ORIGINAL ARTICLE

Exploring the Bidirectional Interactions Between Human Cytomegalovirus and Hepatitis C Virus Replication After Liver Transplantation

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Recurrence of Hepatitis C (HCV) post-liver transplantation (LT) is universal and its course is more aggressive than in immunocompetent individuals. Human cytomegalovirus (CMV) infection is a common post-LT infection and has immunomodulatory effects that could adversely affect the outcome of HCV. To date, the effect of HCV replication on the dynamics of CMV have not been investigated. From 2000 to 2004, a cohort of 69 HCV-infected liver transplant recipients and 188 HCV-negative liver transplant recipients (NON-HCV cohort) were monitored for CMV infection twice weekly by CMV polymerase chain reaction (PCR) with preemptive therapy initiated after 2 consecutive positive results. None of the patients received CMV prophylaxis. A subset of 18 HCV-infected patients had their HCV viral load monitored regularly post-LT by quantitative PCR. CMV DNAemia (>200 genomes/mL blood) did not influence the level of HCV replication within 150 days posttransplantation or the stage of liver fibrosis in liver biopsies at 1 yr post-LT. There were no differences in the incidence of CMV DNAemia or replication dynamics in the HCV cohort compared to the NON-HCV cohort. In conclusion, short term CMV viremia does not enhance the replication of HCV after LT, while HCV replication does not alter the replication dynamics of CMV. ***Liver Transpl 13:130-135, 2007.*** **© 2006 AASLD.**

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Currently, chronic hepatitis C virus (HCV)-related cirrhosis is the most common indication for liver transplantation (LT) in Europe and the United States,[1,2] accounting for 40 to 50% of individuals on the waiting list. Unfortunately, the recurrence of HCV infection, defined by detectable HCV ribonucleic acid (RNA) posttransplantation, is nearly universal, and can been detected as early as 48 hours posttransplantation.[3,4] Several studies have shown that the HCV RNA increases rapidly from the second week posttransplantation, reaching a peak between the third and fourth month after transplantation. The level of HCV RNA at this stage is a good predictor of subsequent histological activity index and fibrosis stage.[2,5] The histological progression of HCV is more aggressive after LT than in chronic HCV-infected nontransplant patients, with a cumulative probability of developing cirrhosis of 30% at 5 yr.[6] Human cytomegalovirus (CMV) infection is a common and important cause of morbidity in liver transplant recipients. CMV infection not only causes direct effects in target organs (e.g., hepatitis), but it has also a number of indirect effects, including a general immunosuppressive syndrome.[7] This enhanced immunosuppressive effect could influence the recurrence and/or the severity of HCV replication in the posttransplantation setting. CMV load has been shown to be an important factor in CMV disease progression,[8,9] with high viral loads being associated with an increased probability of disease.[9]

Abbreviations: HCV, hepatitis C virus, LT, liver transplantation, CMV, cytomegalovirus; PCR, polymerase chain reaction; AUC, area under the curve; RNA, ribonucleic acid; DNAemia, >200 genomes/mL blood; D, donor; R, recipient.

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To date, the impact of CMV infection post-LT in HCV infected individuals has led to contradictory findings. Three studies have shown an association between CMV infection and occurrence of liver cirrhosis in the graft.[10-12] However, another study was unable to demonstrate that CMV viremia or disease was associated with a poorer histological outcome.[13] Since CMV can impact adversely on the net state of immunosuppression[14] and HCV infection alters the phenotype of CMV CD8 + T cells and has a pervasive influence on the circulating CD8 + T cell population,[15] we investigated the bidirectional interaction between HCV and CMV at the level of viral replication post-LT.

PATIENTS AND METHODS

Patient Cohorts

From January 2000 to December 2004, a total of 69 patients with end-stage HCV-related cirrhosis that underwent orthotopic LT at the Royal Free Hampstead NHS Trust, London (HCV group), were assigned in the context of an ongoing randomized controlled trial to receive an immunosuppressive regime with either tacrolimus (FK) monotherapy (0.1 mg/kg daily) or triple therapy with FK (0.1 mg/kg daily), azathioprine at 1 mg/kg daily, and prednisolone at 20 mg daily tapered over 3 months. During the same time period a total of 188 patients with end-stage-related liver cirrhosis (excluding HCV-related cirrhosis) underwent LT at the Royal Free Hampstead NHS Trust, London (NON-HCV group). According to the clinical protocol these patients received individualized combinations of the following immunosuppressive drugs: prednisolone, azathioprine, cyclosporine, FK, and mycophenolate mofetil. Although the majority of patients received either FK monotherapy or triple therapy comprising FK, prednisolone, and azathioprine as their immunosuppressive regimen.

Quantitative Detection and Genotyping of HCV

A total of 18 HCV LT patients were analyzed in details for HCV replication. HCV RNA levels in frozen sera samples (mean number of samples per patient = 7) were measured weekly for the first month post-LT and then once a month up to day 150 by quantitative polymerase chain reaction (PCR) (Abbott RealTime HCV Assay; Abbott Diagnostic, Maidenhead, United Kingdom) with a lower limit of detection of 12 IU/mL. The HCV genotype was determined by reverse transcription PCR followed by reverse hybridization of the amplified sequence using the Versant HCV Genotype (LiPA) assay, (Bayer Healthcare, Newbury, United Kingdom).

Liver Histology

Of the 69 patients in the HCV group, 56 who had at least 1 yr of follow-up, had a protocol liver biopsy available for analysis at 1 yr. There were 17 CMV DNAemia-positive patients and 39 CMV DNAemia-negative patients. In the subgroup were biopsies were available, the histological evaluation of recurrent HCV was performed by using the Ishak score.[16] Briefly each liver biopsy was evaluated for the stage of the disease (fibrosis = 0-6) and the degree of the necroinflammatory activity (grade = 0-18), resulting from the sum of the piecemeal necrosis score (0-4), confluent necrosis score (0-6), focal necrosis score (0-4), and portal inflammation (0-4). A stage score ≥4 was considered as severe fibrosis. In 3 patients' biopsies the histology showed characteristics consistent with chronic rejection and therefore the biopsies could not be staged. For the remaining 13 patients, 1 liver biopsy was not available, 9 patients died, and 4 had missed their first-year liver biopsy. Among the 9 patients who died, 3 were CMV DNAemia-positive and 6 CMV DNAemia-negative, and none of deaths were due to HCV-related causes. Four patients died due to septicemia, 3 had multiorgan failure, 1 had pulmonary hypertension, and another 1 died because of graft failure.

CMV Replication Dynamics

Patients were monitored for CMV DNAemia twice weekly while they were inpatients and whenever they attended the outpatient clinic. DNA was extracted from whole blood using blood DNA extraction column (Qiagen, Crawley, United Kingdom) and then analyzed by using a qualitative in-house CMV PCR (from January 2000 to August 2002). The limit of detection of this assay was 200 genomes/mL of whole blood. From August 2002, a quantitative CMV PCR TaqMan-based assay, using the same primers as the qualitative assay, was used as described previously[17] (lower level of sensitivity = 200 genomes/mL whole blood). A significant level of replication was defined as a CMV load above 200 genomes/mL of blood. CMV replication dynamics, including the doubling time and half-life of decline following antiviral chemotherapy or peak CMV virus load, were computed using standard exponential growth and decay equations applied to the CMV load datasets following linear regression as described extensively elsewhere.[17]

The area under the curve (AUC) for viral load was calculated by integrating the viral load over time (trapezium method) and expressed as AUC per day after logarithmic transformation. CMV replication dynamics and AUC were calculated for the patients who had serial quantitative measurements of CMV available (from 2002 onward).

Preemptive Treatment of Human CMV Infection

None of the patients included in the study received CMV prophylaxis. From 2000 to 2002, preemptive antiviral therapy for CMV with either intravenous ganciclovir (5 mg kg/twice daily) or oral valganciclovir (900 mg twice daily) was started after 2 consecutive blood samples with a CMV virus load greater than 200 genomes/mL. After 2002, patients were treated preemptively only if CMV loads rose above 3000 genomes/mL.[18,19] Patients

TABLE 1. Characteristics of Liver Transplant Recipients With and Without CMV DNAemia

	HCV (n = 69)		NON HCV (n = 188)	
	CMV PCR+	CMV PCR−	CMV PCR+	CMV PCR−
Number	21 (100%)	48 (100%)	68 (100%)	120 (100%)
Age (median)	51	52	50	51
Gender				
Male	15 (71%)	42 (87.5%)	35 (51.4%)	65 (54%)
Female	6 (28.5%)	6 (12.5%)	33 (48.5%)	55 (45.8%)
D−/R−	0 (0%)	7 (14.6%)	2 (3%)	21 (17.5%)
D-/R+	8 (38%)	25 (52%)	14 (20.6%)	47 (39%)
D+/R+	12 (57%)	12 (25%)	41 (60.2%)	41 (34%)
D+/R−	1 (4.7%)	4 (8.3%)	11 (16%)	9 (7.5%)
D/R NA*				2 (1.6%)
Rejections				
0	6 (28.6%)	23 (37.5%)	23 (33.8%)	51 (42.5%)
1	11 (52.4%)	18 (37.5%)	25 (36.7%)	34 (28.3%)
2	4 (19%)	5 (10.4%)	13 (19%)	24 (20%)
≥3	0 (0%)	2 (4.2%)	7 (10.2%)	11 (9%)

*Not available.

were treated until 2 consecutive CMV PCR-negative samples were obtained (<200 genomes/mL) irrespective of the assay used.

Treatment of Acute Rejection

Acute rejection detected in protocol liver biopsies between day 5 and day 8 post-LT or in biopsies taken when rejection was suspected, was treated with methylprednisolone (1 gm daily intravenously for 3 days and repeated if necessary).

Statistical Analysis

Cox proportional hazards regression models were used to identify factors such as age, gender, calendar year of transplant, donor (D)/recipient (R) CMV status, associated with significant CMV replication (>200 copies/mL) within 100 days post-LT. Categorical variables were compared using chi-squared test. Comparison of continuous variables, such as virus load, between groups was achieved by use of the Mann-Whitney U test. In all analyses P values of 0.05 or below were regarded as statistically significant.

RESULTS

Baseline Characteristics

In the NON-HCV cohort 68 of 188 (36%) became CMV DNAemic by day 100 post-LT, and 21 of 69 (30%) in the HCV group. In univariable analysis, neither age, gender, nor calendar year of transplant were significantly associated with the probability of high-level replication (>200 genomes/mL). However, CMV D/R status was a significant risk factor for high-level replication (relative hazard for D+R−/D+R+ compared to D−R−/D−R+: 2.83, 95% confidence interval [1.17-6.83], P = 0.02 in the HCV group and RH for D−R+ compared to D−R−: 6.81 [0.90-51.52], P = 0.06; for D+R−/D+R+ compared to D−R−: 16.47 [2.27-119.32], P = 0.006 for the NON-HCV group). No significant difference between the study groups regarding the incidence of acute cellular rejection (P = 0.28 in the HCV cohort and P = 0.59 in the NON-HCV cohort) were observed (Table 1).

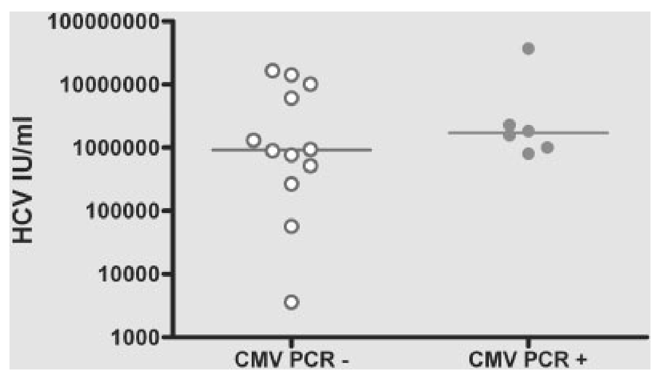

Figure 1. Maximum HCV viral loads (IU/mL) within 150 days post-LT in patients who had detectable CMV DNAemia (CMV PCR+, closed circle) compared to those who had no detectable CMV DNAemia (CMV PCR−, open circle).

Influence of Human CMV DNAemia on HCV Replication

Detailed analysis of 18 HCV-transplanted patients from January 2004 showed that CMV infection occurred in 6 patients (33%). The maximum HCV viral load within 150 days post-LT was not significantly higher in patients with simultaneous human CMV replication (median HCV viral loads = 1.7×10^6 IU/mL (8×10^5 to 3.7×10^7) vs. those who remained CMV PCR-negative (median HCV viral loads = 9.1×10^5 IU/mL (3.6×10^3 to 1.7×10^7) (P = 0.28) (Fig. 1). In addition, an alternative analysis was undertaken to capture all the viral

TABLE 2. Histological Grade and Stage of Liver Biopsy at 1 Year Posttransplantation in HCV Patients With and Without CMV Viremia

	HCV CMV PCR+	HCV CMV PCR−	*P* value*
Grade	3.5 (0–9)	4 (1–8)	0.3
Stage	1.5 (0–6)	2 (0–5)	0.24

*Chi-squared test.

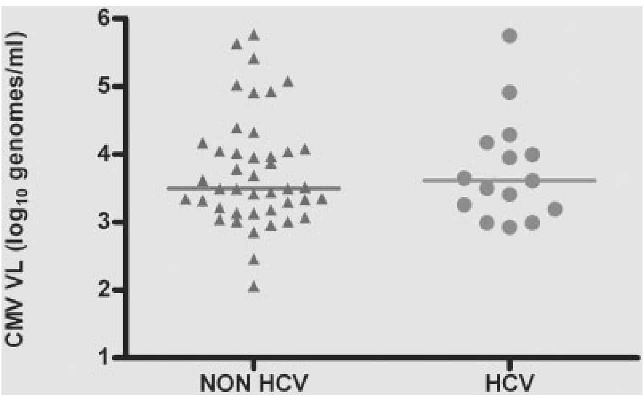

Figure 2. Maximum CMV viral load (CMV viral loads in log_{10} genomes/mL) within 100 days post-LT in the cohort of NON-HCV transplant patients (triangles) compared to HCV-infected liver transplant patients (circles).

load data, using the mean area under the HCV viral load-time curve expressed per day. Using this approach there was no significantly difference between the 2 groups ($P = 0.42$).

Histological Outcome of HCV at 1 Yr Post-LT

One-yr liver biopsies were performed in 56 of the HCV-infected patients (CMV DNAemia-positive group, n = 17; CMV DNAemia-negative group, n = 39), although the Ishak score was available only for 53 because 3 patients' biopsies had shown features consistent with chronic rejection and not recurrent HCV infection. A stage score ≥4 was found in 2 of 17 (12%) in the CMV DNAemia-positive group and in 4 of 39 (10.2%) in the CMV DNAemia-negative group. There was no difference in either histological grading or stage between the 2 groups ($P = 0.3$; $P = 0.24$, respectively) (Table 2).

Influence of HCV Replication on CMV DNAemia

Sequential CMV load quantified by real-time PCR was available in 33 of 68 (48.5%) of CMV-positive patients in the NON-HCV cohort and in 15 of 21 (71%) in the HCV population. A comparison of CMV loads expressed as maximum viral load within 100 days post-LT (Fig. 2) and log_{10} daily AUC (Fig. 3) in patients with and without HCV infection showed no difference between these 2

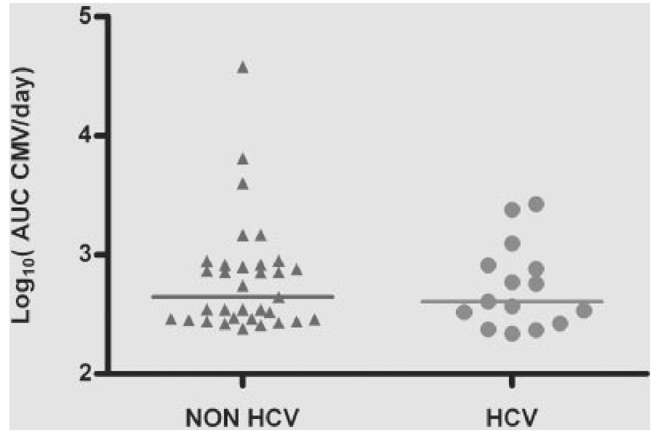

Figure 3. Total CMV viral burden expressed as the log of AUC per day during the first 100 days post-LT in the NON-HCV transplant cohort (triangles) compared to HCV-infected LT patients (circles).

groups ($P = 1$ for the maximum CMV viral load; $P = 0.7$ for the log_{10} daily AUC).

CMV replication dynamics, including doubling time and half-life of decline ($t_{1/2}$) from peak CMV load were not significantly different in the HCV cohort compared to the NON-HCV group (Table 3).

DISCUSSION

The recurrence of HCV infection post-LT is not only universal, but its course is more aggressive than in immunocompetent individuals. Several studies have sought to determine whether CMV infection adversely affects the outcome of HCV recurrence with discordant results. To our knowledge, there have not been extensive investigations on the effect of HCV replication on CMV replication post-LT.

In this study we could not show any influence of CMV DNAemia on the level of HCV replication measured within 3 months posttransplant. However, it is interesting to note that HCV RNA levels in the CMV-positive patients appeared to have a trend toward higher viral load, although this difference was not statistically significant. In addition, HCV-infected patients with CMV DNAemia were not at increased risk of liver fibrosis at 1 yr posttransplantation. It is unlikely that donor age was a confounding variable in our analysis since a previous study of 193 LT patients from our center showed that donor age did not influence survival following LT.[20] Given data showing that HCV can modulate CD8 T-cell activity, we investigated the effects of HCV replication on CMV replication dynamics. Interestingly, there was no difference in either the frequency of CMV DNAemia or replication dynamics (maximum viral load, AUC, doubling time, and decline rate after therapy) of CMV when we compared the HCV cohort with the NON-HCV cohort.

The results of our study are consistent with those of Humar et al.,[21] who found no correlation between CMV and HCV viral load and with Texeira et al.,[13] who found

TABLE 3. CMV Replication Dynamics in the HCV Group and NON-HCV Group

	HCV	NON HCV	*P* value*
Log CMV maximum viral load	3.61 (2.92–5.75) n = 15	3.49 (2.05–3.76) n = 43	1
Doubling time (days)	2.23 (1–8) n = 12	2 (0.1–69) n = 44	0.96
Decline rate after CMV therapy (days)	2 (0.8–12.6) n = 11	2.8 (–0.7–19) n = 43	0.11

*Mann-Whitney test.

that CMV viremia measured qualitatively did not influence the histological outcome of HCV recurrence. Other studies[10-12] have shown an association between CMV DNAemia and the severity of recurrence of HCV in the liver biopsy, although in studies[11,12] the association between CMV viremia and histological recurrence of HCV was observed only in the long-term outcome. In contrast Razonable et al.[22] have shown that CMV DNAemia was associated with higher fibrosis stage and higher HCV viral loads at 16 weeks but not at 1 yr. Although some of these studies suggest that a possible influence of CMV DNAemia influences long-term HCV recurrence, the effects are frequently not seen within 1 yr after transplantation consistent with our observations.

It is important to emphasize that in our study CMV viremia was detected by regular blood CMV PCR and that preemptive treatment with ganciclovir or valganciclovir was also initiated promptly. In contrast, the other studies have either used prophylaxis against CMV or have not specified the antiviral management used for CMV. Such rapid intervention in our study could have reduced the influence of CMV DNAemia on HCV replication and disease progression.

The immunomodulatory effects of both CMV and HCV appear not to exert themselves by influencing viral replication of either CMV or HCV after LT. Nevertheless, increased bacterial and fungal infections have been observed in patients with active CMV infection and can be reduced in patients receiving CMV prophylaxis.[23] However, further more detailed analyses of the effects of both infections on T-cell function following LT are warranted.

In conclusion, this study has shown that there is no significant unidirectional or bidirectional interactions between HCV and CMV operating at the level of viral replication in vivo following LT.

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