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Hepatic Stellate Cells Attenuate the Immune Response in Renal Transplant Recipients With Chronic Hepatitis

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ABSTRACT

Background. Chronic viral hepatitis is no longer a contraindication to renal transplantation (RT), owing to our better understanding of the hepatitis virus. Hepatitis patients may receive RT depending on their response to viral therapy. RT patients with hepatitis generally do not have an inferior prognosis compared with RT patients without the disease. Hepatic stellate cells (HSCs) are activated during chronic viral hepatitis. The role of HSCs in immunoregulatory effects in RT recipients has not been fully elucidated.

Methods. We recruited 22 RT recipients with chronic viral hepatitis, who composed the chronic liver disease (CLD) group, and 25 disease-free recipients, who served as the control group. We retrieved their clinical data and collected serum to measure cytokine levels. To investigate the immunoregulatory effect of HSCs, we cocultured HSCs with allogeneic antigen-presenting cell–activated T cells (mixed lymphocyte reaction [MLR]) in Transwell plates.

Results. The liver biopsy disclosed activation HSCs in 1 chronic hepatitis C virus recipient without treatment. Serum monocyte chemoattractant protein-1 (MCP-1) levels in the CLD group (41.6 ± 27.4 pg/mL) were significantly higher than those in the control group (28.1 ± 12.8 pg/mL; $P = .008$). There were similar levels of transforming growth factor-β1 (TGF-β1). In allogeneic MLR, HSCs inhibited T-cell activation through the soluble factors in the Transwell assays. There was a high level of MCP-1 in the supernates of the HSC group in the allogeneic MLR, but TGF-β1 was lower in HSCs cocultured with MLR than in the control group, except in the early period.

Conclusions. HSCs may play an immunoregulatory role in chronic viral hepatitis recipients to minimize the effect of immunosuppressants without affecting rejection. The immunomodulatory effects may be attributed to soluble factors in HSCs.

There are high prevalence rates of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in Taiwan. Chronic liver disease and its complications may affect graft function and patient survival among renal transplant (RT) recipients. Several studies have shown that the prognosis in RT recipients with chronic hepatitis are not

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worse than that in disease-free recipients.[1–5] Hepatic tolerance has been demonstrated in liver transplantation, because the liver is a specific milieu to regulate the immune response.

In a previous study, we reported that activated hepatic stellate cells (HSCs) suppressed T-cell responses in allogeneic mixed lymphocyte reaction (MLR) cultures associated with enhanced T-cell apoptosis[6] and that cotransplantation of HSCs prevented islet allograft rejection without systemic immunosuppression.[7] In an in vitro study, activated HSCs more frequently induced lymphocyte apoptosis in MLR than quiescent HSCs.[8] Chronic liver infection or inflammation is a cytokine-rich environment in which inflammatory cells are recruited and HSCs are activated to respond to an injured region, which contributes to hepatic fibrosis.[9,10] In the present study, we demonstrate that HSCs were activated in chronic hepatitis recipients with a high serum level of monocyte chemoattractant protein-1 (MCP-1), and that the activated HSC-suppressed T-cell responses in MLR correlated with high levels of MCP-1 in supernates.

MATERIALS AND METHODS

Patients

We recruited 22 RT recipients with chronic viral hepatitis and elevated liver function twice above the normal limit for 3 months, assigning them to a chronic liver disease (CLD) group. Twenty-five disease-free RT recipients served as the control group. Their clinical data were retrieved and serum collected for cytokine analysis. The study was approved by our Institutional Review Board (IRB No. CE11155). The liver biopsies were reviewed by a pathologist.

Animals

BALB/c and C57BL/6 mice were obtained from the National Laboratory Animal Center, Taipei, Taiwan. All of the animals were kept at our Animal Center. They received mouse chow and tap water ad libitum. The effector T cells were from BALB/c, and the HSCs and allogenic antigen-presenting cells (alloAPCs) were from C57BL/6.

Mixed Lymphocyte Reaction

To investigate the soluble immunoregulatory effects of HSCs, we cultured HSCs in alloAPCs activated T cells in Transwell plates. In these assays, carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled responder T cells (2×10^5/well) and alloAPCs (2×10^4/well) were first added to the compartments of 96-well plates. Nunc CC Inserts with a 0.2-μm pore size were then inserted into wells, to which HSCs were added (2×10^4/well). After 6 days of culture, the inserts were removed. The supernates collected after centrifugation were stored at −80°C for further detection of cytokines. The cells in each well were then harvested to analyze CFSE fluorescence using flow cytometry.

Detection of Cytokines by Enzyme-Linked Immunosorbent Assay

MCP-1 and TGF-β1 concentrations in patient sera and MLR supernates were analyzed by sandwich enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (Biosource for MCP-1; R&D Systems for TGF-β1).

RESULTS

Clinical Manifestations and Outcomes

In the CLD group, there were HBV infections in 9 recipients (41%) and HCV infections in 13 (59%), and neither in the control group. Table 1 shows that there were no differences in gender, causes of end-stage renal disease (ESRD), duration of dialysis therapy, age of transplantation, and duration of follow-up between patients with vs without hepatitis. After follow-up of 5.3 ± 4.5 years (range 0.6–17.8), the dosages of immunosuppressive agents were significantly lower in the CLD than in the control group. Furthermore, there were no significant differences between the groups in time to first rejection and acute rejection episodes. Serum creatinine levels, which represented graft function, were nonsignificantly different in the CLD compared with the non-disease group.

HSCs Activated in Chronic Hepatitis

The liver biopsy from 1 chronic HCV hepatitis recipient disclosed minimal portal and interlobular mononuclear infiltrates and swollen hepatocytes (stage 1) at 6 months after RT, and only scanty anti–smooth muscle actin–positive HSCs were found in the perisinusoidal space (space of Disse) (Fig 1A and 1C). A second liver biopsy was performed owing to

Table 1. Comparison of Immunosuppressive Agents and Clinical Outcomes Between Chronic Liver Disease (CLD) and Control Groups

	CLD (n = 22)	Control (n = 25)	*P* Value
Male (%)	13 (59)	11 (46)	.485
Cause of ESRD			
CGN	18 (82)	17 (68)	.200
SLE	2 (9)	0 (0)	
DM	1 (5)	4 (16)	
Congenital	1 (5)	2 (8)	
Obstructive	0 (0)	2 (8)	
Dialysis duration (y)	2.6 ± 3.6	2.3 ± 3.5	.754
Age of renal transplant (y)	44.1 ± 10.7	49.4 ± 1.3 10.7	.098
Follow-up duration (y)	6.6 ± 5.2	4.1 ± 3.4	.061
sCr (mg/dL)	1.5 ± 0.4	1.5 ± 0.4	.727
GOT (U/L)	97.9 ± 49.9	19.0 ± 4.2	.000
GPT (U/L)	124.4 ± 40.8	19.4 ± 7.0	.000
Immunosuppressive agents			
Prednisolone (mg/d)	3.7 ± 1.3	5.6 ± 2.1	.002
Tacrolimus (mg/d)	2.2 ± 1.0	6.0 ± 3.8	.004
Cyclosporine (mg/d)	100 ± 20.0	139.3 ± 40.5	.313
Mycophenolic mofetil (mg/d)	795.5 ± 245.4	1361.1 ± 287.3	.000
Outcomes			
Time to first acute rejection	2.6 ± 2.1	2.0 ± 3.5	.801
Acute rejection (%)	3 (13.6)	8 (32.0)	.170

Abbreviations: ESRD, end-stage renal disease; CGN, chronic glomerular nephritis; SLE, systemic lupus erythematosus; DM, diabetes mellitus; sCr, serum creatinine; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase.

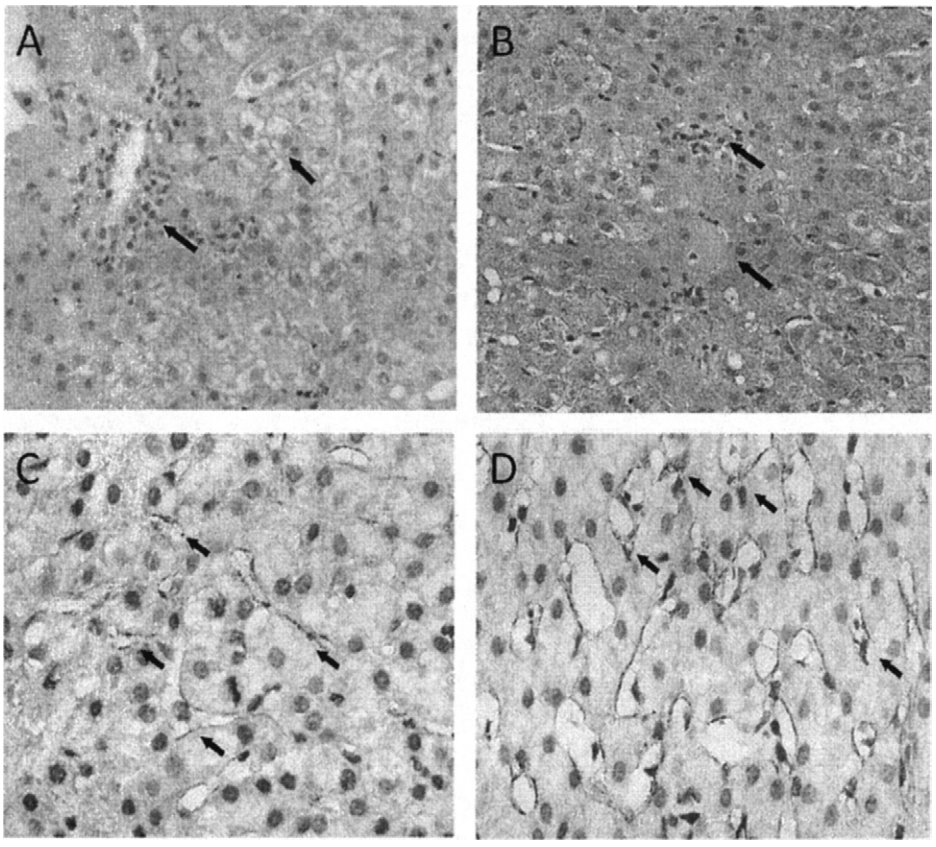

Fig 1. Changes in the liver parenchyma in the liver biopsy from recipient with chronic HCV-related hepatitis. **(A)** Minimal portal and interlobular mononuclear infiltrates and swollen hepatocytes were found 6 months after renal transplantation. **(B)** The second biopsy was performed 1 year later, revealing a mild increase of interlobular mononuclear infiltration, acidophilic necrosis, and swollen hepatocytes with mild portal fibrosis and mild piecemeal necrosis. **(C)** Scanty stain of anti–smooth muscle actin–positive hepatic stellate cells (HSCs) located in the perisinusoidal space (space of Disse) in the interlobular region. **(D)** More active and proliferative HSCs were found in the second liver biopsy.

persistent liver dysfunction at 1 year after the first biopsy. The pathology disclosed a mild increase in interlobular mononuclear infiltration, acidophilic necrosis, and swollen hepatocytes with mild portal fibrosis and mild piecemeal necrosis with more active and proliferative HSCs (Fig 1B and 1D). Chronic HCV hepatitis damaged hepatocytes and induced inflammatory cell infiltration simultaneously; HSCs were activated to induce liver fibrosis.

HSCs Suppressed Lymphocyte Activation Through Soluble Factors

In previous reports, we demonstrated HSCs to suppress allogenic T-cell proliferation.[6,7,22] When activated HSCs were cocultured inside inserts, decreased T-cell proliferative response was observed in Transwell MLR, indicating that soluble HSC factors may play an important roles in immunosuppression. In Fig 2, the soluble factors of HSCs in the milieu suppressed the CFSE-labeled allogenic T-cell response in MLR by 59%.

High Level of MCP-1 in the Serum and Supernate

The serum level of MCP-1 in CLD recipients (41.6 ± 27.4 pg/mL) was significantly higher than that in control subjects (28.1 ± 12.8 pg/mL; $P = 0.008$); the serum level of TGF-β1 in CLD recipients also was significantly higher than that in disease-free recipients (Fig 3A). We also measured cytokines in the MLR supernates over time, detecting extraordinarily high levels of MCP-1 in the presence of HSCs in allogenic MLR. The distribution of TGF-β1 level was progressively elevated in allogenic MLR. Interestingly, TGF-β1 was lower in HSCs than in the control group, except in the early stage (2 days; Fig 3B). The level of TGF-β1 varied corresponding to the level of MCP-1 in the supernate on the observation day.

DISCUSSION

Poor outcomes in patients with HBV or HCV infection in the early era of RT prompted many workers to consider hepatitis virus infections to be contraindications for RT. The liver biopsy from a chronic HCV hepatitis recipient demonstrated progressive destruction of normal tissue architecture, showing that activation of HSCs caused liver fibrosis. In RT recipients with untreated chronic HCV hepatitis, 41% had progressive liver disease, 39% had stable histologic findings, and 20% actually showed improvement in liver disease ≥6 years after RT.[12] In the present study, we demonstrated RT recipients with chronic viral hepatitis to receive lower dosages of immunosuppressant without an increased risk of acute rejection episodes and with similar graft function at various follow-up periods. Thanks to recent research efforts, the hepatitis virus is now better understood. Depending on the response to viral therapy,

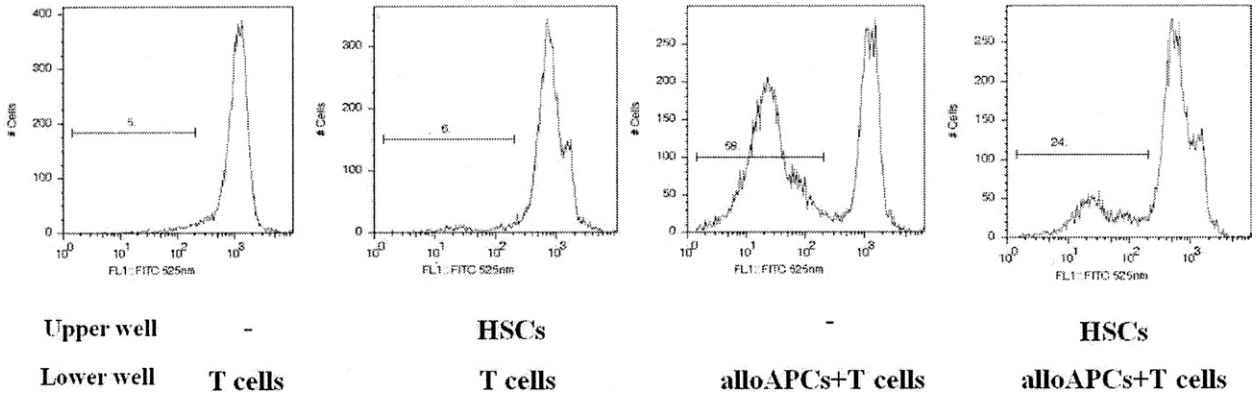

Fig 2. When activated HSCs were cocultured inside inserts, decreased CFSE-labeled T-cell proliferative response was observed, indicating that soluble HSC factors may play an important role in immunosuppression.

chronic HBV or HCV infection is no longer a contraindication for RT, especially because patients with hepatitis are now known to not have inferior survival rates compared with disease-free RT patients.[13]

Liver tolerance is well known. Liver transplantations show a lower susceptibility to rejection than other organs. Immunosuppressive drugs have been completely withdrawn in a select group of recipients.[14] The liver possesses unusual interactions between the hepatic milieu and immunologic cells. It is also the graveyard for trapping, apoptosis, and phagocytosis of activated T cells.[15] In our previous reports, HSC-induced T-cell hyporesponsiveness was associated with enhanced T-cell apoptosis.[6,7,11] Activated HSCs in hepatic fibrotic tissue induced apoptosis of extravasated and infiltrated T cells in vivo.[5] In the present Transwell assays, soluble HSC factors appeared to play an important role in the allogeneic T-cell hyporesponsiveness. The supernates from the HSC-cocultured MLR showed high levels of MCP-1, which activates T cells in the inflammatory region and has been shown to promote T_H2-phenotype inflammation in a murine model by enhancing the expression of interleukin (IL) 4 and inhibiting the production of IL-12. These observations indicate that MCP-1 is a protective cytokine in lethal murine endotoxemia, shifting the balance in favor of anti-inflammatory cytokine expression in endotoxin-challenged animals.[16] The distribution of TGF-β1 levels was progressively elevated in allogenic MLR. Although TGF-β1 is one of the major secretory cytokines activated by HSCs,

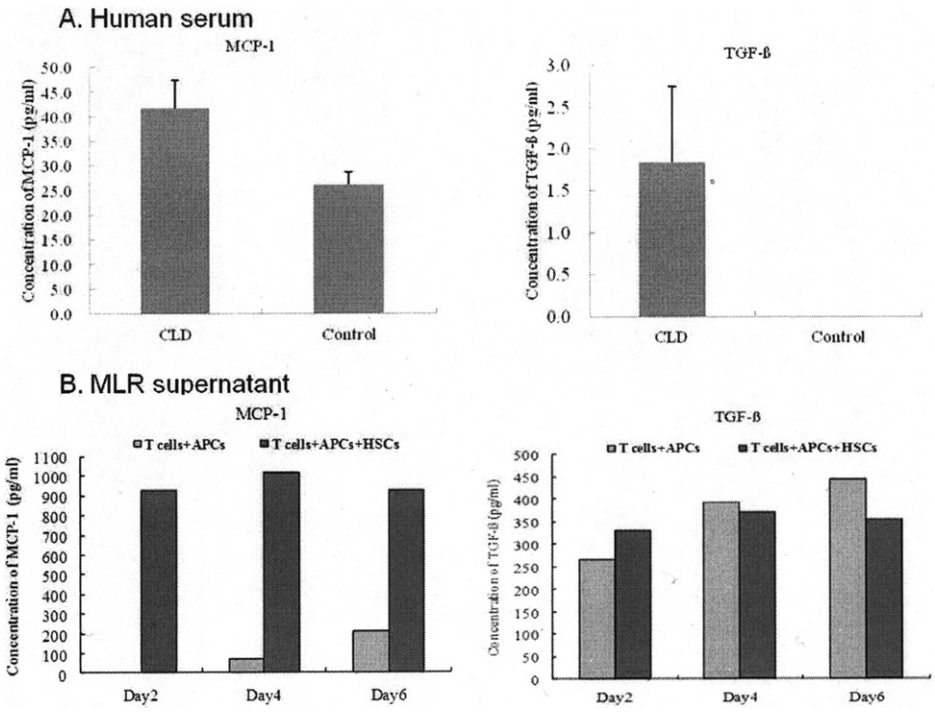

Fig 3. Comparison of cytokines in serum of recipients and supernate from mixed lymphocyte reaction. **(A)** MCP-1 and TGF-β1 levels were significantly higher in chronic liver disease (CLD) recipients than those in the disease-free control group. **(B)** The MCP-1 and TGF-β1 levels were measured in the supernates over time; when HSCs were cocultured inside inserts, there were higher levels of MCP-1 than in the control.

our results showed that it was lower in HSC-cocultured MLR than in the control group and varied according to the level of MCP-1 except in the early period. This phenomenon may be explained by the low TGF-β1 levels in HSC-cocultured MLRs caused by HSC suppression of allogenic T-cell proliferation, while most immune cells, including T cells, also secrete TGF-β1. Thus, we observed high levels of TGF-β1 in initial cultured periods, followed by lower amounts than in the control. Another possibility is that activated HSCs secrete TGF-β1 initially, which undergoes uptake by immune cells to down-regulate proliferative effects of T cells. Further studies are needed to evaluate these hypotheses.

In this study, we demonstrated that HSCs were activated during chronic viral hepatitis infection with significantly reduced doses of immunosuppressive agents and similar renal function without an increased occurrence of acute rejection episodes on long-term follow-up in RT recipients. There were significantly higher serum levels of MCP-1 and TGF-β1 than in control subjects, which suggests an immunoregulatory role of HSCs in chronic viral hepatitis. The Transwell assay also showed that soluble factors might suppress allogeneic T-cell proliferation. The immunomodulatory activity of HSCs may contribute to negative regulation of immune responses in the liver, resulting in hepatic tolerance.

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