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Original Article

Splenectomy increases the number of circulating hematopoietic stem/progenitor cells in patients with hepatitis C virus-associated liver cirrhosis

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Aim: The spleen is not believed to contribute to hematopoiesis in healthy adults. However, several reports have demonstrated that the spleen in adults contains a large number of hematopoietic stem/progenitor cells (HSC). Although splenectomy increases platelet and leukocyte counts, the effects of splenectomy on circulating HSC have not been elucidated. In this study, we evaluated the association between the number of circulating HSC and splenectomy in patients with hepatitis C virus (HCV)-associated liver cirrhosis (LC).

Methods: In 48 patients with various stages of HCV-associated chronic liver disease and seven patients with LC who underwent splenectomy, and 10 healthy volunteers, we determined the numbers of circulating $CD34^+$ cells and colony-forming unit culture by flow cytometry and methylcellulose culture, respectively. Plasma stromal cell-derived factor-1α (SDF-1α) concentrations were measured using an enzyme-linked immunosorbent assay.

Results: The numbers of circulating $CD34^+$ cells and colony-forming unit culture decreased but the plasma SDF-1α concentration increased with the progression of liver disease. There was an inverse correlation between the number of circulating HSC and the plasma SDF-1α concentration. The numbers of circulating HSC and platelets were determined before and after splenectomy in seven patients with LC. In these patients, the numbers of circulating HSC and platelets increased significantly after splenectomy and the enhancing effect persisted for a long time.

Conclusion: Our data suggest that the spleen plays an important role in modulating HSC dynamics in patients with HCV-associated chronic liver disease. Our results also imply that splenectomy may improve liver function in patients with LC.

Key words: hematopoietic stem/progenitor cells, hepatitis C virus, liver cirrhosis, splenectomy

INTRODUCTION

FOR PATIENTS WITH end-stage liver disease, orthotopic liver transplantation is the only therapeutic option with curative effects. However, alternative therapeutic approaches are still necessary because of limited donor availability, the need for long-term immunosuppression after liver transplantation and the high cost of the procedure. There is accumulating evidence showing that hematopoietic stem/progenitor cells (HSC) participate in liver regeneration in animal models,[1–4] and based on observation of liver biopsies from recipients of sex-mismatched therapeutic bone marrow (BM) and orthotopic liver transplantations.[5,6] Some authors have reported that autologous BM cell infusion therapy improved the clinical symptoms and biochemical data by activating the progenitor cell compartment and

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enhancing hepatocyte proliferation in patients with decompensated liver cirrhosis (LC).[7,8]

Although HSC are a potential source of cells for liver repopulation, the mechanisms and kinetics of HSC mobilization in patients with chronic liver disease (CLD) are poorly understood.[9,10] To clarify whether the number of circulating HSC in CLD patients is higher or lower than that in healthy controls, we determined the numbers of $CD34^+$ cells and colony-forming unit culture (CFU-C) using flow cytometry and colony assays, respectively, in peripheral blood (PB) samples from patients with hepatitis C virus (HCV)-associated CLD. We found that both of these factors decreased with the progression of liver disease unlike in previous reports.[9,10]

In humans, the spleen plays a principal role in blood formation during fetal development, but this function rapidly diminishes after birth. Therefore, the spleen is not believed to contribute to hematopoiesis in healthy individuals.[11] Recently, however, several reports have demonstrated that the spleen in adults contains a significant number of HSC.[12,13] Splenectomy was reported to increase the number of platelets and leukocytes, and to reduce the number of long-lived memory B cells.[14–16] Splenectomy is performed to improve thrombocytopenia in cirrhotic HCV patients being treated with pegylated interferon (IFN)-α and ribavirin.[17] However, the effects of splenectomy on circulating HSC have not been determined. Therefore, in this study, we determined the number of circulating HSC before and after splenectomy in patients with LC, and confirmed that the number of HSC increased significantly after splenectomy, an effect that persisted for a long time.

METHODS

Patients

FORTY-EIGHT PATIENTS (22 men, 26 women; mean ± standard deviation age, 56 ± 12 years) with HCV-associated CLD, who were followed up at the Mie University Hospital between February and December 2004, were included in this study to assess the association between the number of circulating HSC and CLD stage. The presence of HCV was confirmed by a positive reverse transcription polymerase chain reaction for HCV RNA at diagnosis. The patients were subdivided into the following four groups using a combination of laboratory tests, abdominal ultrasonography and computed tomography: (i) nine patients with an asymptomatic carrier state (ASC); (ii) nine patients with chronic active hepatitis (CAH); (iii) 15 patients with LC; and (iv) 15 patients with LC and hepatocellular carcinoma (LC + HCC). Patients with other causes of liver injury, including co-infection with hepatitis B virus or HIV, autoimmune liver disease or alcoholic cirrhosis were excluded. Ten healthy adults (eight men, two women; age, 48 ± 17 years) with no blood biochemical abnormalities were included as a control group. Between November and December 2005, 60 patients (33 men, 27 women; age, 63 ± 12 years) with HCV-associated CLD, including 13 patients enrolled in the former study, were included in a study designed to assess the correlation between plasma stromal derived factor-1α (SDF-1α) concentrations and CLD stage. Additionally, seven patients with HCV-associated LC, who had adequate liver function and underwent splenectomy between February 2005 and May 2007 (three men, four women; age, 55 ± 9 years), were enrolled in this study. Blood samples were collected preoperatively, at 1 week after surgery and at 1–3 months after surgery. Spleen samples were obtained from another three LC patients, who underwent splenectomy within 1 year. Written informed consent was obtained from all patients and healthy volunteers. The study protocol was approved by the Human Studies Subcommittee of Mie University Graduate School of Medicine (approval no. 287) and conformed to the ethical guidelines of the Declaration of Helsinki, 1975.

Cell preparation

Peripheral blood samples were drawn from patients with HCV-associated CLD and from healthy volunteers. Erythrocytes were lysed using ACK lysis buffer, and the remaining cells were washed twice with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS; Gibco, Grand Island, NY, USA) and resuspended in PBS containing 0.1% de-ionized fraction V bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA). We used the remaining cells as PB total nucleated cells (TNC) for flow cytometry. PB mononuclear cells (MNC) were prepared by density gradient centrifugation over Ficoll-Paque PLUS (GE Healthcare Bio-Sciences, Uppsala, Sweden) and were used for the CFU-C assay.

Flow cytometry

Peripheral blood TNC were stained with fluorescein isothiocyanate (FITC)-conjugated antihuman CD34 (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and phycoerythrin (PE)-conjugated antihuman CD90 (Becton Dickinson Pharmingen, San Diego, CA, USA) or PE-conjugated antihuman CD117 (Becton Dickinson Pharmingen). Relevant isotype-

matched control antibodies were included in the staining to exclude non-specific binding. After adding 1 μg/mL of propidium iodide (Sigma-Aldrich) to eliminate dead cells from the analysis, the cells were washed and resuspended in PBS containing 0.1% BSA. At least 200 000 events in live leukocyte populations were acquired by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA, USA) and were analyzed using CellQuest software (BD Biosciences). The number of $CD34^+$ cells in living PB-TNC was determined using a CD34-FITC versus side-scatter dot plot.

In some samples, PB-TNC were double stained with FITC-conjugated anti-human CD34 antibody and allophycocyanin-conjugated anti-human CD45 antibody (BioLegend, San Diego, CA, USA).

CFU-C assay

The methylcellulose culture was performed in 35-mm Falcon suspension culture dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA). One milliliter of the culture contained 50 000 MNC, α-modified Eagle's medium, 1.2% 1500-centipoise methylcellulose (Shinetsu Chemical, Tokyo, Japan), 30% fetal bovine serum, 1% BSA, 1×10^{-4} M 2-mercaptoethanol (Wako Pure Chemical Industries, Osaka, Japan), and cytokines including 100 ng/mL recombinant human (rh) stem cell factor, 10 ng/mL rh interleukin-3, 10 ng/mL rh granulocyte macrophage colony-stimulating factor, 10 ng/mL rh granulocyte colony-stimulating factor and 2 U/mL rh erythropoietin. All cytokines were gifts from Kirin Brewery (Tokyo, Japan). The dishes were then incubated in a humidified atmosphere with 5% CO_2 in air. On day 14 of culture, colonies consisting of 40 cells or more were scored on an Olympus CK40 inverted microscope (Olympus, Tokyo, Japan).

Measurement of plasma SDF-1α

Plasma SDF-1α was measured using a human CXCL12/SDF-1α Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA). The intra- and inter-assay coefficients of variations were 3.5% and 10.3%, respectively. SDF-1α concentrations were measured in duplicate for each sample and standard solution.

Immunohistochemistry and immunofluorescence analysis of spleen

Formalin-fixed, paraffin-embedded 5-μm tissue sections of spleen specimens obtained from three splectomyzed LC patients were used for immunohistochemical staining for SDF-1α. Deparaffinized sections were heated for 5 min at 100°C in a pressure cooker to reactivate the antigen and treated with 0.3% H_2O_2 in methanol for 30 min to abolish endogenous peroxidase activity. Sections were blocked with 1% goat serum in PBS, covered with rabbit anti-SDF-1α polyclonal antibody (PeproTech, Rocky Hill, NJ, USA; dilution 1:200) overnight at 4°C, washed, covered with a second-step biotinylated antibody for 30 min, and incubated with peroxidase-labeled streptavidin for 30 min. After washing, sections were incubated with 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.15% H_2O_2, and counterstained with 10% hematoxylin (Wako Pure Chemical Industries). Controls were performed by omitting the primary SDF-1α antibody.

Immunofluorescence studies were performed with similar methods using rabbit anti-human CD34 and mouse anti-human CD45 (both Abcam, Cambridge, MA, USA; dilution 1:200). The following secondary antibodies were used: FITC-conjugated goat anti-mouse IgG and PE-conjugated goat anti-rabbit IgG $F(ab')_2$ (both Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:400). Nuclei were stained with 4′,6-diamidino-2-phenylindole (Molecular Probes Invitrogen, Eugene, OR, USA). Staining with appropriate FITC- or PE-conjugated isotype controls was performed as negative controls. All sections were examined under a fluorescence microscope (BZ-X700; Keyence, Osaka Japan).

Statistics

Statistically significant differences between groups were analyzed by Student's *t*-test and the Mann–Whitney *U*-test. Correlations were determined by the linear regression test. Differences were considered significant at $P < 0.05$.

RESULTS

Complete blood counts in patients with HCV-associated liver disease

IT IS WELL known that cytopenia, especially thrombocytopenia, usually affects patients with advanced liver disease.[18,19] Table 1 shows the complete blood counts in patients with HCV-associated liver disease. The white blood cell and platelet counts, and the hemoglobin (Hb) level decreased significantly with disease progression. Patients with LC and LC + HCC exhibited particularly severe thrombocytopenia.

Circulating HSC count in patients with HCV-associated liver disease

The number of circulating HSC, which express CD34 antigen, is very low in the PB in steady state.[20,21] We first

Table 1 Complete blood counts in patients with hepatitis C virus-associated chronic liver disease

Subjects	*n*	WBC (/μL)	Hb (g/dL)	PLT ($\times 10^4$/μL)
Healthy volunteers	10	5520 ± 728	14.5 ± 1.0	21.4 ± 2.9
ASC	9	5167 ± 1533	13.5 ± 1.3	17.5 ± 3.3*
CAH	10	4200 ± 856**	13.4 ± 1.4	14.5 ± 3.5***
LC	15	4213 ± 1804*	12.9 ± 1.2**	9.4 ± 4.4***
LC + HCC	14	3693 ± 1017***	10.8 ± 2.1***	7.3 ± 3.5***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs healthy volunteers.
Values are means ± standard deviation.
ASC, asymptomatic carrier state; CAH, chronic active hepatitis; Hb, hemoglobin; LC, liver cirrhosis; LC + HCC, liver cirrhosis and hepatocellular carcinoma; PLT, platelet; WBC, white blood cell.

analyzed the number of circulating $CD34^+$ cells, by flow cytometry, in 48 patients with various stages of HCV-associated CLD. As shown in Figure 1(a), the number of circulating $CD34^+$ cells decreased significantly with the progression of liver disease: healthy controls, 2.4 ± 1.1 cells/μL; ASC, 1.2 ± 0.5 cells/μL; CAH, 1.0 ± 0.2 cells/μL; LC, 0.7 ± 0.4 cells/μL; and LC + HCC, 0.5 ± 0.2 cells/μL.

As shown in Figure 1(b), the number of circulating CFU-C, which was determined by methylcellulose assay, decreased with the progression of liver disease: healthy controls, 851 ± 370 colonies/mL; ASC, 286 ± 125 colonies/mL; CAH, 277 ± 143 colonies/mL; LC, 115 ± 61 colonies/mL; and LC + HCC, 62 ± 37 colonies/mL. The number of $CD34^+$ cells was significantly and positively correlated with CFU-C (Fig. 1c). These results suggest that the number of circulating HSC is significantly associated with liver conditions.

As shown in Figure 2, the number of circulating $CD34^+$ cells was positively correlated with the leukocyte and platelet counts, and Hb in PB. In particular, the correlation between the numbers of circulating $CD34^+$ cells and platelets was very prominent in patients with CLD (Fig. 2c).

Table 2 shows the correlation between the number of circulating $CD34^+$ cells and various blood test scores in LC and LC + HCC patients. Serum albumin concentration, serum cholinesterase activity and platelet count were positively correlated with the number of circulating $CD34^+$ cells.

Plasma SDF-1α concentrations in patients with HCV-associated liver disease

As shown in Figure 3(a), the plasma SDF-1α concentrations in healthy volunteers, ASC, CAH, LC and LC + HCC patients were 1851 ± 326, 2202 ± 220, 2203 ± 384, 2811 ± 422 and 3340 ± 212 pg/mL, respectively. The plasma SDF-1α concentration was positively correlated with the CLD stage but was negatively correlated with the number of circulating HSC (Fig. 3b). In seven LC patients, the plasma SDF-1α concentration was negatively correlated with serum cholinesterase activity (Fig. 3c). Furthermore, the plasma SDF-1α concentration in chronic hepatitis C patients who achieved sustained virological response (SVR) after treatment with IFN-α was similar to that in healthy volunteers (Table 3). Based on these findings, we think that the plasma SDF-1α concentration may be used as a biomarker to determine the severity of liver injury.

Effects of splenectomy on the numbers of circulating HSC and platelets in patients with LC

Next, we determined the numbers of circulating $CD34^+$ cells and platelets before and after splenectomy in seven patients with LC who underwent splenectomy to treat thrombocytopenia at our institute. Table 4 presents the clinical features of the splenectomized LC patients. Laboratory data indicate that the patients had mild to moderate liver dysfunction. As shown in Figure 4, the numbers of circulating $CD34^+$ cells and platelets before splenectomy were 0.6 ± 0.3 cells/μL and $4.9 \pm 1.6 \times 10^4$ cells/μL, respectively. These cell numbers increased significantly to 2.5 ± 1.3 cells/μL and $26.0 \pm 12.3 \times 10^4$ cells/μL, respectively, at 1 month after splenectomy. In four patients, the numbers of HSC and platelets remained high (1.3 ± 0.7 cells/μL and $16.8 \pm 1.7 \times 10^4$ cells/μL, respectively) at 3 months or more after splenectomy. IFN-α therapy was started in two patients at 3 months after splenectomy, and both patients achieved SVR. Both patients are still alive without relapse at 5 years or more after splenectomy.

There were some clusters of SDF-1α-expressing cells (Fig. 5a) and some $CD34^+CD45^+$ cells (HSC) in the spleen of splenectomized LC patients (Fig. 5b).

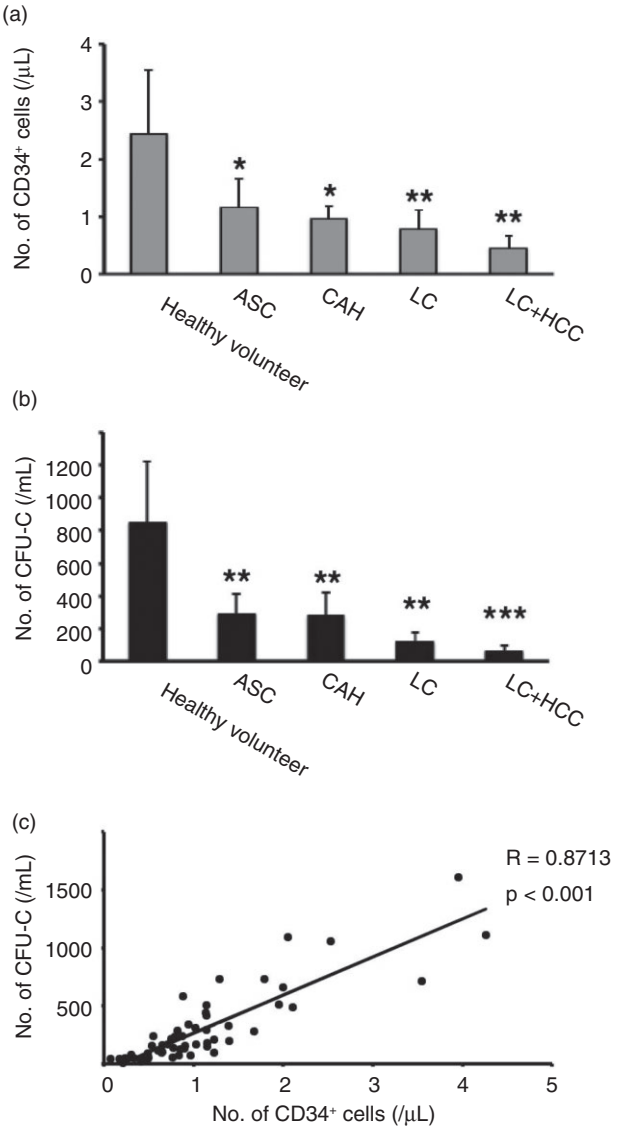

Figure 1 Numbers of circulating hematopoietic stem/progenitor cells in patients with hepatitis C virus (HCV)-associated chronic liver disease (CLD). (a,b) The numbers of $CD34^+$ cells (a) and colony-forming unit culture (CFU-C) (b) in peripheral blood from patients with HCV-associated CLD decreased significantly with the progression of liver disease. $^*P < 0.01$, $^{**}P < 0.001$ and $^{***}P < 0.0001$ vs healthy volunteers. ASC, asymptomatic carrier state; CAH, chronic active hepatitis; LC, liver cirrhosis; LC + HCC, liver cirrhosis and hepatocellular carcinoma. (c) The number of $CD34^+$ cells was significantly and positively correlated with CFU-C. R, correlation coefficient.

DISCUSSION

ALTHOUGH CIRCULATING $CD34^+$ cells comprise HSC and endothelial progenitor cells,[22,23] HSC can be distinguished from endothelial progenitor cells by the expression of CD45. HSC and endothelial progenitor cells are $CD34^+CD45^+$ and $CD34^+CD45^-$ cells, respectively.[24] We simultaneously stained the PB-TNC from five CLD patients and five healthy donors with

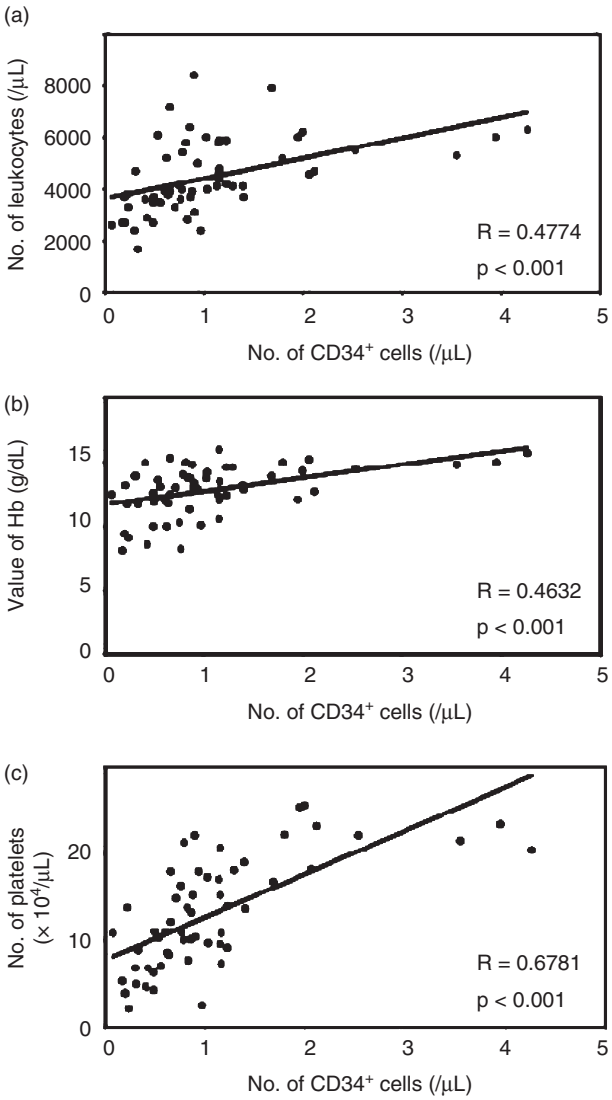

Figure 2 Comparison of complete blood count parameters and circulating $CD34^+$ cell count. (a) Leukocyte count, (b) hemoglobin (Hb) and (c) platelet count were positively correlated with the $CD34^+$ cell count in peripheral blood from patients with hepatitis C virus-associated chronic liver disease and healthy volunteers. R, correlation coefficient.

Table 2 Correlation between circulating CD34⁺ cell count and various blood test scores in LC and LC + HCC patients

Factor	R	*P*
Serum albumin	0.572	<0.01
Serum cholinesterase	0.649	<0.001
Prothrombin time	0.381	NS
Platelet count	0.561	<0.01

LC, liver cirrhosis; LC + HCC, liver cirrhosis and hepatocellular carcinoma; NS, not significant; R, correlation coefficient.

antibodies against CD34 and CD45, and confirmed that 98.5% of CD34⁺ cells were positive for CD45 (data not shown). Because the co-expression of Thy-1 (CD90) and c-kit receptor (CD117) on CD34⁺ cells seems to characterize the true hematopoietic stem cells, we analyzed the expression of them on circulating CD34⁺ cells. As shown in Table 5, approximately 20% and 60% of CD34⁺ cells were positive for Thy-1 and c-kit receptor, respectively. Our results are almost similar to those by Murray *et al.* and D'Arena *et al.*[25,26] Furthermore, we simultaneously determined the numbers of CD34⁺ cells and CFU-C to accurately assess the number of circulating HSC in patients with CLD. This analysis confirmed that there was a significant positive correlation between these factors.

We found that the percentage and the absolute number of circulating HSC decreased with the progression of liver disease in patients with HCV-associated CLD. However, in previous reports, there were no significant differences in the percentage of HSC among patients with LC.[9,27] It is well known that the number of leukocytes in the PB decreases significantly with disease progression in patients with CLD.[18,19] Therefore, even if there is no difference of the percentage of CD34⁺ cells among patients with CLD, the absolute number of these cells is thought to decrease with the progression of liver disease. Because it was previously reported that the BM cellularity in patients with CLD, including patients with LC, is almost normal or increases despite pancytopenia or bicytopenia,[28,29] a decrease in the number of circulating HSC may not be associated with myelosuppression.

Hematopoietic stem/progenitor cells are mobilized in response to stress signals during infection, inflammation and tissue injury.[30,31] This process is tightly regulated by cytokines/chemokines, including granulocyte colony-stimulating factor, stem cell factor, monocyte chemoattractant protein-1 and SDF-1α, as well as by hormones such as growth hormone and parathyroid hormone.[32–35] SDF-1α is a chemokine that was initially described as a key factor for B lymphopoiesis and myelopoiesis, and was shown to induce chemotaxis of CD34⁺ HSC, T lymphocytes, pro- and pre-B lymphocytes, monocytes and megakaryocytes.[36–40] Although

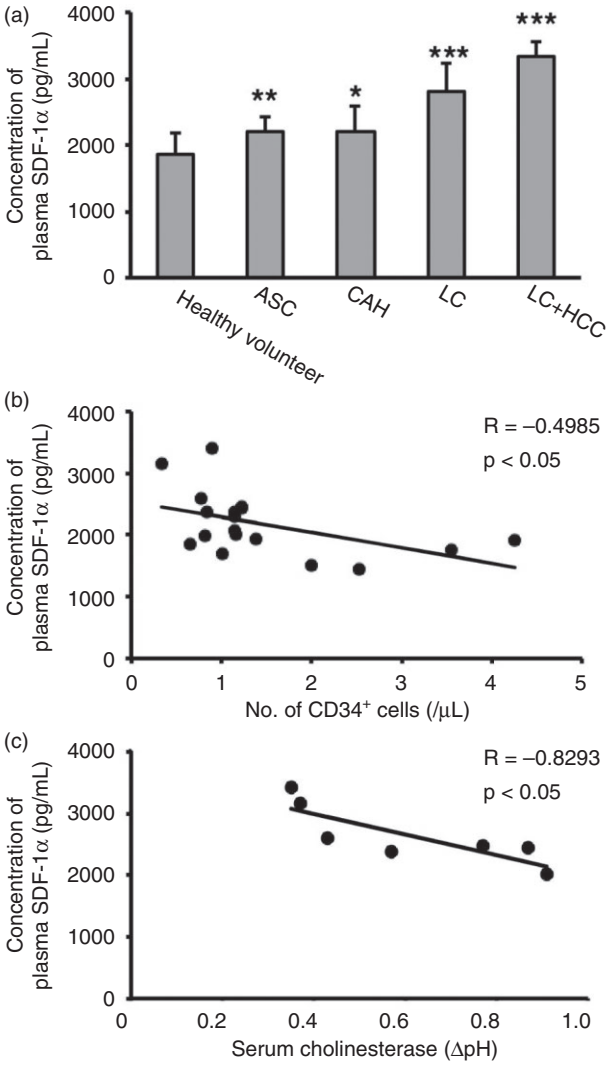

Figure 3 Plasma stromal derived factor-1α (SDF-1α) concentrations in patients with hepatitis C virus (HCV)-associated chronic liver disease (CLD). (a) The plasma SDF-1α concentration increased with progression of liver disease. $*P < 0.05$, $**P < 0.01$ and $***P < 0.0001$ vs healthy volunteers. ASC, asymptomatic carrier state; CAH, chronic active hepatitis; LC, liver cirrhosis; LC + HCC, liver cirrhosis and hepatocellular carcinoma. (b) The number of CD34⁺ cells in the peripheral blood was negatively correlated with the plasma SDF-1α concentration in patients with HCV-associated CLD. (c) Serum cholinesterase was negatively correlated with the plasma SDF-1α concentration in LC patients. R, correlation coefficient.

Table 3 Comparison of SDF-1α, CD34⁺ cell count and CFU-C between healthy volunteers, patients with CAH and patients who achieved SVR after IFN-α treatment

Subjects	*n*	SDF-1α (pg/mL)	CD34⁺ cells (/μL)	CFU-C (/mL)
Healthy volunteers	10	1871 ± 333	2.44 ± 1.11	851 ± 370
Patients with CAH	10	2203 ± 384	0.97 ± 0.21	277 ± 143
Patients with an SVR	7	1895 ± 286*	1.87 ± 0.83*	611 ± 335*

*$P < 0.05$ vs patients with CAH.
Values are means ± standard deviation.
CAH, chronic active hepatitis; CFU-C, colony-forming unit culture; IFN-α, α-interferon; SDF-1α, stromal cell-derived factor-1α; SVR, sustained virological response.

SDF-1α is constitutively expressed in many organs, including BM, liver and spleen, several reports demonstrated that SDF-1α is upregulated after injury in experimental models, including toxic liver injury, myocardial infarction and ischemic renal injury.[41–43] In humans, plasma SDF-1α concentrations are significantly elevated in HCV patients.[44] SDF-1α and its receptor, C-X-C chemokine receptor type 4 (CXCR4), are involved in the recruitment of immune cells and endothelial progenitor cells to the injured liver during chronic HCV and hepatitis B virus infection.[44,45] Because CD34⁺ HSC express CXCR4, the SDF-1α/CXCR4 signaling axis is thought to play an important role in the migration of HSC into the liver during injury. Furthermore, some authors reported that transplanted HSC, acute myeloid leukemia cells and endothelial cells migrate into the spleen via the SDF-1α/CXCR4 axis.[41,42,46] We found some clusters of SDF-1α-expressing cells and some HSC in the spleen of splenectomized LC patients. Therefore, we speculate that many HSC may migrate to and lodge in the spleen of the patients with CLD.

Table 4 Clinical features of seven LC patients who underwent splenectomy

Factor	Means ± standard deviation
Serum albumin (g/dL)	3.2 ± 0.6
AST (U/L)	59 ± 27
ALT (U/L)	56 ± 30
Total bilirubin (mg/dL)	1.5 ± 1.0
Serum cholinesterase (ΔpH)	0.42 ± 0.20
Prothrombin time (%)	66.3 ± 14.2
Hyaluronic acid (ng/mL)	384 ± 249
Platelet count (×10⁴/μL)	4.9 ± 1.6

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LC, liver cirrhosis.

Several studies have found that autologous BM cell infusion therapy improved the clinical symptoms and biochemical data by activating the progenitor cell compartment and enhancing hepatocyte proliferation in patients with decompensated LC.[7,8] Indeed, Iwamoto *et al*. demonstrated that splenectomy enhanced the

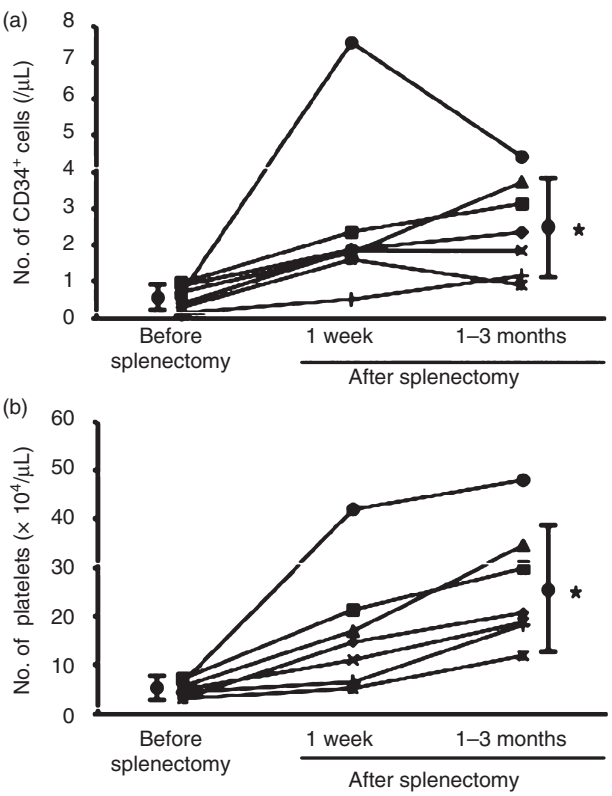

Figure 4 Comparison of the numbers of CD34⁺ cells and platelets in peripheral blood samples obtained from seven patients before and after splenectomy. The numbers of (a) CD34⁺ cells and (b) platelets increased significantly after splenectomy. *$P < 0.01$ vs before splenectomy.

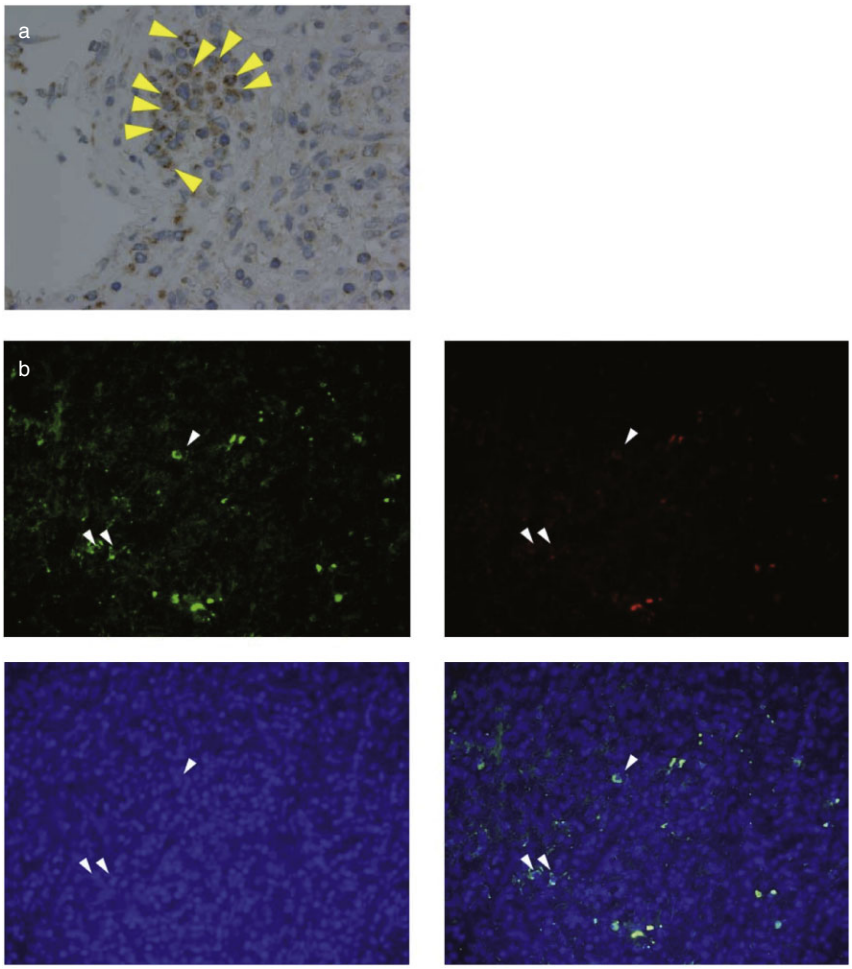

Figure 5 Expression of stromal derived factor-1α (SDF-1α) and localization of hematopoietic stem/progenitor cells (HSC) in the spleen. (a) Immunohistochemical staining of SDF-1α in the spleen. Tissue sections were stained with SDF-1α. The brown staining (yellow triangles) in the spleen indicates SDF-1α protein expression. The figure is representative of three spleen samples (original magnification, ×400). (b) Immunofluorescence staining of HSC in the spleen. Histological sections were stained with 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) to identify cell nuclei. Panels show CD45 as green, CD34 as red, DAPI as blue and the combined merged image. White triangles indicate $CD34^+CD45^+$ HSC. The figure is representative of three spleen samples (original magnification, ×400).

efficacy of autologous BM infusion for improving cirrhosis in a murine model and in a clinical study.[47] They thought that the increase in the migration of BM cells into the liver in splenectomized mice was caused by the absence of captured BM cells in the enlarged spleen after splenectomy. In our study, we found that splenectomy augmented the number of circulating HSC in patients with LC, and that IFN-α treatment could achieve SVR in

Table 5 Expression of Thy-1 and c-kit on circulating $CD34^+$ cells in patients with hepatitis C virus-associated chronic live disease

Subjects	*n*	No. of $CD34^+$ cells (/μL)	Percentage of cells expressing each antigen in $CD34^+$ cells	
			Thy-1	c-kit
Healthy volunteers	5	2.01 ± 0.99	15.0 ± 2.7	68.0 ± 11.2
ASC	8	1.12 ± 0.54	20.4 ± 7.5	69.2 ± 14.6
CAH	7	0.90 ± 0.37	19.1 ± 10.9	57.9 ± 8.0
LC	12	0.54 ± 0.27	20.5 ± 13.5	56.1 ± 13.5
LC + HCC	7	0.39 ± 0.19	20.1 ± 9.5	57.4 ± 13.9

Values are means ± standard deviation.
ASC, asymptomatic carrier state; CAH, chronic active hepatitis; LC, liver cirrhosis; LC + HCC, liver cirrhosis and hepatocellular carcinoma.

some splenectomized patients. These reports and our findings imply that splenectomy may improve liver function by increasing the number of circulating HSC. However, it remains unclear how HSC contribute to the regeneration of hepatocytes in damaged liver.

In conclusion, our data suggest that the spleen plays a principal role in modulating the dynamics of HSC via the SDF-1α/CXCR4 axis in patients with HCV-associated CLD. Our results also demonstrate the usefulness of splenectomy for improving liver function in patients with LC.

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