

LONG-TERM OUTCOME OF HEPATITIS C INFECTION AFTER LIVER TRANSPLANTATION

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Abstract Background. End-stage cirrhosis related to hepatitis C virus (HCV) is a common reason for liver transplantation, although viremia is known to persist in most cases. We investigated the impact of persistent HCV infection after liver transplantation on patient and graft survival and the effects of the HCV genotype and the degree of HLA matching between donor and recipient on the severity of recurrent hepatitis.

Methods. A group of 149 patients with HCV infection who received liver transplants between January 1982 and April 1994 were followed for a median of 36 months; 623 patients without HCV infection who underwent liver transplantation for end-stage chronic liver disease were used as a control group. A total of 528 liver-biopsy specimens from the HCV-infected recipients were reviewed, including 82 obtained one year after transplantation as scheduled and 39 obtained at five years as scheduled. In addition, biopsy specimens were obtained from 91 of the HCV-negative patients five years after transplantation.

Results. Cumulative survival rates for the 149 patients with HCV infection were 79 percent after one year, 74 percent after three years, and 70 percent after five years, as compared with rates of 75 percent, 71 percent,

and 69 percent, respectively, in the HCV-negative transplant recipients ($P=0.12$). Of the 130 patients with hepatitis C infection who survived more than 6 months after transplantation, 15 (12 percent) had no evidence of chronic hepatitis on their most recent liver biopsy (median follow-up, 20 months), 70 (54 percent) had mild chronic hepatitis (median, 35 months), 35 (27 percent) had moderate chronic hepatitis (median, 35 months), and 10 (8 percent) had cirrhosis (median, 51 months). Graft loss occurred after a median of 303 days in 27 of the 149 patients, including 5 with HCV-related cirrhosis and 3 with HCV-related cholestatic hepatitis. Infection with HCV genotype 1b was associated with more severe graft injury, whereas the primary immunosuppressive regimen used and the extent of HLA mismatching between donors and recipients had no significant effect on this variable.

Conclusions. After liver transplantation for HCV-related cirrhosis, persistent HCV infection can cause severe graft damage, and such damage is more frequent in patients infected with HCV genotype 1b than with other genotypes. After five years, the rates of graft and overall survival are similar between patients with and those without HCV infection. (N Engl J Med 1996;334:815-20.)

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CIRRHOSIS related to infection with the hepatitis C virus (HCV) is a common reason for liver transplantation, although viremia is known to persist in over 95 percent of patients.¹ Recurrence of HCV infection in the graft can be demonstrated as early as four weeks after liver transplantation for HCV-induced cirrhosis,² and acute lobular hepatitis will develop in most patients during the first year.³ Because the initial graft dysfunction usually resolves, chronic hepatitis was thought to be a rare sequela.^{4,5} However, recent reports have indicated that liver-graft damage can occur at an accelerated rate, leading to recurrent cirrhosis within five years,⁶⁻⁸ unlike the indolent course of HCV infection seen in patients who have not undergone transplantation.^{9,10} Preliminary data suggested a detrimental effect of matching the liver donor and recipient for the HLA-DQB antigen, because of an association with a recrudescence of chronic hepatitis.¹¹ Certain HCV genotypes may be associated with more severe liver disease after transplantation, but reports are conflicting.^{12,13}

We investigated the natural history of HCV infection in liver-transplant recipients to determine the impact of such infection on the morphologic characteristics of the graft and the long-term outcome. We also analyzed the relation of HLA mismatches between donors and recip-

ients and viral genotypes to the severity of recurrent disease in the graft.

METHODS

Between January 1982 and April 1994, a total of 946 patients underwent orthotopic liver transplantation in the Cambridge and King's College Hospital programs. Neither pretransplantation nor post-transplantation serum samples were available for 85 patients, who were thus excluded from the study. In the remaining 861 patients, pretransplantation serum samples were tested for anti-HCV antibody. If the antibody was detected, post-transplantation serum samples were tested for HCV RNA. In addition, HCV RNA was sought in post-transplantation serum samples from all patients who were negative for anti-HCV who had histologic evidence of hepatitis in a liver-biopsy specimen obtained one year after transplantation or at the time of graft dysfunction. A total of 420 transplant recipients were tested for HCV RNA. The primary immunosuppressive regimens used in all patients consisted of a combination of either cyclosporine, azathioprine, and prednisolone or tacrolimus (FK 506) and prednisolone.

Post-transplantation HCV infection was confirmed in 149 liver-transplant recipients on the basis of persistent HCV RNA in serum (Table 1), and their long-term outcome was assessed. Twenty-five of these patients had been included in a previous prospective analysis of the factors affecting the level of viral replication after recurrent HCV infection.¹⁴

The patients were followed for a median of 36 months after transplantation (range, 1 to 138). Between 1 and 12 biopsy specimens of the graft (median, 4) were reviewed for each patient. Biopsies were performed at one year as part of studies of new immunosuppressive agents between 1990 and 1992 and as part of routine post-transplantation management since then, and the biopsies were performed as scheduled in 82 of the 115 patients with at least one year of follow-up. Biopsies were also performed at five years in all patients not lost to follow-up (39 of 52). A total of 528 biopsy specimens were assessed by one of us in a nonblinded fashion.

Chronic viral hepatitis was diagnosed on the basis of the presence

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Table 1. Characteristics of 149 Patients with Persistent HCV Infection after Liver Transplantation.*

CHARACTERISTIC	VALUE
Age (yr)	
Median	50
Range	22–66
Male sex — no. (%)	114 (77)
Seropositive for HBsAg — no. (%)	12 (8)
Seropositive for anti-HBs or anti-HBc — no. (%)	33 (22)
Hepatocellular carcinoma — no. (%)	44 (30)
Racial or ethnic origin — no. (%)†	
Mediterranean	63 (42)
Northern Europe	37 (25)
Arabic countries	30 (20)
Israel	10 (7)
India	4 (3)
South America	3 (2)
Asia	1 (1)
Caribbean	1 (1)

*Anti-HBs denotes antibody against hepatitis B surface antigen, and anti-HBc antibody against hepatitis B core antigen.

†Because of rounding, percentages do not total 100 percent.

of focally intense, often aggregated, lymphocytic infiltrates in portal areas with few if any eosinophils, focal infiltration of and damage to a single interlobular bile duct without duct loss, and a variable degree of piecemeal necrosis and the absence of serious portal or hepatic venular endotheliitis. The changes were subjectively graded as mild if the cellular infiltrate was mostly confined to the portal areas, or moderate to severe when extensive piecemeal necrosis was present.¹⁵

The diagnosis of chronic rejection was based on findings of overt ductopenia with loss of more than 50 percent of the interlobular bile ducts, perivenular cholestasis and hepatocyte loss, and inconspicuous ductular proliferation and was confirmed on biopsy of a hepatectomy specimen by a finding of associated foam-cell arteriopathy.

Patient and graft survival was analyzed for the 149 liver-transplant recipients with confirmed HCV infection after transplantation and 623 other patients who received liver transplants for chronic liver disease (primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis) during the same period. Eighty-nine HCV-negative patients who underwent transplantation for acute liver failure were not included in the analysis. Among the 623 patients, 91 underwent a liver biopsy five years after transplantation and were found to be seronegative for HCV RNA. These 91 patients were used as a control group for the analysis of liver-biopsy specimens.

Serologic Tests

Serum samples were tested for anti-HCV antibody with a second-generation enzyme-linked immunoassay (United Biomedical, New York) and for hepatitis B surface antigen (HBsAg) and antibody against hepatitis B surface antigen and core antigen with commercial kits (Ausria II, Ausab, Corab, or IMX, Abbott, North Chicago, Ill.). Active cytomegalovirus infection was excluded on the basis of the absence of specific histopathological features (including immunostaining for immediate early antigen), negative blood cultures, and negative serologic findings. Serum levels of aspartate aminotransferase were measured.

Serum was analyzed for HCV RNA with the Amplicor assay (Roche Diagnostics, Hoffmann-LaRoche, Basel, Switzerland),^{16,17} Genotyping of HCV, of which there are 6 major genotypes and 18 subtypes, was performed in serum obtained after transplantation. Briefly, a nested polymerase chain reaction (PCR) was performed with biotinylated primers from the 5' untranslated region, and the second-round product was genotyped with a second-generation line probe assay (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium).¹⁸

Lymphocytes were obtained from donors and recipients for HLA typing. Typing of HLA-A and B antigens was performed with a standard complement-dependent microcytotoxicity assay.¹⁹ Typing of HLA-DR was performed with a combination of serologic techniques and either analysis involving restriction-fragment-length polymorphisms or

PCR-based sequence-specific oligonucleotide typing, whereas only PCR-based oligonucleotide typing was used for the typing of HLA-DQB.²⁰ For HLA-A and B, only mismatches at broad specificities were considered, whereas for HLA-DR and DQ, mismatches of subspecificities (split) of DR1 through 18 and DQ1 through 9 were identified. Matches of broad antigens with mismatches of split antigens were rare.

The results of genotyping of each donor and recipient were compared to identify mismatches in the pair. For each locus, the number of mismatches was scored as 0, 1, or 2. In cases in which data on two loci were considered, the score ranged from 0 to 4, and in cases in which four loci were considered, the score ranged from 0 to 8. The mean mismatch scores were calculated for each group of patients.

Statistical Analysis

The results were compared by nonparametric tests where appropriate: the chi-square or Fisher's exact test, the Mann-Whitney test, Wilcoxon's matched-pairs test, or Kruskal-Wallis probability tests. Kaplan-Meier survival curves were calculated with the BMDP statistical package, and the groups were compared with the Mantel-Cox and Breslow log-rank methods.²¹ The independent effects of host and viral factors on histologic outcome were determined with multiple logistic-regression analysis.²²

RESULTS

In 93 of the 149 patients with confirmed HCV infection after liver transplantation (62 percent), acute lobular hepatitis developed between 23 and 469 days after transplantation (median, 77) and subsequently resolved. Analysis of the most recent liver-graft specimen from the 130 patients who survived more than 6 months postoperatively demonstrated no evidence of chronic hepatitis in 15 patients (12 percent; median follow-up, 20 months; range, 6 to 103), mild chronic hepatitis in 70 (54 percent; median follow-up, 35 months; range, 6 to 130), moderate chronic hepatitis in 35 (27 percent; median follow-up, 35 months; range, 6 to 127), and cirrhosis in 10 (8 percent; median follow-up, 51 months; range, 24 to 138). As of the most recent follow-up, 4 of the 10 patients with recurrent HCV-induced cirrhosis remained well between 62 and 118 months after transplantation, and 1 patient had peripheral edema that was controlled with diuretics (follow-up, 138 months). Liver failure developed in the remaining five patients between 32 and 106 months after transplantation; two underwent a second, successful transplantation after 34 months in one case and 108 months in the other; one patient died of decompensated cirrhosis at 61 months and another at 84 months; and one patient was awaiting a second transplant procedure (follow-up, 39 months).

Liver-biopsy specimens were obtained as scheduled from 82 HCV-infected patients one year after transplantation and from 39 patients five years after transplantation. When the biopsy specimens obtained at one year were compared with those obtained at five years, there were no significant differences in the numbers of grafts without hepatitis (7 of 82 vs. 2 of 39), grafts with mild chronic hepatitis (51 of 82 vs. 20 of 39), and grafts with moderate chronic hepatitis (24 of 82 vs. 9 of 39). None of the patients had cirrhosis after one year, whereas eight had cirrhosis at five years ($\chi^2 = 18$, $P < 0.001$). For 30 patients biopsy specimens obtained at both one and five years were available. Of the 21 patients with mild chronic hepatitis at one year, 4 had moderate chronic hepatitis at five years and 1 had cirrhosis. In compari-

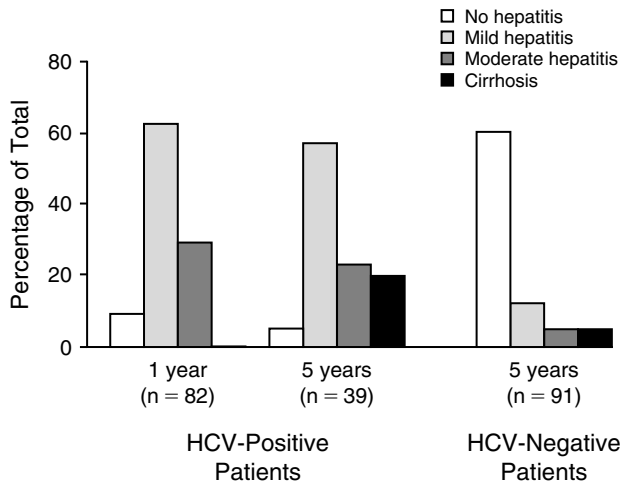


Figure 1. Biopsy Findings One and Five Years after Liver Transplantation in Recipients with HCV Infection after Transplantation and in Those without HCV Infection.

The number of patients in each group is given in parentheses. $P < 0.001$ for the comparison of each variable between the HCV-positive and HCV-negative groups at five years.

son, six of the nine patients with moderate chronic hepatitis at one year had cirrhosis at five years ($P = 0.001$ by Fisher's exact test).

Of the 91 HCV-negative liver-transplant recipients who underwent liver biopsy at five years, 11 had mild chronic hepatitis, 5 had moderate chronic hepatitis, and 5 had cirrhosis. Thus, there was a significant difference at five years between the HCV-infected transplant recipients and the control group with respect to the incidence of cirrhosis and of mild and moderate chronic hepatitis ($P < 0.001$ by the Mann-Whitney test) (Fig. 1). In 7 of the 10 HCV-negative patients with moderate chronic hepatitis or cirrhosis, the disease was caused by recurrent infection with hepatitis B virus.

Serum aspartate aminotransferase levels were a poor indicator of the severity of HCV-related graft injury. Fifty-six percent of the HCV-positive patients with mild chronic hepatitis had values in the normal range, as did 40 percent of those with moderate chronic hepatitis and 50 percent of those with cirrhosis (Fig. 2).

Of the 130 patients with hepatitis C infection who survived more than six months after transplantation, 109 received cyclosporine, azathioprine, and prednisolone as primary immunosuppressive therapy and 21 received tacrolimus and prednisolone. There was no significant difference between these two groups with respect to the distribution of histologic findings: 12 of 109 did not have hepatitis, as compared with 3 of 21; 59 of 109 had mild chronic hepatitis, as compared with 11 of 21; 30 of 109 had moderate chronic hepatitis, as compared with 5 of 21; and 8 of 109 had cirrhosis, as compared with 2 of 21 ($P > 0.1$ for all four comparisons by the Mann-Whitney test). Similarly, there was no significant difference in the distribution of histologic findings between the 59 patients who required adjuvant high-dose corticosteroids for the treatment of acute rejection and the 71 who did not: 4 of

the 59 had no evidence of hepatitis, as compared with 11 of the 71; 32 had mild chronic hepatitis, as compared with 38; 18 had moderate chronic hepatitis, as compared with 17; and 5 had cirrhosis in each group ($P > 0.1$ for all four comparisons by the Mann-Whitney test).

Among the 149 patients with HCV infection after transplantation, chronic rejection was diagnosed in 15 (10 percent) between 28 and 1470 days after transplantation (median, 99), a value that was similar to the incidence of chronic rejection in HCV-negative patients (12 percent). Six of these 15 patients were successfully treated with tacrolimus, 6 received a second liver transplant, and 3 died of graft failure related to chronic rejection.

Graft loss occurred in 27 of the 149 patients (18 percent) between 93 and 2436 days after transplantation (median, 303) and was due to chronic rejection in 9 patients, intractable acute rejection in 1, recurrent hepatitis B virus infection in 2, recurrent hepatocellular carcinoma in 2, hepatic-artery thrombosis in 3, biliary complications in 2, HCV-related cirrhosis in 5, and HCV-related severe cholestatic hepatitis in 3. The last three patients presented with jaundice 4, 8, and 16 weeks after liver transplantation; their condition deteriorated steadily; and graft failure developed at 3, 8, and 9 months, respectively. Serial liver biopsies demonstrated a rapid progression from acute lobular hepatitis to diffuse hepatocytic ballooning, with severe intrahepatic cholestasis but only minimal inflammatory infiltrate.

The cumulative survival rates for the 149 patients with HCV infection were 79 percent after one year, 74 percent after three years, and 70 percent after five years. The corresponding rates for the 623 HCV-negative transplant recipients were 75 percent, 71 percent, and 69 percent

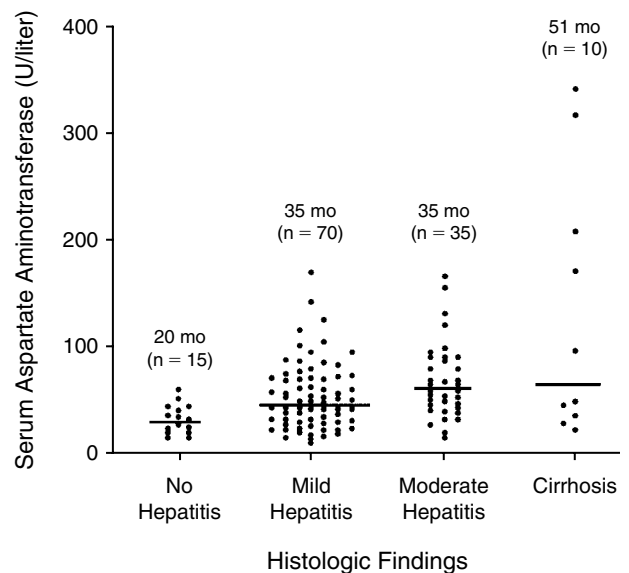


Figure 2. Serum Aspartate Aminotransferase Levels at the End of Follow-up in Relation to the Severity of Graft Injury.

The median length of follow-up and the number of patients are shown for each group. Bars indicate the median values. The normal range of values for aspartate aminotransferase is 0 to 50 U per liter.

($P=0.12$ by the Mantel–Cox test and $P=0.14$ by Breslow’s test for the difference between groups) (Fig. 3).

HLA Matching between Donors and Recipients

Lymphocytes from both members of 125 pairs of donors and recipients were available for HLA-A, B, and DR typing, including 14 cases involving a second transplantation. DNA samples from both members of 72 donor–recipient pairs were available for HLA-DR and DQ typing. No relation was found between the number of HLA-A, B, DR, or DQ mismatches and the extent of graft changes — whether absent or mild (no hepatitis or mild chronic hepatitis) or more severe (moderate chronic hepatitis or cirrhosis) at the end of follow-up. There was also no relation between the combined mismatch scores for HLA class I antigens (mean [\pm SD] mismatch score for HLA-A and B in the group with no hepatitis or mild chronic hepatitis, 2.88 ± 0.90 , vs. 2.94 ± 0.86 in the group with moderate chronic hepatitis or cirrhosis) or for class II antigens (HLA-DR and DQ) (2.44 ± 1.16 vs. 2.50 ± 1.22) or for all four loci (5.38 ± 1.34 vs. 5.30 ± 1.75). There was also no association between the rate of graft loss and the extent of mismatching at individual loci or all four loci combined.

Influence of HCV Genotype

The HCV genotype was assessed in 100 patients. The most prevalent genotype was 1b, found in 43 patients. Genotype 4 was found in 14 patients, all but 2 of whom were from the Middle East. Three patients were infected with more than one subtype. The HCV genotype could not be determined in one patient. Twenty of the 43 patients who were infected with genotype 1b (46 percent) had progressive liver disease (moderate chronic hepatitis or cirrhosis) at the end of follow-up, as compared with 13 of the 53 patients (24 percent) infected with other HCV genotypes ($\chi^2=5.1$, $P=0.02$) (Fig. 4).

When the independent effects of the HCV genotype,

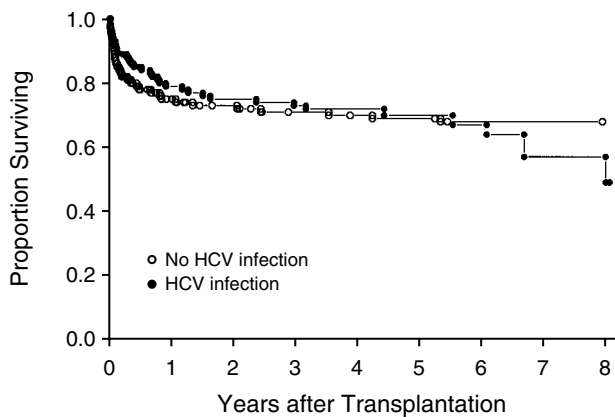


Figure 3. Kaplan–Meier Analysis of Survival According to HCV-Infection Status after Liver Transplantation. The number of patients in each group at each point is indicated.

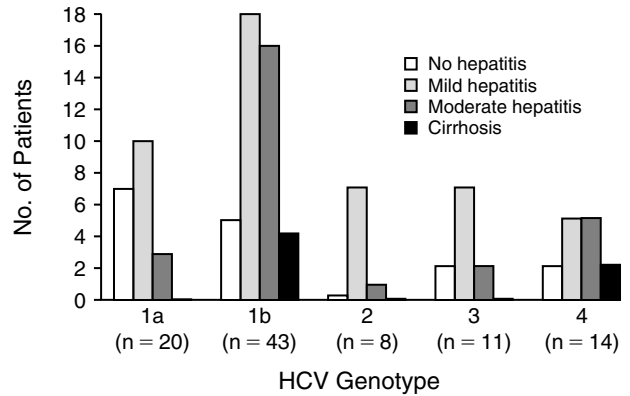


Figure 4. Effect of HCV Genotype on the Severity of Graft Injury in 96 Liver-Transplant Recipients with HCV Infection after Liver Transplantation.

The number of patients with each genotype is given in parentheses. The HCV genotype was determined in 100 patients. Three patients who were infected with two subtypes and one patient with no clear genotype pattern were excluded from the analysis.

the age at transplantation, sex, the type of HCV infection (recurrent or acquired), the length of time since transplantation, HBsAg status before transplantation, primary immunosuppressive regimen, use of adjuvant therapy for acute rejection, and the degree of HLA mismatching on histologic outcome were determined with multiple logistic-regression analysis, only the HCV genotype had a significant effect. Infection with HCV genotype 1b (HCV-1b) was more frequently associated with progressive graft damage than was infection with the other genotypes ($P=0.01$; odds ratio, 3.4; 95 percent confidence interval, 1.4 to 8.5).

DISCUSSION

Unlike previous reports suggesting that HCV infection is a relatively benign condition after liver transplantation,^{1,2,4} our study found that moderate chronic hepatitis developed in 27 percent of the patients after a median of 35 months and that the disease progressed to cirrhosis in 8 percent after a median of 51 months. Although the rates of both graft survival and overall survival in patients infected with HCV were similar to those in patients without HCV infection up to five years after liver transplantation, our findings suggest that with longer follow-up the HCV-infected patients may experience more problems than the control patients.

The rapid clinical and histologic progression in the three patients in whom subacute liver failure developed within nine months after liver transplantation is similar to that described in two case reports of severe HCV infection in transplant recipients.^{23,24} Although fibrosis was not a predominant feature in any of our patients, their clinical course resembled that of fibrosing cholestatic hepatitis in recurrent hepatitis B infection.²⁵ Serum aspartate aminotransferase levels were not correlated with the severity of chronic hepatitis in the graft, as has been observed in patients with chronic hepatitis C who have not undergone liver transplantation.^{10,26}

Because cellular immune reactions restricted by both

HLA class I^{27,28} and II^{29,30} antigens are involved in the recognition of HCV peptides, HLA matching between donor and recipient could potentially increase damage to the graft from recurrent viral infections by facilitating host recognition of viral antigens. Recently, a beneficial effect of a complete HLA-DQ mismatch was reported in 14 patients after transplantation for hepatitis C cirrhosis.¹¹ We found no relation between histologic outcome and the extent of matching of either HLA-DR or DQ. This difference may be due to the higher resolution achieved through the use of PCR-based sequence-specific oligonucleotide typing, as compared with the serologic methods used in the previous study. Nonetheless, cellular immune responses restricted by HLA class II antigens may be involved in the pathogenesis of HCV-induced graft damage. The graft is repopulated by the recipient's antigen-presenting cells and effector cells (including CD4+ lymphocytes) within weeks of transplantation, allowing cellular immune responses restricted by HLA class II antigens to operate independently of the donor's HLA class II antigens.

The finding that infection with HCV-1b was associated with more severe graft injury is consistent with the more aggressive liver disease caused by this genotype in patients who have not undergone transplantation.³¹⁻³³ This finding has been attributed to an increased replicative potential of HCV-1b,^{34,35} increased heterogeneity of HCV quasispecies,¹² and increased expression of viral antigen in liver tissue.³⁶ Alternatively, HCV-1b may be more immunogenic than other genotypes.³⁷ There are quantitative^{17,38} and qualitative³⁹ differences in antibody profiles between patients infected with different HCV genotypes.

Since prednisolone enhances HCV replication in patients with chronic HCV infection who have not undergone transplantation,^{40,41} our practice is to discontinue corticosteroid therapy within six months after transplantation in patients with HCV infection. High-dose therapy with intravenous methylprednisolone for acute allograft rejection is associated with a massive increase in the level of hepatitis C viremia¹⁴ and earlier recurrence of hepatitis.⁴² The need for supplemental intravenous corticosteroids and the cumulative dose of oral corticosteroids are both significantly lower in patients receiving tacrolimus-based immunosuppression than in those given cyclosporine-based therapy,^{43,44} suggesting that the former regimen may have advantages in patients with HCV infection. However, we found no difference between the two regimens in either the incidence or the severity of recurrent hepatitis in the graft. The use of OKT3 in patients who underwent transplantation for HCV-induced cirrhosis was recently associated with an earlier onset of lobular hepatitis in the graft and with more severe chronic hepatitis than are seen with other immunosuppressive drugs.⁴⁵ We could not investigate this association, because none of our patients received OKT3 as induction or adjuvant immunosuppressive therapy and because tacrolimus is used in our facility as rescue therapy for episodes of rejection that do not respond to corticosteroid therapy.

In conclusion, we found that HCV infection frequent-

ly recurs after liver transplantation for HCV-induced cirrhosis and may be associated with accelerated rates of graft damage in some patients, especially those infected with HCV-1b. Liver biopsies to detect and grade the extent of HCV-related graft damage may help identify patients who would benefit from antiviral therapy.

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