 deduced amino acids representing hypervariable region 1 (HVR1) of E2 gene, spanning map positions 384–414 of prototype HCV strain, obtained by direct sequencing from serial serum samples from patient and chimpanzee 1422, which was inoculated with serum from patient. Remaining sequences of 144 amino acids, spanning map position 326–500 (not shown), are available on request. When microheterogeneity was detected, conserved nucleotide was used for sequence comparison. Dots indicate amino acid identity with sequence obtained from first sample of patient. OLT, orthotopic liver transplantation.
confirmed by sequence analysis of 9 molecular clones performed on the same sample.

Discussion

Our study provides evidence that HCV can be a causative agent of fulminant hepatic failure in humans and can be transmitted from a patient with fulminant hepatitis C to a chimpanzee. As we described in a recent case report [4], this study confirms that fulminant hepatitis C is characterized by high levels of HCV replication, both in serum and in liver, that persist throughout the course of the disease and correlate with the degree of hepatocellular necrosis. The sustained levels of viremia detected by PCR in the course of fulminant hepatitis C strongly suggest that, in patients without detectable serum HCV RNA, even when only a single sample is available for testing, HCV is not etiologically involved. Thus, the lack of serum HCV RNA in most patients with non-A, non-B fulminant hepatic failure thus far reported in Western countries may explain the failure to transmit hepatitis from patients with non-A, non-B fulminant hepatitis to chimpanzees.

The pathogenetic mechanisms of virus-related fulminant liver failure are still unknown. In fulminant hepatitis B, traditional views suggest an enhanced immune response to viral antigens as a possible mechanism leading to extensive hepatocellular necrosis [10]. Our study suggests that viral factors are important in the pathogenesis of fulminant hepatitis C. The most striking indication that an unusually virulent strain of HCV was implicated in the pathogenesis of liver failure is the observation of two episodes of fulminant hepatitis C in a single patient, due to the same HCV strain, each occurring after a separate liver transplantation. The possible role of an unusually pathogenic HCV strain leading to fulminant hepatic failure was further suggested by the experimental transmission of hepatitis C from our patient to a chimpanzee. The acute hepatitis seen in this animal was characterized by the highest serum ALT peak and the most-severe histopathologic changes ever seen in a chimpanzee infected in our laboratory. In >30 chimpanzees infected with different HCV strains (Purcell RH, unpublished data), the mean ALT peak was 214.9 ± 122.1 IU/L (mean ± SD). Moreover, in none of these chimpanzees was severe acute hepatitis ever diagnosed. Sequence analysis indicated that the virus recovered from the chimpanzee was the same as that in the inoculum from the patient. Of interest, in contrast to the high degree of viral diversity reported in chronic hepatitis C [11], a low genetic heterogeneity appears to be the rule in fulminant hepatitis C, as also reported previously [4].

Although our data strongly suggest that HCV can be a causative agent of fulminant hepatitis, we cannot exclude, in principle, the role of other viral agents, such as HGV [9]. However, HGV does not appear to be a hepatotropic agent that causes liver disease in humans [12]. All clinical studies thus far reported have failed to show a causative association between the acquisition of HGV infection and the development of acute, chronic, or fulminant hepatitis [9, 12, 13].

In summary, our study provides evidence that HCV can cause fulminant hepatic failure, demonstrates that HCV—when present—can be successfully transmitted to a chimpanzee from a patient with fulminant hepatitis C, and sheds new light on the pathogenesis of fulminant hepatitis. The observation that the same HCV strain induced two episodes of fulminant hepatitis in a single patient, as well as an unusually severe acute hepatitis in a chimpanzee, strongly suggests that the pathogenicity or virulence of a specific HCV strain may play an important role in the pathogenesis of fulminant hepatitis C.

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References