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Recurrence of hepatitis C after liver transplantation is associated with increased systemic IL-10 levels

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BACKGROUND: Recurrence of hepatitis C after liver transplantation is an almost universal occurrence. T-cell derived cytokines have an important role in the development of liver damage associated with chronic hepatitis C, their post-transplant levels, however, have not been correlated with histologic recurrence of the disease.

***Aims:* We sought to analyze levels of TNF-α, soluble IL-2 receptor, IL-4 and IL-10 at 1 month, 6 months and 1 year after transplantation in 27 patients undergoing transplantation for hepatitis C related end-stage liver disease.**

***Methods:* HCV RNA levels were monitored by a branched-chain DNA signal amplification assay. Diagnosis of recurrent hepatitis was based on 1-year protocol biopsies and on biopsies performed for liver enzyme elevations.**

***Results:* Recurrent hepatitis C was detected in 52% (n=14) of the 27 patients. HCV RNA levels rose over time in all patients regardless of histologic recurrence. TNF-α, and IL-4 levels, although elevated, did not show specific patterns over time or in correlation with recurrence. Similarly, the early elevation followed by a gradual decrease over the first year in the amount of soluble IL-2 receptor was not related to histologic recurrence. We observed a significant increase in circulating IL-10 levels over the first year in patients with biopsy-proven recurrence, while patients with no signs of histologic recurrence displayed increased, but steady levels.**

***Conclusions:* These results suggest that while these cytokines are associated with post-transplant recurrence of hepatitis C, their production may be altered by additional factors.**

Key words: Liver transplantation, Hepatitis C, Recurrence, Cytokines

Introduction

Hepatitis C is a progressive disease that leads to cirrhosis in approximately 20% of affected individuals[1–3] and has emerged as the most common indication for liver transplantation in both Europe and the USA.[4] Serologic recurrence of hepatitis C infection after transplantation is virtually universal.[5,6] Histologic recurrence has been observed in 30–70% of patients within one year following transplantation for hepatitis C related end-stage liver disease.[6–8] A subset of these patients will develop allograft failure as a result of hepatitis C recurrence requiring re-transplantation.[9]

The nature of the injury induced by chronic hepatitis C infection has been extensively investigated. Cytokines appear to play an important role in the liver damage associated with chronic hepatitis C via a variety of mechanisms, including activation of T lymphocytes,[10] direct hepatocellular damage,[11] and by modulation of both the viral-specific immune response[12,13] and viral replication.[14,15] The relation between changes in cytokine patterns and post-transplant recurrence of hepatitis C, however, is unclear.

This study was designed to evaluate cytokine profiles (TNF-α, IL-4, IL-10, and the soluble receptor of IL-2 [sIL-2R]) over the first year following primary orthotopic liver transplantation (OLTx) for liver failure secondary to hepatitis C and to correlate these profiles with hepatitis C RNA viral levels and with biopsy-proven histologic recurrence.

Patients and methods

Patients

Twenty-seven patients transplanted for liver failure secondary to hepatitis C cirrhosis between February 1994 and December 1996 were included in this study.

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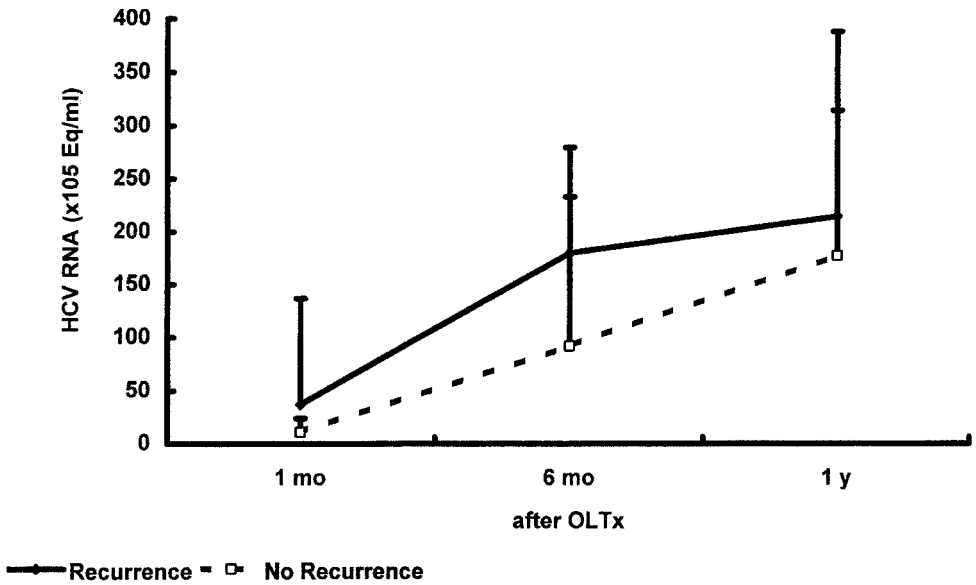

FIG. 1. HCV RNA levels in patients transplanted for hepatitis C related liver failure.

Patients undergoing transplantation for hepatitis C with hepatocellular carcinomas requiring adjuvant chemotherapy (i.e., tumors >3 cm or with vascular invasion), or with positive serology for hepatitis B surface antigen were excluded. Informed consent was obtained from all patients prior to enrollment.

OLTx

All patients received induction immunotherapy with OKT3 followed by triple immunosuppression therapy with cyclosporine, azathioprine and steroids. Patients were converted to tacrolimus for recurrent or severe rejection episodes or for side effects of cyclosporine.

Sample collection

Serum samples were obtained from each patient preoperatively and at 1 month, 6 months and 1 year after transplantation. All samples were aliquoted into cryotubes and stored at –80°C until analyzed.

Hepatitis C virus RNA levels

RNA levels were determined in duplicate by a branched-chain DNA signal amplification assay (Chiron HCV RNA 1.0, Chiron Diagnostics, Emeryville, CA). Actual hepatitis C viral concentrations were computed for each sample using a standard curve provided by the manufacturer.

Cytokine levels

Serum cytokines were measured using commercially available ELISA kits for IL-10, IL-4, sIL-2R and TNF-α (Immunotech, France) following the manufacturer's instructions. Actual cytokine levels were calculated for each cytokine using a specific standard curve supplied by the manufacturer. According to the manufacturer, there are no detectable levels of these cytokines in normal human sera.

Diagnosis of recurrence

The diagnosis of recurrent hepatitis was made histologically by examination of 1-year protocol biopsies and/or examination of biopsies performed for liver enzyme elevations. Histologic diagnosis was based on the presence of: (1) diffuse necroinflammatory lesions within the parenchyma in the absence of acute cellular rejection and other causes of acute hepatitis (e.g., CMV, hepatitis B, drugs) along with dense lymphoid aggregates and lymphoid follicles in the portal tracts, or (2) piecemeal necrosis and bridging necrosis in the absence of other causes.

Statistical analysis

All data were expressed as the mean±SD. Data were analyzed with ANOVA. Only p values <0.05 following Bonferroni correction were considered statistically significant.

Results

Recurrent hepatitis

Biopsy-proven histologic evidence of hepatitis recurrence was evident in 52% (n=14) of the 27 patients.

Hepatitis C viral RNA levels

Serum levels of hepatitis C viral RNA are shown in Fig. 1. All patients had evidence of hepatitis C viremia after transplantation, regardless of evidence of histologic recurrence of hepatitis. Over the first year

Table 1. Serum cytokine levels in patients transplanted for hepatitis C related liver failure

	After OLTx in patients					
	1 month		6 months		1 year	
	Recurrence	No recurrence	Recurrence	No recurrence	Recurrence	No recurrence
TNF-α (pg/ml)	300 ± 144	324 ± 1136	312 ± 1115	351 ± 1151	315 ± 1122	341 ± 1139
sIL-2R (pg/ml)	102 ± 197	91 ± 1123	68 ± 151	65 ± 166	58 ± 155	57 ± 161
IL-4 (pg/ml)	199 ± 1195	331 ± 1329	267 ± 1165	286 ± 1233	250 ± 1290	315 ± 1317

following transplantation, viral RNA levels rose significantly in patients with and without recurrence. No significant differences in viral levels were observed between the two patient groups at any time point.

TNF-α levels

Serum levels of TNF-α were considerably elevated following transplantation and remained elevated over the first year in both patient groups. No significant differences were observed over time in either group or between the 2 patient groups (Table 1).

sIL-2R levels

A moderate elevation in levels of sIL-2R was observed in both patient groups at 1 month following transplantation. In both groups, there was a decreasing trend over the first year regardless of histologic recurrence, with no differences between the 2 patient groups (Table 1).

IL-4 levels

No significant differences in IL-4 levels were observed over time in either group or between the 2 patient groups (Table 1).

IL-10 levels

Changes in serum levels of IL-10 are shown in Fig. 2. In patients without histologic evidence of recurrence, levels of IL-10 were elevated after transplantation but did not vary significantly over the first year. On the other hand, in patients with histologic evidence of recurrence, IL-10 levels dramatically increased over time, with a statistically significant difference between 1 month and 1 year ($p<0.01$), as well as between 6 months and 1 year ($p<0.01$). Additionally, there was a considerably significant difference between IL-10 levels in the 2 patient groups at 1 year ($p<0.05$).

Discussion

The immunologic mechanisms involved in chronic hepatitis C-related liver injury are not fully understood, and these events may be even more complex in recurrent hepatitis C following transplantation. Intense immunosuppression,[16,17] pre-transplantation viral load, post-transplantation changes in viral level,[9,18] and the viral genotype[19] have all been postulated to affect hepatitis C recurrence following liver transplantation.

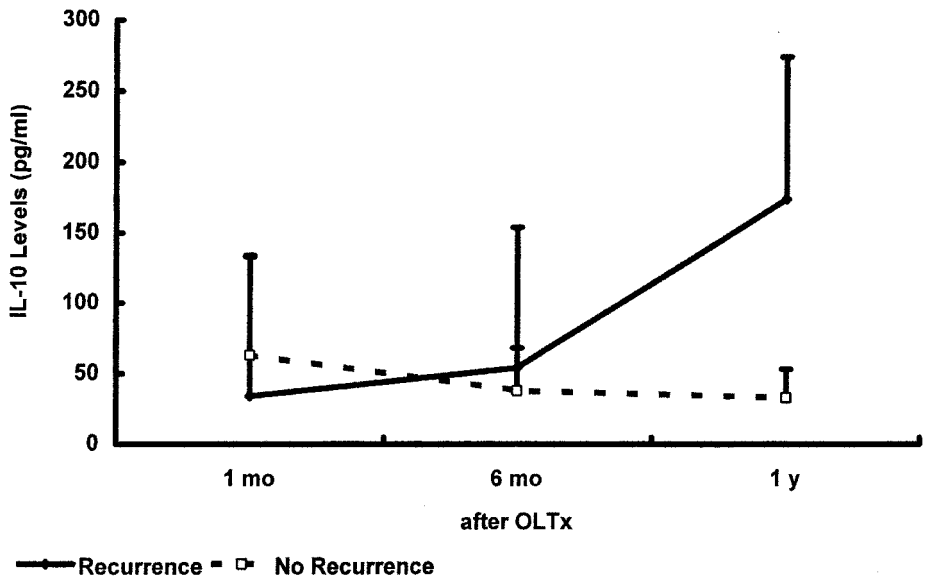

FIG. 2. IL-10 levels in patients transplanted for hepatitis C related liver failure.

In the present study, we observed that in patients who undergo liver transplantation for hepatitis C infection, viral levels rise over the first year regardless of histologic recurrence. Furthermore, the quantity of circulating virus did not correlate with the finding of histologic recurrence. These observations support those of previous studies, which have found that viremia develops in almost all patients after liver transplantation for hepatitis C and that the severity of recurrent hepatitis C does not correlate with viral levels.[11,20,21]

Cytokines are crucial mediators of tissue homeostasis. As important co-stimulators of T-cell activation, cytokines function to broaden the allograft immune response by recruiting other cells and stimulating the expression of adhesion molecules. In chronic liver diseases, cytokine profiles are known to vary according to the specific etiology as well as disease severity.[22–24] The T-helper type 1 (Th-1)/T-helper type 2 (Th-2) paradigm is based upon distinct cytokine secretion patterns from 2 sub-populations of CD4+ T-helper lymphocytes.[25,26] Th-1 cells are involved in cellular immunity, and Th-1 related cytokines (IL-2, TNF-α, IFN-γ) are important in the host antiviral immune response. Th-2 cells are central to humoral immunity, while Th-2 related cytokines (IL-4, IL-10) have major negative immunoregulatory functions. A shift in Th-1/Th-2 balance might be involved in disease progression.[10,22,26,27]

The cytokines chosen for evaluation in this study all play important roles in humoral and cell-mediated immunity, as they represent both the Th-1 (sIL-2R, TNF-α) and Th-2 (IL-4, IL-10) arms of T-cell response. Since all these cytokines are known to be elevated in chronic hepatitis C, monitoring their levels following transplantation offers a unique opportunity to investigate their possible association with recurrent hepatitis C. The increased levels of these mediators following OLTx in patients with liver failure secondary to hepatitis C represent an activated T lymphocyte response.

In patients with chronic hepatitis C, TNF-α has been observed to regulate hepatocyte damage and to be present in increased levels.[2,23,28–31] While we observed significantly elevated levels of TNF-α over the first year following liver transplantation in patients with and without recurrence, there was no correlation between TNF-α levels and histologic recurrence or viral RNA levels.

Increased release of sIL-2R, a marker of T cell activation, has also been reported by several groups in patients with chronic hepatitis.[2,15,32–35] The moderate increase in sIL-2R levels described in the present study 1 month following liver transplantation was irrespective of outcome and may reflect the effects of the intense immunosuppression on activated T cells. Indeed, earlier we reported that sIL-2R levels increased during the first week after OLTx in patients with hepatitis C, with the highest levels measured in patients whose primary immune suppression was OKT3, likely denoting the marked degree of cytolysis which occurs following OKT3 administration.[36]

IL-4 is involved in the humoral immune response. Its effects have been observed to be associated with the progression of viral infections, and its production is known to be enhanced in patients with chronic hepatitis C.[10,12,22] Increased expression of IL-4 has been linked to the development of hepatitis C chronicity.[37] In this study, we observed significantly elevated levels of IL-4 that were sustained over the first year following transplantation, but we saw no specific patterns that correlated with histologic recurrence or viral load.

IL-10 is known to inhibit many effector functions of the immune system.[12,13,38] Its production has also been observed to be significantly enhanced in patients with chronic active hepatitis C.[10,39] We found increased levels of IL-10 in both patient groups following transplantation. An immediate/early post-transplantation increase in systemic IL-10 levels, which quickly returned to normal values, has been reported and was associated with possible activation of the graft's macrophages.[40] The changes in the post-transplant pattern of systemic IL-10 levels reported here for the first time – a dramatic increase over the first year only in those patients with histologic evidence of recurrence – support that IL-10 may be related to the development of hepatitis C chronicity.[37] Increased levels of IL-10 inhibit the development of Th-1 effector mechanisms, leading to decreased IFN-γ production and antiviral response.[33] While persistent HCV replication was shown to be associated with the release of IL-10 in other studies,[39] the fact that in our patients HCV-RNA levels rose over the first year regardless of histological evidence of recurrence suggest a more complex, yet unclear role for IL-10 in post-transplant recurrent hepatitis C.

Our findings indicate that after liver transplantation for hepatitis C, production of these 4 cytokines is not exclusively driven by disease recurrence but may be altered by confounding factors, including graft damage, allo-specific immune response and immunosuppression. While induction immunotherapy was uniformly achieved with OKT3 among these patients, the sampling schedule did not allow us to correlate cytokine levels to important clinical variables such as ischemia/reperfusion damage, frequency and treatment of rejection episodes, or infection. Since these mediators may serve as possible targets for treatment of post-transplant recurrent hepatitis C, further investigations are warranted.

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