A 51-year-old heart transplant recipient who developed subfulminant hepatic failure because of organ-transmitted hepatitis C virus (HCV) infection is described. He presented with a predominantly cholestatic liver damage after heart transplantation. An extensive evaluation, including abdominal ultrasound and computed tomography scan and endoscopic retrograde cholangiopancreatography was unrevealing. Liver biopsy, however, was suggestive of a large duct obstruction with prominent portal and pericellular fibrosis, marked cholestasis, pericholangitis with marked ductular proliferation, and diffuse hepatocyte degeneration. Antibody to HCV (anti-HCV) was initially negative. He deteriorated in the ensuing 3 months. A repeat enzyme immunoassay—2 test for anti-HCV 4 months after initial presentation was weakly positive. Quantitation of serum HCV RNA by branched DNA assay revealed high level viremia, $547 \times 10^6$ genome equivalents per milliliter. Using in situ polymerase chain reaction, HCV RNA was detected in the cytoplasm in >80% of the hepatocytes. The patient underwent interferon alfa therapy, and serum HCV RNA levels were reduced 20-fold after four doses. Unfortunately, the patient developed pulmonary aspergillosis and died. This case illustrates that in immunosuppressed patients anti-HCV is not a good marker for the diagnosis of HCV infection, and HCV can cause a progressive form of cholestatic liver disease mimicking a large duct obstruction.

Hepatitis C virus (HCV) infection is common in organ transplant recipients.1–3 HCV infection present before transplant persists, even after liver transplant, and de novo infection acquired from either transfusion or the donor organ occurs frequently.4–7 Between 28% and 48% of recipients of organ from HCV infected donors acquire HCV infection.5,7 HCV infection in organ recipients is usually subclinical or mild.4,7 The resulting liver disease is typically a hepatocellular type of injury. Herein, we report a heart transplant recipient who acquired HCV infection from the donor organ and developed rapidly progressive cholestatic hepatic failure with a histological pattern mimicking large bile duct obstruction.

Case Report

A 51-year-old white man was referred to the Section of Hepatobiliary Diseases, University of Florida, in December 1992 for evaluation of progressive cholestasis. In September 1992, he underwent a heart transplantation for ischemic cardiomyopathy. He had neither a history of nor risk factors for liver disease, and he was seronegative for hepatitis B virus (HBV), HCV, and human immunodeficiency virus (HIV) markers before transplantation. However, the heart donor was seropositive for antibody to HCV (anti-HCV, enzyme immunoassay—2, Abbott Laboratories, Chicago, IL). Three weeks after transplantation, the patient had a mild elevation of his serum alanine aminotransferase (ALT) (54 IU/L, normal 0–40 IU/L) and total bilirubin (1.6 mg/dL, normal 0.3–1.5 mg/dL). Serum albumin, alkaline phosphatase, and aspartate transaminase (AST) levels were normal. His immunosuppressive regimen consisted of prednisone 150 mg daily, cyclosporin A 375 mg daily, and methotrexate 10 mg weekly. His cyclosporin A parent compound level was maintained between 2000 and 3000 mg/L (within the therapeutic range) throughout his course. In late November 1992, his liver biochemistry further deteriorated [total bilirubin, 1.7 mg/dL; ALT, 246 IU/L; AST, 488 IU/L (normal, 0–40 IU/L), alkaline phosphatase, 138 IU/L (normal, 30–120 IU/L)]. In the next 3 weeks, his serum aminotransferases remained at approximately the same levels, but there was a progressive increase in his alkaline phosphatase and bilirubin. Three months after transplantation, his blood tests revealed the following: total bilirubin increased to 15.0 mg/dL (indirect bilirubin, 11.4 mg/dL), alkaline phosphatase increased to 328 IU/L, ALT decreased to 219 IU/L, AST decreased to 421 IU/L, and albumin was 27 g/L. He was receiving the four immunosuppressives listed above and magnesium oxide only. Cotrimoxazole prophylaxis was discontinued 2 months after transplantation. Serum markers for hepatitis A virus (HAV), HBV, HCV, and HIV remained negative. Results of abdominal ultrasound, computerized tomography, and radioisotope scanning were normal. Endoscopic retrograde cholangiopancreatography showed a normal biliary tree. Multiple blood, urine,
Liver biopsy showed prominent periportal and pericellular fibrosis, marked cholestasis, pericholangitis with marked ductular proliferation, diffuse hepatocyte degeneration with only mild inflammatory cell infiltrates, and histological features suggestive of a large duct obstruction. Rapid immunofluorescence of the liver section subsequently showed a weak staining suggestive of a large duct obstruction. Rapid immunofluorescence of the liver section subsequently showed a weak staining for cytomegalovirus early antigens. In view of a normal endoscopic retrograde cholangiopancreatography, cholestasis of sepsis and drug-induced cholestasis were considered. Therefore, the patient was given a 14-day course of ganciclovir and imipenam. Azathioprine was also discontinued. Right-sided heart catheterization, which showed normal findings, was also performed.

The patient became increasingly icteric during the subsequent 2 months and was readmitted 5 months after transplantation. His liver profile still showed a prominent cholestatic picture (total bilirubin, 39.1 mg/dL; direct bilirubin, 24.6 mg/dL; alkaline phosphatase, 328 IU/L; AST, 623 IU/L; ALT, 206 IU/L; albumin, 20 g/L) (Figure 1). Repeat abdominal ultrasonography and endoscopic retrograde cholangiopancreatography showed no abnormality of the biliary tree. Multiple blood cultures were negative for bacteria, viruses, and fungi. Another 14-day course of imipenam for presumed cholangitis did not improve his cholestasis. A second liver biopsy, which showed histological features similar to but more pronounced than those in the first biopsy, was performed (Figure 2A). Viral serologies for HAV, HBV, and HIV were repeated and remained negative. Anti-HCV was, however, weakly positive (enzyme immunoassay—2) at this time. Quantitation of serum HCV RNA by the branched DNA assay in samples that were centrifuged, separated, and frozen within 1 hour of venipuncture. We have shown that HCV RNA is best preserved under these conditions (Davis GL, et al., unpublished data). This quantitation method is a unique solid-phase sandwich hybridization assay coupled with signal amplification using branched DNA (Chiron Corporation, Emeryville, CA). The details of this assay have been given previously. Detection and localization of HCV RNA in liver tissue was performed in formalin-fixed, paraffin-embedded liver biopsy sections by in situ reverse transcription polymerase chain reaction with direct incorporation of the digoxigenin labeled dUTP (Boehringer Mannheim, Indianopolis, IN) incorporation using a protocol described by Nuovo et al. with minor modifications: proteinase K (40 μg/mL) was used for proteolytic digestion instead of pepsinogen, and primer 321 (antisense, 5'-GCACGGTCTACGAGACCT-3') was used for reverse transcription and primers 126 (sense, 5'-GTGGTCTCAGGAGGACCA-3') and 299 (antisense, 5'-GGGACTCCGAGGA-3') were used for the polymerase chain reaction. Positive controls included sections from patients with chronic HCV infection known to be positive for HCV RNA by in situ reverse transcription polymerase chain reaction. Specificity controls included liver sections from patients with chronic HBV infection, in situ polymerase chain reaction without the reverse transcription step, and omission of the detection antibody.

Laboratory Methods

Serum HCV RNA was detected and quantitated by the branched DNA assay in samples that were centrifuged, separated, and frozen within 1 hour of venipuncture. We have shown that HCV RNA is best preserved under these conditions (Davis GL, et al., unpublished data). This quantitation method is a unique solid-phase sandwich hybridization assay coupled with signal amplification using branched DNA (Chiron Corporation, Emeryville, CA). The details of this assay have been given previously. Detection and localization of HCV RNA in liver tissue was performed in formalin-fixed, paraffin-embedded liver biopsy sections by in situ reverse transcription polymerase chain reaction with direct incorporation of the digoxigenin labeled dUTP (Boehringer Mannheim, Indianopolis, IN) incorporation using a protocol described by Nuovo et al. with minor modifications: proteinase K (40 μg/mL) was used for proteolytic digestion instead of pepsinogen, and primer 321 (antisense, 5'-GCACGGTCTACGAGACCT-3') was used for reverse transcription and primers 126 (sense, 5'-GTGGTCTCAGGAGGACCA-3') and 299 (antisense, 5'-GGGACTCCGAGGA-3') were used for the polymerase chain reaction. Positive controls included sections from patients with chronic HCV infection known to be positive for HCV RNA by in situ reverse transcription polymerase chain reaction. Specificity controls included liver sections from patients with chronic HBV infection, in situ polymerase chain reaction without the reverse transcription step, and omission of the detection antibody.

Discussion

This is the first documented report of subfulminant hepatic failure caused by HCV acquired via transplantation of a nonliver solid organ. The absence of anti-HCV for 4 months in the presence of high level HCV RNA suggests that testing for anti-HCV alone is not adequate for excluding the diagnosis of HCV in this clinical setting. We have also shown previously the insensitivity of anti-HCV assays in renal transplant recipients. Thus, HCV RNA should be sought in immunosuppressed patients with suspected HCV infection.

The predominantly cholestatic picture in this patient...
is atypical for HCV infection. Cholestatic hepatitis with atypical histological features, such as ductular proliferation and acute pericholangitis mimicking large bile duct obstruction, has recently been reported in liver transplant recipients with HCV infection. However, most patients in that series had other complications like rejection and stenosed biliary anastomosis, making an accurate assessment of the role of HCV in the mediation of liver damage in these patients difficult. Two patients with subfulminant liver failure have been reported in non-A, non-B hepatitis acquired through solid organ transplantation. However, details were not provided with respect to the patients' HCV status before transplantation or their subsequent clinical course.

The very high serum and tissue levels of HCV RNA suggested that HCV may be directly cytopathic to the hepatocytes in this condition. This hypothesis may explain the rapid progression to hepatic failure and the histological features of prominent cholestasis and hepatocyte degeneration. In liver transplant recipients who develop fibrosing cholestatic hepatitis following recurrence of HBV infection, fibrosis and marked cholestasis are prominent features together with ballooning degeneration of the hepatocytes packed with HBV. The high level of expression of HCV RNA in hepatocytes and the hepatocytic dysfunction and marked cholestasis, by analogy with fibrosing cholestatic hepatitis, suggested that a similar mechanism for hepatocellular dysfunction and enhancing fibrogenesis might have been present in this patient. The slower downhill course in this patient compared with patients with fibrosing cholestatic hepatitis is consistent with lower replication rate of HCV compared with HBV. The cause for the apparent histological appearance of large duct obstruction in this patient remains unknown. Large bile duct damage is a known feature of chronic HCV infection, intralobular bile duct damage was not apparent on liver biopsy, and HCV RNA was not detected in the bile duct epithelium by in situ reverse-transcription polymerase chain reaction in this patient.

This patient's unusual presentation and clinical course may have been modified by immunosuppression. In renal transplant recipients, antirejection immunosuppressive therapy has been shown to be associated with an increase in serum HCV RNA levels but only with mild clinical disease. Serum HCV RNA levels have also been noted to be high in liver transplant recipients. In chimpanzees acutely infected with HCV, the degree of ALT elevation and histological inflammation was reduced by immunosuppression. Such a suppression of immune response may allow unusually high levels of HCV replication to develop, thereby inducing hepatocyte injury.

IFN-α has been used to treat liver transplant recipients with HCV infection. The marked reduction of serum HCV RNA with IFN-α therapy in our patient suggests an antiviral effect of IFN-α in this condition. If our hypothesis that HCV is directly cytopathic in this clinical setting is true, a reduction of HCV load in the liver...
might be beneficial. It is also possible that an early institution of IFN-α therapy may be more effective. Whether IFN-α is capable of suppressing HCV replication to a level that is not harmful to hepatocytes remains to be established.

References


Received July 16, 1993. Accepted September 14, 1993.
Address requests for reprints to: Johnson Y. N. Lau, M.D., Section of Hepatobiliary Diseases, University of Florida, P.O. Box 100214 JHMHC, Gainesville, Florida 32610.
The authors thank the Chiron Corporation (Emeryville, CA) for providing the branched DNA assay and Ke-Ping Qian, PhD, for performing the assay. They also thank Dr. Gerald Nuovo, MD, for his invaluable advice on the in situ reverse-transcription polymerase chain reaction assay. This study was supported by the following grants: DSR-D-1-15 and DSR-RDA-1-15 from the Division of Sponsored Research, University of Florida, the American Liver Foundation Hans Popper Liver Scholar Award, and the Glaxo Institute of Digestive Health Clinical Investigator Award (to Dr. Lau).