to evaluate the regional hepatic damage. In the case of both AH and FHF, a separate analysis of function in the right and left hepatic lobes would enable us to evaluate regional pathobiologic differences in the liver, which would not have been possible previously. It is therefore necessary to establish an objective and convenient diagnostic method for this purpose.

The asialoglycoprotein receptor (ASGPR) is expressed abundantly on the sinusoidal surface of hepatocytes and its function is to mediate the rapid clearance of serum glycoproteins containing terminal galactose residues from the circulation [4]. Recently, we have developed an analytical method of analyzing asialoglycoprotein receptors (ASGPRs) based on technetium-99m-diethylenetriaminepentaacetic acid-galactosyl human serum albumin (^{99m}Tc-GSA) single photon emission computed tomography (SPECT) that can be performed readily and allows the evaluation of regional liver function or damage using an arbitrary region of interest [5–7]. Intravenously administered ^{99m}Tc-GSA is taken up by ASGPRs, which are expressed abundantly on the sinusoidal surface of hepatocytes [8–10]. The extent of ^{99m}Tc-GSA incorporation into the liver can be calculated objectively from the SPECT analysis, and the hepatic regional expression and density of ASGPRs are obtained. Regional liver function and damage are thus determined quantitatively using this novel tool [7]. The study presented here shows that ^{99m}Tc-GSA SPECT analysis of the liver is a useful and reliable tool for assessing the severity of regional liver damage and monitoring regional liver regeneration in AH and FHF patients. Moreover, we have showed that the expression of ASGPRs in the right hepatic lobe is particularly reduced in patients with FHF.

2. Patients and methods

2.1. Patients

Forty-two patients with AH and 10 patients with FHF were admitted to our hospital between July 1997 and August 2003. The patients with AH were diagnosed on the basis of biochemical profiles and prodromal symptoms. The criteria for a diagnosis of FHF were: (i) hepatic encephalopathy of grade 2 or more within 2 months of the onset of signs and symptoms of hepatitis, (ii) a plasma prothrombin level of less than 40% or (iii) evidence of massive or submassive necrosis of the liver found in either biopsy or necropsy specimens [11]. In all participating patients in the AH and FHF groups, SPECT analysis was performed within 1 week after admission to our hospital.

The subjects comprised 42 patients with AH (25 men and 17 women; average age 52.3 years; range 17–81 years) and 10 with FHF (6 men and 4 women; average age 50.9 years; range 25–67 years). In 4 of the 42 AH patients, the disease was caused by hepatitis A virus, in 7 by hepatitis B virus, in 4 by hepatitis C virus, in 1 by hepatitis E virus, in 2 by Epstein-Barr virus, in 5 by drugs and in the remaining 19 by unknown etiology. In 3 of the 10 FHF patients the disease was caused by HBV, and in the remaining 7 by unknown etiology. The controls who participated in this study were 18 healthy volunteers (11 men and 7 women: mean age: 54.6 years; range, 35–65 years). All of the controls had normal liver function test results. Informed consent to participate in this study was obtained from each subject before its commencement. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institution's Human Research Review Committee.

2.2. ^{99m}Tc-GSA SPECT analysis

^{99m}Tc-GSA SPECT was performed using a triple-headed camera (MULTISPECT 3; Siemens Medical Systems, Erlangen, Germany). SPECT images of the injection syringe were obtained for 20 s before injection of the ^{99m}Tc-GSA. Subsequently, the ^{99m}Tc-GSA (185 MBq, Nihon Medi-Physics, Nishinomiya, Japan) was injected as an intravenous bolus into a cubital vein, and SPECT data (72 steps, 360°) were obtained from 12 min 30 s to 17 min 30 s after the injection and stored in 64 × 64 matrix. The acquired data were processed on a dedicated computer system (ICON; Siemens). The projection data sets were prefiltered with a two-dimensional Butterworth filter (order, eight; cut off frequency, 0.61 cycle/pixel) and reconstructed with filtered-back projection (Ramp filter). Chang's attenuation collection (0.12 cm^{-1} of attenuation coefficient) was added to the reconstructed data in order to obtain more accurate SPECT counts (Fig. 1A). The liver margin was extracted with 34% of the maximum as the cut off value [4,6].

Liver uptake of ^{99m}Tc-GSA and functional liver volume (FLV; cm^3) were calculated by summation of the radioactivity of sequential slices (thickness of 5.8 mm). The total SPECT counts for the whole liver were divided by the total preinjection SPECT counts for the syringe (i.e., the percentage of the hepatic SPECT value relative to the syringe value was calculated), and this was termed the liver uptake ratio (LUR; %). Using this procedure, the actual percentage of the administered dose of ^{99m}Tc-GSA that was incorporated into the liver was quantified objectively. To lessen the influence of liver volume, the LUR was then divided by the FLV to obtain the liver uptake ratio per unit of volume (liver uptake density [LUD; %/cm^3]). The borderlines between the liver lobes were determined manually using the gallbladder bed, the inferior vena cava, and the medial margin of the right lobe (Fig. 1B). We assessed the SPECT images for the right and left lobes based on these borderlines (Cantlie's line), and obtained separate FLV, LUR and LUD values for each lobe. We also calculated the ratio of the left lobe to that of the right lobe (L/R ratio) for FLV, LUR and LUD in individual cases.

2.3. Statistical analysis

All results are expressed as the mean ± S.D. Statistical analyses were performed using the Stat View 4.0 software

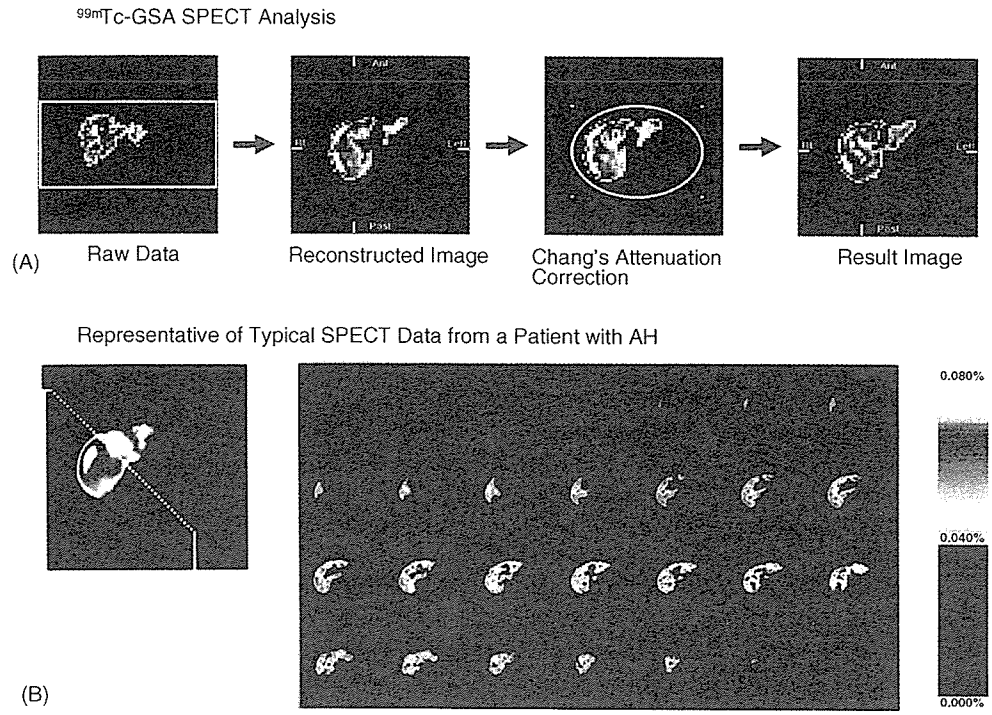

Fig. 1. Method used to measure the ^{99m}Tc-GSA counts in the whole liver, and separately in the right and left lobes: (A) SPECT image processing and (B) representative of the typical SPECT data from a patient with AH.

package (Abacus Concepts, Berkeley, CA, USA). Comparisons of values between any two groups were performed using the Mann–Whitney *U*-test. Correlations between LUR and LUD values and the results of each liver function test were analyzed using standard Pearson correlation analysis. The level of statistical significance was set at $P < 0.01$.

3. Results

3.1. Liver volume measured by ^{99m}Tc-GSA SPECT analysis

Fig. 2(A–C) shows the mean values of FLV calculated from ^{99m}Tc-GSA SPECT analysis. There were no significant differences in the whole liver FLV among the control, AH and FHF groups. There were also no differences between the control, AH and FHF groups in the FLV in the right and left hepatic lobes. However, FLV L/R ratio in the FHF group was significantly higher than those in the control and AH groups ($P < 0.01$ and $P < 0.005$, respectively; Fig. 2D).

3.2. Analysis of ASGPRs expression in the right and left hepatic lobes

We measured the LUR for ^{99m}Tc-GSA in the control, AH and FHF groups. The mean LUR value for the whole liver in the AH group was significantly lower than in the control group ($P < 0.01$). In addition, the mean LUR value for the whole liver in the FHF group was significantly lower than in both the control and AH groups ($P < 0.000001$ and $P < 0.00001$, respectively; Fig. 3A).

The mean LUR value for the right lobe in the AH group was significantly lower than in the control group ($P < 0.005$). In the FHF group, the mean LUR value for the right lobe was significantly lower than in both the control and AH groups ($P < 0.000001$ and $P < 0.00001$, respectively; Fig. 3B).

In terms of the left lobe, the mean LUR value in the AH group was significantly lower than in the control group ($P < 0.01$). In the FHF group, the mean LUR value was significantly lower than in both the control and AH groups ($P < 0.00001$ and $P < 0.01$, respectively; Fig. 3C).

To investigate quantitative differences in ASGPRs between the right and left hepatic lobes in individual patients, we calculated the LUR ratio of the left lobe to that of the right lobe (LUR L/R ratio) in the control, AH and FHF groups. In the control and AH groups, there was a significantly greater number of ASGPRs in the right lobe compared with the left. However, the LUR L/R ratio in the FHF group increased to 0.742 ± 0.255, which is significantly greater than that of both the control and AH groups ($P < 0.001$ and $P < 0.001$, respectively; Fig. 3D).

3.3. Analysis of ASGPRs density in the right and left hepatic lobes

We also examined whether the density of ASGPRs in the right and left hepatic lobes changed according to the severity

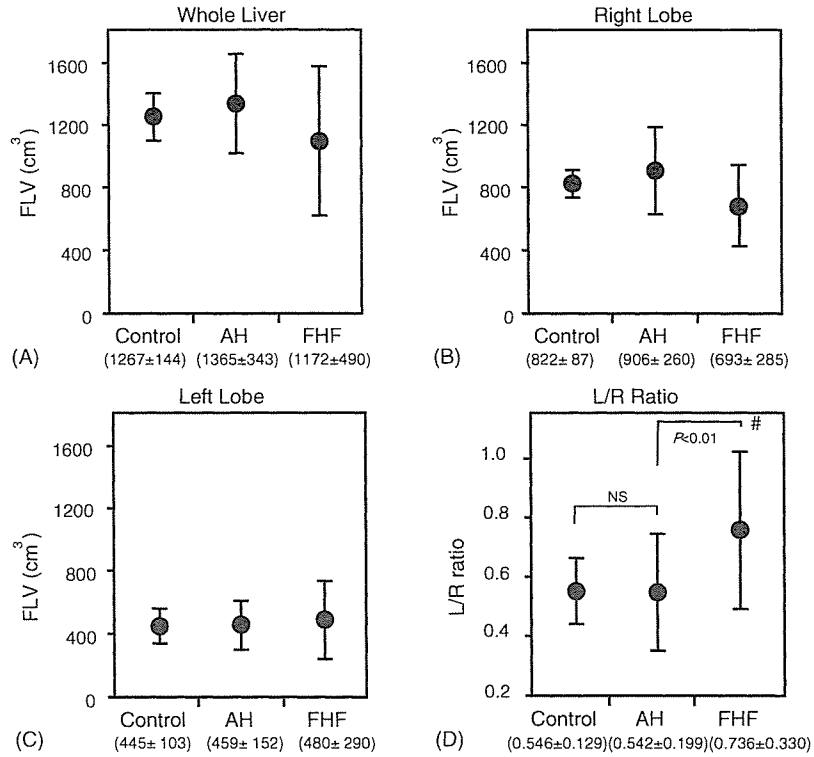

Fig. 2. Functional liver volume (FLV) calculated from the ^{99m}Tc-GSA SPECT analysis: (A) mean FLV value for the whole liver; (B) mean FLV value for the right lobe; (C) mean FLV value for the left lobe. There were no significant differences in FLV of the whole liver and the right and left hepatic lobes between the control, AH and FHF groups. (D) Mean FLV L/R (left lobe to right lobe) ratio. The L/R ratio for the FLV increased significantly in the FHF group. $^{*}P < 0.005$ vs. control; NS, not significant.

of acute hepatic damage. The mean LUD value for the whole liver in the AH group was significantly lower than in the control group ($P < 0.00001$). The mean LUD value for the whole liver in the FHF group was significantly lower than that in both the control and AH groups ($P < 0.000001$ and $P < 0.00001$, respectively; Fig. 4A).

The mean LUD value for the right hepatic lobe in the AH group was significantly lower than in the control group ($P < 0.00001$). The mean LUD value for the right hepatic lobe in the FHF group was significantly lower than in both the control and AH groups ($P < 0.000001$ and $P < 0.00001$, respectively; Fig. 4B).

The mean LUD value for the left lobe in the AH group was significantly lower than in the control group ($P < 0.00001$). The mean LUD value for the left lobe in the FHF group was significantly lower than in both the control and AH groups ($P < 0.000001$ and $P < 0.0001$, respectively; Fig. 4C).

To explore the differences in the density of ASGPRs between the right and left lobes, we examined the LUD ratio of the left lobe to that of the right lobe (LUD L/R ratio). There was no significant difference between the LUD L/R ratios of the control and AH groups. However, the mean LUD L/R ratio value for the FHF group was significantly higher than for both the control and AH groups ($P < 0.0001$ and $P < 0.0001$, respectively; Fig. 4D).

3.4. Correlation between indices obtained using ^{99m}Tc-GSA SPECT analysis and those obtained using conventional liver function tests

We examined any correlations between the results of conventional liver function tests and LUR and LUD values in the patients with AH and FHF. There was no significant correlation between aspartate aminotransferase and alanine aminotransferase levels, and either LUR or LUD. However, significant correlations were observed between whole liver LUR data and conventional indicators of hepatic functional reserve, including serum albumin levels, cholinesterase activity and the prothrombin time. The whole liver LUR data also correlated strongly with total bilirubin levels. When we compared the separate LUR values for the right and left lobes, we found that the data for the right lobe correlated strongly with the results of conventional liver function tests (Table 1). Although the correlation between the LUR for the left lobe and the results of conventional liver function tests was also significant, the degree of correlation was not as strong as for the right lobe (Table 1).

LUD values for the whole liver were also significantly correlated with the results of conventional liver function tests and with total bilirubin levels. These correlations remained significant when the right and left LUD were analyzed separately (Table 1).

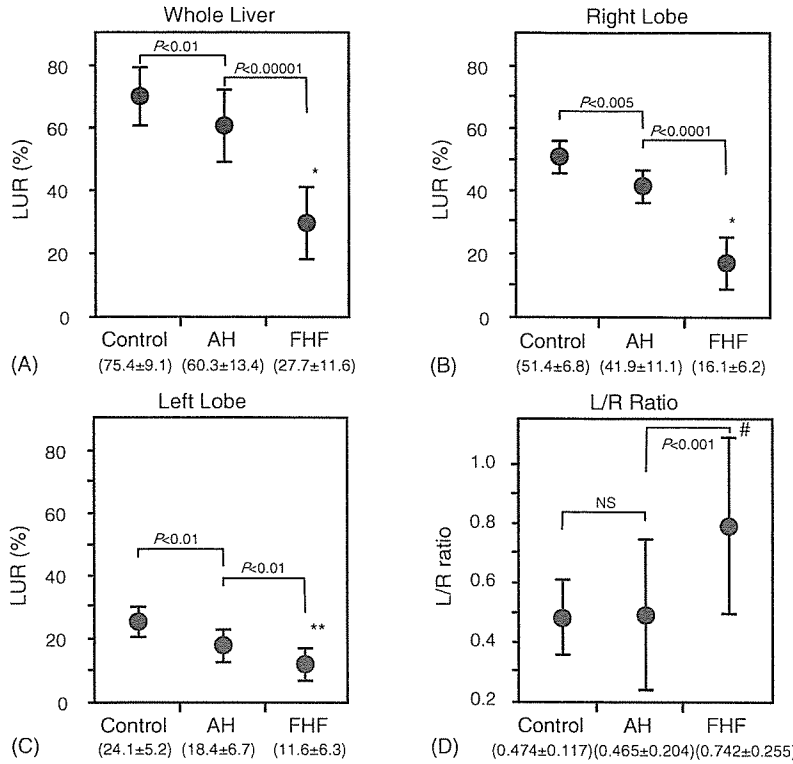

Fig. 3. Liver uptake ratio (LUR) values for the whole liver and the right and left lobes. The mean LUR value decreased with the severity of acute liver damage in the whole liver, and individually in the right and left lobes: (A) mean LUR value for the whole liver; (B) mean LUR value for the right lobe; (C) mean LUR value for the left lobe; (D) mean LUR L/R ratio. $^{*}P<0.000001$ vs. control; $^{**}P<0.01$ vs. control; $^{\#}P<0.001$ vs. control; NS, not significant.

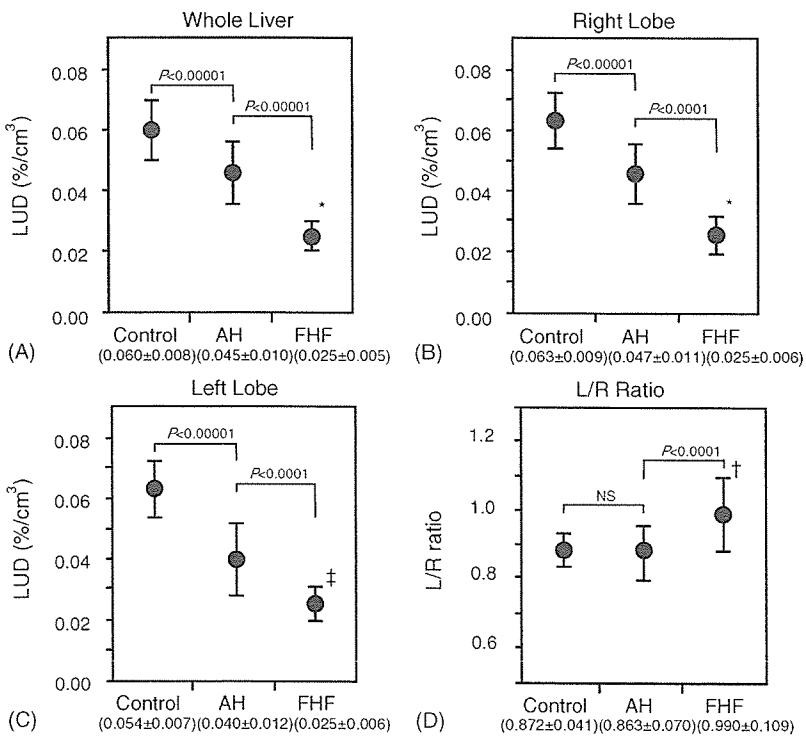

Fig. 4. Liver uptake density (LUD) values for the whole liver, and for the right and left lobes: (A) mean LUD value for the whole liver; (B) mean LUD value for the right lobe; (C) mean LUD value for the left lobe; (D) mean LUD L/R ratio. $^{*}P<0.000001$ vs. control; $^{\ddagger}P<0.000001$ vs. control; $^{\dagger}P<0.0001$ vs. control; NS, not significant.

Table 1
Correlation between the conventional liver functional test and indices from ^{99m}Tc-GSA SPECT

	LUR			LUD		
	Whole liver	Right lobe	Left lobe	Whole liver	Right lobe	Left lobe
ALB	0.667[a]	0.649[a]	0.395[b]	0.476[b]	0.486[b]	0.400[b]
ZTT	−0.122	0.003	−0.319	−0.110	−0.102	−0.160
T.Bil	−0.431[b]	−0.475[b]	−0.141	−0.559[a]	−0.559[a]	−0.509[b]
AST	−0.070	−0.092	0.008	−0.143	−0.149	−0.122
ALT	0.066	0.020	0.127	−0.077	−0.073	−0.070
ChE	0.550[a]	0.558[a]	0.280	0.439[b]	0.440[b]	0.391[c]
PT	0.548[a]	0.522[b]	0.347	0.442[b]	0.456[b]	0.374[c]
HPT	0.604[a]	0.526[b]	0.446[b]	0.448[b]	0.473[b]	0.390[c]

ALB, albumin; ZTT, zinc sulfate turbidity; T-Bil, total bilirubin; AST, asparate aminotransferase; ALT, alanine aminotransferase; ChE, cholinesterase activity; PT, prothrombin time, HPT, hepaplastin test. [a]$P < 0.0001$; [b]$P < 0.0005$; [c]$P < 0.005$.

3.5. Evaluation of liver regeneration by ^{99m}Tc-GSA SPECT

We were able to use ^{99m}Tc-GSA SPECT analysis to monitor the liver regeneration process from the acute phase to the recovery phase in nine patients with AH and four surviving patients with FHF. FLV, LUR and LUD in the whole liver, right lobe and left lobe were measured 1 month after the acute phase of AH or FHF. FLV did not change significantly during the 1-month follow-up period in either disease group (Fig. 5A). On the other hand, LUR in the whole liver and the right lobe increased considerably along with the recovery from acute hepatic damage (Fig. 5B). LUD in the whole liver and the right lobe increased considerably along with the recovery from acute hepatic damage (Fig. 5C).

Fig. 6 shows typical images of ASGPR expression in patients with AH or FHF whose clinical course was monitored by ^{99m}Tc-GSA SPECT analysis. As shown in Fig. 6, the number of ASGPRs in the right lobe appears to be higher than in the left lobe during the recovery from either AH or FHF.

4. Discussion

The ASGPR is composed of two subunits, termed human hepatic lectins 1 and 2 and exhibits a high-affinity binding to asialoglycoproteins [12]. The expression of ASGPRs appears to be regulated at both the transcriptional and posttranscriptional levels, and is controlled by the cell cycle as well as various other factors including biotin and the cytokines [13–17]. In addition to these factors, the number of living hepatocytes is also positively correlated with ^{99m}Tc-GSA binding activity. In acute hepatic damage, the number of living hepatocytes is reduced due to hepatocyte death. The analysis of ASGPR expression therefore offers information on the pathobiological condition of the diseased liver including hepatocyte death.

To date, the indices that have been used when assessing hepatic function by ^{99m}Tc-GSA are: $[R]_0$, which reflects the receptor concentration; HH15, which reflects the ratio of disappearance of ^{99m}Tc-GSA from the blood; LHL15, which reflects the hepatic uptake ratio of ^{99m}Tc-GSA; GSA-Rmax, which reflects the maximal receptor binding rate of ^{99m}Tc-GSA; and Ro, which reflects the amount of ASGPRs present

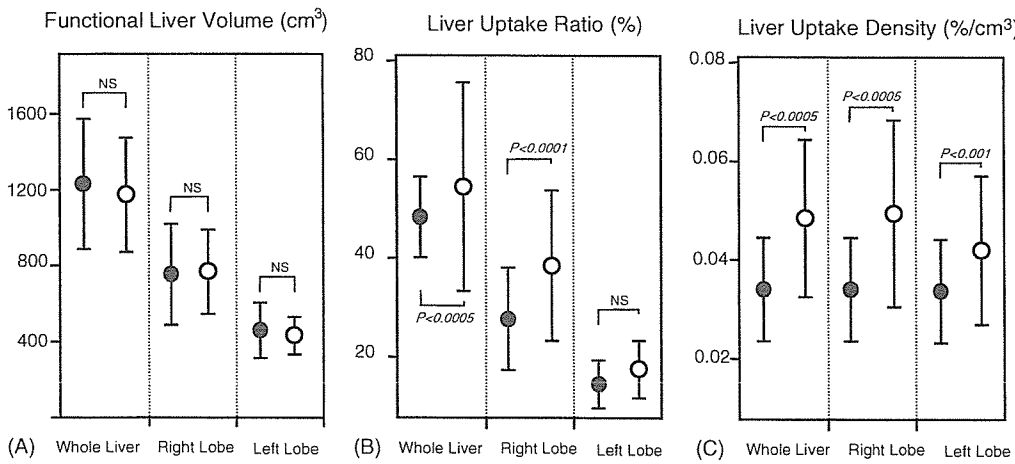

Fig. 5. Evaluation of liver regeneration for the whole liver, and individually for the right and left lobes. LUR and LUD values for the whole liver, and for the right and left lobes increased considerably during the recovery from acute hepatic damage. Closed circle, acute phase; open circle, recovery phase. (A) Mean LUD value for the whole liver; (B) mean LUD value for the right lobe; (C) mean LUD value for the left lobe. NS, not significant.

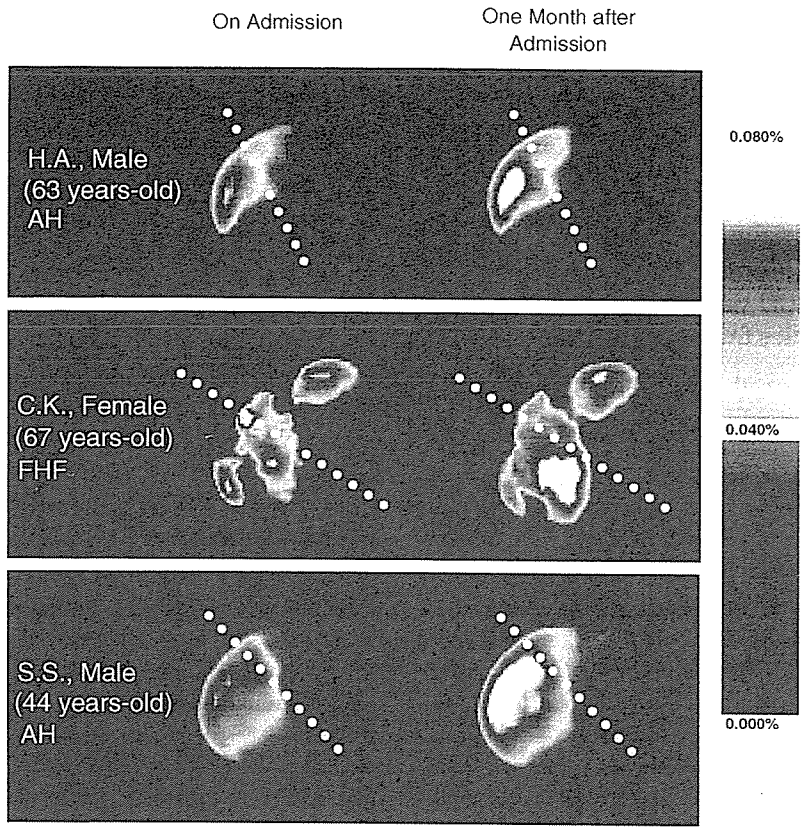

Fig. 6. Typical liver SPECT images taken during the acute and recovery phases in patients with AH and FHF. The liver SPECT images were taken 0 and 4 weeks after admission, showing the increase in the liver uptake of ^{99m}Tc-GSA. The yellow dotted lines indicate the borderline (Cantlie's line) between the right and left hepatic lobes.

[18–23]. These indices are obtained by measuring of the isotope count, with the region of interest set over the heart, the whole liver and the right and middle lung areas. However, these indices have several shortcomings, such as the complexity of the calculations involved; considerable variability in the data obtained, even from a single patient, due to the manufacturer's selection of the region of interest; and measurement limits in patients with severely impaired liver functional reserve. Furthermore, although a method capable of evaluating the right and left hepatic lobes separately would contribute to both the understanding of pathobiological conditions of the liver, indices which can evaluate regional liver function objectively have not been established until now. In our ^{99m}Tc-GSA SPECT analysis, we avoided the manufacturer's selection as far as possible. By ^{99m}Tc-GSA SPECT analysis, we obtained three novel indices: FLV, which reflects the actual hepatic volume, LUR, which reflects the number of hepatic ASGPRs, and LUD, which reflects the density of hepatic ASGPRs. Our ^{99m}Tc-GSA SPECT analysis was convenient and showed good reproducibility of the FLV, LUR and LUD in the whole liver, right lobe and left lobe (data not shown). As shown in the present study, using LUR and LUD, ASGPR dynamics could be evaluated quantitatively in the right and left lobes, even in patients with impaired hepatic functional reserve such as those with FHF, for whom correct evaluation of hepatic functional reserve was previously considered to be difficult.

It was reported that hepatic volumetric analysis by CT scan was not able to predict the outcome of acute hepatic failure in the early stage of acute hepatic damage [24]. Our present study revealed that there was no significant difference of FLV among the control, AH and FHF groups. Our basic examination showed that volumetric analysis by ^{99m}Tc-GSA SPECT and CT scan corresponded well ($r = 0.948$). We routinely perform ^{99m}Tc-GSA SPECT analysis immediately after admission to our hospital, and the liver volume reduction does not occur during this period even in FHF. Although the severity of acute hepatic damage was difficult to assess by FLV alone, the increased FLV L/R ratio suggested that the degree of decrease in the right hepatic lobe in comparison with that in the left hepatic lobe in each case depended on the severity of acute hepatic damage. This implies that the ratio of left hepatic lobe to whole liver volume increases in accordance with the severity of acute hepatic damage, suggesting a difference in pathobiologic condition between the right and left lobes.

In the present study, the LUR and LUD values of the AH and FHF groups were significantly lower than those of the

control group. In addition, the LUR and LUD of the FHF group were significantly lower compared with those of the AH group. These results suggest that LUR and LUD are reliable for evaluating the severity of acute hepatic damage, and particularly for making a clear distinction between AH and FHF. In the present study, there were several AH patients with reduced LUR and LUD values. The severe AH patients presented with marked hyperbilirubinemia and elongation of the prothrombin time less than 40%. Therefore, AH patients with reduced LUR and LUD values should be followed up carefully so that the transition from AH to FHF can be monitored, because the liver in such patients is severely damaged. In the FHF group, ^{99m}Tc-GSA SPECT analysis revealed a significant reduction of both the LUR and LUD in the hepatic lobes from both sides, as well as a significant increase of the L/R ratio for both LUR and LUD. This implies that the number and density of ASGPRs had decreased in FHF group, and were particularly predominant in the right lobes. So far, no reports have analyzed the ASGPR expression of the right and left lobes separately in patients with AH and FHF. Our present finding of a difference in the expression of ASGPR between the right and left lobes in patients with acute hepatic damage is considered to be a novel one.

From comparisons with conventional blood tests, the LUR and LUD values obtained from ^{99m}Tc-GSA SPECT analysis correlated well with total bilirubin and parameters that reflect hepatic functional reserve, including serum albumin levels, cholinesterase activity, prothrombin time and hepaplastin test. Individual analyses of the right and left hepatic lobes demonstrated that the correlation between the results of laboratory examinations and either LUR or LUD was stronger for the right than for the left lobes. Because the L/R ratios for LUR and LUD increased significantly in the FHF group, a decreased ASGPR expression in the right lobes is more significant than in the left lobes. Concomitant consideration of the correlation with the results of laboratory examinations and L/R ratios suggests that (i) conventional laboratory examinations indicating hepatic functional reserve reflect mainly the hepatic functional reserve of the right lobes and (ii) the right lobes are more severely damaged in the FHF group.

In the present study, we were able to show clearly that ASGPR expression increased with recovery from either AH or FHF. The increased expression of ASGPRs in patients with AH or FHF may reflect the increased number of functional hepatocytes that occurs as a consequence of liver regeneration. Separate analyses of ASGPR expression in the right and left hepatic lobes showed that the increased LUR and LUD values for the right lobes were significantly higher than for the left. It has been reported that the blood supply to the right hepatic lobes originates primarily from the superior mesenteric vein, whereas that to the left lobes originates primarily from the splenic vein [25]. We speculate that the different speeds of recovery observed for the right and left hepatic lobes may be attributable to this difference in their blood supply.

5. Conclusion

We were able to use ^{99m}Tc-GSA SPECT analysis to evaluate the severity of acute hepatic damage in individual hepatic lobes in cases of both AH and FHF. In the present study, we have shown for the first time the feasibility of analyzing pathophysiological condition of the acute hepatic damage including hepatocyte death and regeneration separately in the right and left lobes of patients with AH and FHF.

References

[1] Sherlock S, Dooley J. Acute liver failure. In: Sherlock S, Dooley J, editors. Diseases of the liver and biliary system. 11th ed. London: Blackwell Publishing; 2001. p. 111–24.
[2] Kurtz AB, Rubin CS, Cooper HS, et al. Ultrasound findings in hepatitis. Radiology 1980;136:717–23.
[3] Kumahara T, Muto Y, Moriwaki H, Yoshida T, Tomita E. Determination of the integrated CT number of the whole liver in patients with severe hepatitis: as an indicator of the functional reserve of the liver. Gastroenterol Jpn 1989;24:290–7.
[4] Marshall JS, Green AM, Pensky J, Williams S, Zinn A, Carlson DM. Measurement of circulating desialylated glycoproteins and correlation with hepatocellular damage. J Clin Invest 1974;54:555–62.
[5] Onodera Y, Takahashi K, Sugai Y, et al. Evaluation of the liver uptake rate measured by 99mTc-GSA SPECT to assess liver function. Kaku Igaku 1999;36:37–44 [abstract in English].
[6] Sugai Y, Komatani A, Hosoya T, Yamaguchi K. Response to percutaneous transhepatic portal embolization: new proposed parameters by 99mTc-GSA SPECT and their usefulness in prognostic estimation after hepatectomy. J Nucl Med 2000;41:421–5.
[7] Sugahara K, Togashi H, Takahashi K, et al. Separate analysis of asialoglycoprotein receptors in the right and left hepatic lobes using Tc-GSA SPECT. Hepatology 2003;38:1401–9.
[8] Ashwell G, Morell AG. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. Adv Enzymol Relat Areas Mol Biol 1974;41:99–128.
[9] Sawamura T, Kawasato S, Shiozaki Y, Sameshima Y, Nakada H, Tashiro Y. Decrease of a hepatic binding protein specific for asialoglycoproteins with accumulation of serum asialoglycoproteins in galactosamine-treated rats. Gastroenterology 1981;81:527–33.
[10] Sugai Y, Komatani A, Hosoya T, Takahashi K. Analysis of the early blood kinetics of 99mTc-GSA and its verification: new one-compartment model and regression equation. Nucl Med Commun 2001;22:773–8.
[11] Takahashi Y, et al. Aetiology and prognosis of fulminant viral hepatitis in Japan: a multicentre study. J Gastroenterol Hepatol 1991;6:159–64.
[12] Stockert RJ. The asialoglycoprotein receptor: relationships between structure, function, and expression. Physiol Rev 1995;75:591–609.
[13] Huber BE, Glowinski IB, Thorgeirsson SS. Transcriptional and post-transcriptional regulation of the asialoglycoprotein receptor in normal and neoplastic rat liver. J Biol Chem 1986;261:12400–7.
[14] McAbee DD, Clarke BL, Oka JA, Weigel PH. The surface activity of the same subpopulation of galactosyl receptors on isolated rat hepatocytes is modulated by colchicine, monensin, ATP depletion, and chloroquine. J Biol Chem 1990;265:629–35.
[15] Stockert RJ, Paietta E, Racevskis J, Morell AG. Posttranscriptional regulation of the asialoglycoprotein receptor by cGMP. J Biol Chem 1992;267:56–9.
[16] Nakaya R, Kohgo Y, Mogi Y, Nakajima M, et al. Regulation of asialoglycoprotein receptor synthesis by inflammation-related cytokines in HepG2 cells. J Gastroenterol 1994;29:24–30.

[17] Ise H, Sugihara N, Negishi N, Nikaido T, Akaike T. Low asialoglycoprotein receptor expression as markers for highly proliferative potential hepatocytes. Biochem Biophys Res Commun 2001;285:172–82.
[18] Kudo M, Todo A, Ikekubo K, et al. Quantitative assessment of hepatocellular function through in vivo radioreceptor imaging with technetium 99m galactosyl human serum albumin. Hepatology 1993;17:814–9.
[19] Kwon AH, Ha-Kawa SK, Uetsuji S, Kamiyama Y, Tanaka Y. Use of technetium 99m diethylenetriamine-pentaacetic acid-galactosyl-human serum albumin liver scintigraphy in the evaluation of preoperative and postoperative hepatic functional reserve for hepatectomy. Surgery 1995;117:429–34.
[20] Shiomi S, Kuroki T, Enomoto M, et al. Fulminant hepatic failure monitored by technetium-99m-DTPA-galactosyl-human serum albumin scintigraphy. J Nucl Med 1996;37:641–3.
[21] Kwon AH, Ha-Kawa SK, Uetsuji S, et al. Preoperative determination of the surgical procedure for hepatectomy using technetium-99m-galactosyl human serum albumin (99mTc-GSA) liver scintigraphy. Hepatology 1997;25:426–9.
[22] Shuke N, Okizaki A, Kino S, et al. Functional mapping of regional liver asialoglycoprotein receptor amount from single blood sample and SPECT. J Nucl Med 2003;44:475–82.
[23] Monoe T, Nomura T, Nakao H, et al. Prognostic indicators estimated by (99m)Tc-GSA in acute liver damage. Hepatol Res 2005;31:153–9.
[24] Shakil AO, Jones BC, Lee RG, et al. Prognostic value of abdominal CT scanning and hepatic histopathology in patients with acute liver failure. Dig Dis Sci 2000;45:334–9.
[25] Kashiwagi T, Kamada T, Abe H. Dynamic studies on the portal hemodynamics of scintiphotosplenoportography. Streamline flow in the human portal vein. Gastroenterology 1975;69:1292–6.

doi:10.1111/j.1440-1746.2006.04497.x

HEPATOLOGY

Serum levels of stem cell factor and thrombopoietin are markedly decreased in fulminant hepatic failure patients with a poor prognosis

Kazuo Okumoto, Takafumi Saito, Motoyuki Onodera, Ayuka Sakamoto, Mamiko Tanaka, Etsuko Hattori, Hiroaki Haga, Jun-Itsu Ito, Kazuhiko Sugahara, Koji Saito, Hitoshi Togashi and Sumio Kawata

Department of Gastroenterology, Faculty of Medicine, Yamagata University, Yamagata City, Yamagata, Japan

Key words
bone marrow, fulminant hepatitis, growth factor, liver regeneration, stem cell.

Accepted for publication 17 February 2006.

Correspondence
Dr Takafumi Saito, Department of Gastroenterology, Faculty of Medicine, Yamagata University, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan.
Email: tasaitoh@med.id.yamagata-u.ac.jp

Abstract

Background and Aim: Hematopoietic growth factors including stem cell factor (SCF), thrombopoietin (TPO) and granulocyte colony stimulating factor (G-CSF) have a potential role in inducing bone marrow hematopoietic stem cells to move into the circulation, and the association of these factors with liver regeneration has received a lot of attention recently. The aim of this study was to determine the serum levels of such factors in patients with acute liver injury.

Methods: The subjects were 25 patients with acute hepatitis (AH) who had a favorable prognosis and 26 patients with fulminant hepatitis (FH), of whom 11 were alive and 15 had died. Sixty-six healthy subjects matched for age and sex served as controls. Serum samples were collected before treatment, and the levels of SCF, TPO and G-CSF were measured using enzyme-linked immunosorbant assays.

Results: The levels of SCF and TPO were significantly lower in FH patients than in AH patients and the controls, and were also significantly lower in the FH patients who died, compared to the surviving patients. The G-CSF levels did not differ among them.

Conclusions: These results suggest that low serum levels of SCF and TPO may be linked to poor prognosis in patients with severe liver injury.

Introduction

Many types of liver injury induce biological responses that promote recovery and subsequent reconstruction of the injured liver. Hence, hepatocytes can proliferate independently by division after the loss of liver cells, as is often observed after partial hepatectomy,[1,2] and an alternative mechanism, in which liver stem cells are produced and subsequently differentiate into liver cells, is involved in reconstruction after severe liver damage.[3,4] Experimentally, oval cells that appear around the portal tract of the liver in models of severe liver damage have been demonstrated to be one such type of liver stem cell.[5–8] Bone marrow cells may also be a candidate for stromal multipotent stem cells differentiating into liver cells,[9,10] and we have shown in a previous study that bone marrow cells can differentiate into a liver-cell lineage.[11–13]

Several hematopoietic growth factors including stem cell factor (SCF), thrombopoietin (TPO) and granulocyte colony stimulating factor (G-CSF) have a potential role in inducing bone marrow hematopoietic stem cells to move into the systemic circulation.[14–16] Furthermore, SCF has an important role in development of oval cells[17,18] and G-CSF accelerates liver regeneration in a rat fulminant hepatitis model,[19,20] while TPO has been shown to contribute to induction of megakaryocytes, and thus play a role in regulation of platelet count in disease.[21,22] However, the association of such hematopoietic stem cell factors with acute liver diseases has yet to be elucidated. Although acute hepatitis (AH) has a favorable prognosis, liver diseases involving severe injury, such as fulminant hepatitis (FH), have a high mortality rate, and thus it is of interest to investigate the serum levels of hematopoietic stem cell factors in cases of acute liver injury. The aim of this study was to perform liver function tests and investigate the serum levels of SCF, TPO and G-CSF in patients with acute liver injury, and compare the serum levels of these factors between AH and FH patients and between surviving and deceased FH patients.

Methods

The subjects comprised 51 patients, 26 of whom were diagnosed with FH and 25 with AH (Table 1). The FH cases include 11 surviving patients and 15 deceased patients (Table 2). None of the

Table 1 Characteristics on admission of patients with fulminant hepatitis and acute hepatitis

Characteristic	Fulminant hepatitis ($n = 26$)	Acute hepatitis ($n = 25$)	Control ($n = 66$)	*P*-value
Sex (Male : Female)	12:14	13:12	32:34	NS
Age (years)	49.9 ± 15.6	46.8 ± 21.3	49.9 ± 4.5	NS
Total bilirubin (mg/dL)	18.4 ± 9.6	4.5 ± 8.6	NT	<0.0001
AST (IU)	616.3 ± 701.9	819.9 ± 2323.8	20.1 ± 4.1	NS
ALT (IU)	565.6 ± 459.4	664.8 ± 993.7	19.0 ± 7.0	NS
WBC ($\times 10^3/\mu L$)	7.95 ± 3.89	6.21 ± 3.46	NT	NS
Platelet ($\times 10^4/\mu L$)	13.4 ± 6.6	23.1 ± 9.2	NT	<0.001
Hemoglobin (g/dL)	12.1 ± 1.9	13.1 ± 1.8	NT	NS
PT (%)	21.9 ± 7.4	97.2 ± 25.4	NT	<0.0001
HGF (ng/mL)	16.3 ± 11.3	2.2 ± 2.9	0.8 ± 0.2	<0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HGF, hepatocyte growth factor; NS, not significant; NT, not tested; PT, prothrombin time; WBC, white blood cell. Values are shown as mean ± SD.

Table 2 Characteristics on admission of patients with fulminant hepatitis according to survival

Characteristic	Survived ($n = 11$)	Died ($n = 15$)	*P*-value
Sex (Male : Female)	4:7	8:7	NS
Age (years)	48.2 ± 16.5	51.2 ± 15.4	NS
Encephalopathy (Grade 2/3/4)	10/1/0	6/3/6	<0.01
Total bilirubin (mg/dL)	15.1 ± 9.47	21.7 ± 9.3	NS
AST (IU)	607.1 ± 668.4	623.4 ± 753.7	NS
ALT (IU)	543.1 ± 397.8	582.9 ± 517.2	NS
WBC ($\times 10^3/\mu L$)	7.16 ± 3.97	8.73 ± 3.87	NS
Platelet ($\times 10^4/\mu L$)	15.0 ± 7.9	11.7 ± 4.2	NS
Hemoglobin (g/dL)	11.6 ± 2.0	12.3 ± 1.9	NS
PT (%)	26.2 ± 7.4	18.8 ± 5.8	<0.01
HGF (ng/mL)	9.8 ± 5.6	21.0 ± 12.2	<0.01

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HGF, hepatocyte growth factor; NS, not significant; PT, prothrombin time; WBC, white blood cell. Values are shown as mean ± SD.

AH patients had died. FH was diagnosed according to Japanese diagnostic criteria,[23] which require encephalopathy of greater than class II to occur within 8 weeks after the onset of liver disease, and a prothrombin time (PT) activity of lower than 40%. The etiology of FH was due to hepatitis B virus infection ($n = 5$), autoimmune hepatitis ($n = 2$) and unknown origin ($n = 19$), and the etiology of AH was due to hepatitis A virus infection ($n = 5$), hepatitis B virus infection ($n = 4$), drug-induced hepatitis ($n = 6$) and unknown origin ($n = 10$). Blood cell counts and liver function tests for total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), PT and hepatocyte growth factor (HGF) were performed on admission (Table 1). Sixty-six healthy subjects matched for age and sex served as controls.

Serum was collected when the patients were admitted to the hospital and before treatment, and the serum concentrations of three hematopoietic factors, SCF, TPO and G-CSF, were assayed. Three different ELISA kits were used for the assays according to the manufacturers' instructions: the Quantikine human SCF immunoassay Kit (R & D Systems, Minneapolis, MN, USA), the Quantikine human TPO immunoassay Kit (R & D Systems), and the Immunoassay Kit Human G-CSF (BioSource International, Camarillo, CA, USA). Microplates were coated with manufacturer-provided monoclonal antibodies against HGF, SCF and TPO, and following the enzyme reaction the plates were measured using a microplate manager (BIO-RAD Laboratories, Hercules, CA, USA) and the optical density was determined at 450 nm.

Statistical analysis was performed using Student's *t*-test and the Mann–Whitney *U*-test, with $P < 0.05$ considered to be significant.

Results

Serum levels of SCF

The serum levels of SCF in FH patients were significantly lower than those in AH patients (685.3 ± 506.6 pg/mL *vs* 926.6 ± 318.7 pg/mL, $P < 0.05$) There was no significant difference in serum SCF levels between AH patients and the controls (870.3 ± 202.9 pg/mL) (Fig. 1a). Among the FH patients, the serum levels of SCF in deceased patients were significantly lower than those in surviving patients (517.3 ± 360.4 pg/mL *vs* 914.4 ± 599.8 pg/mL, $P < 0.05$) There was no significant difference in serum SCF levels between surviving patients and the controls (Fig. 1b).

Serum levels of TPO

The serum levels of TPO in FH patients (43.3 ± 18.3 pg/mL) were significantly lower than those in AH patients (144.4 ± 96.1 pg/mL,

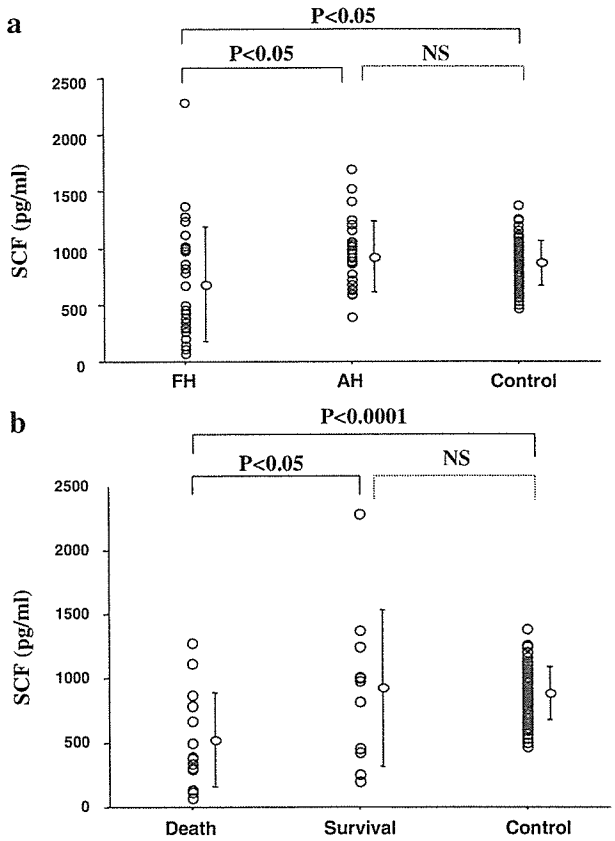

Figure 1 Serum levels of stem cell factor (SCF) in patients with acute liver injury. (a) The serum levels of SCF in fulminant hepatitis (FH) patients were significantly lower than those in acute hepatitis (AH) patients and the controls. (b) Among the patients with FH, the serum levels of SCF in patients who died were significantly lower than those in patients who survived.

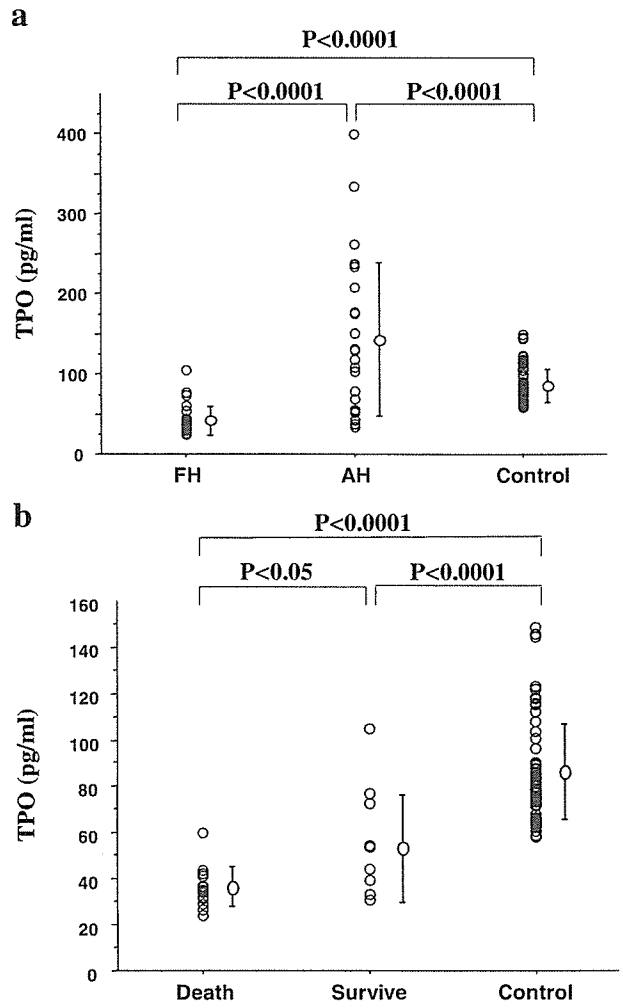

Figure 2 Serum levels of thrombopoietin (TPO) in patients with acute liver injury. (a) The serum levels of TPO in fulminant hepatitis (FH) patients were significantly lower than those in acute hepatitis (AH) patients and the controls. (b) Among the patients with FH, the serum levels of TPO in patients who died were significantly lower than those in patients who survived.

$P < 0.0001$) and the controls (86.7 ± 21.0 pg/mL, $P < 0.0001$). The serum levels of TPO in AH patients were significantly higher than those in the controls ($P < 0.0001$) (Fig. 2a). Among the FH patients, the serum levels of TPO in deceased patients were significantly lower than those in surviving patients (36.2 ± 8.5 pg/mL *vs* 53.0 ± 23.5 pg/mL, $P < 0.05$) (Fig. 2b).

Serum levels of G-CSF

Serum levels of G-CSF did not differ significantly among the FH patients (146.1 ± 522.5 pg/mL), AH patients (98.2 ± 361.8 pg/mL) and the controls (76.7 ± 97.7 pg/mL). There was no significant difference in serum G-CSF levels between the deceased and surviving FH patients (231.3 ± 684.9 pg/mL *vs* 29.8 ± 12.2 pg/mL).

Relationship of the serum levels of TPO and SCF to those of HGF

A clear significant inverse correlation between the serum level of TPO and that of HGF was found in all the patients with acute liver injury ($r = 0.134$, $P < 0.01$) (Fig. 3). Furthermore, although there was no significant correlation between the serum levels of SCF and those of HGF, there was a tendency for an inverse correlation ($r = 0.072$, $P = 0.057$) (Fig. 4).

Discussion

Multipotent stem cells in bone marrow have recently been shown to differentiate into a variety of organ-specific cells, including hepatocytes.[9,10] We have shown that bone marrow cells differentiate into a liver-cell lineage both *in vitro* and *in vivo*,[11–13] and thus these cells are considered to be a potential candidate for stromal liver stem cells that play a role in regeneration of the severely injured liver. However, the potential role of hematopoietic growth

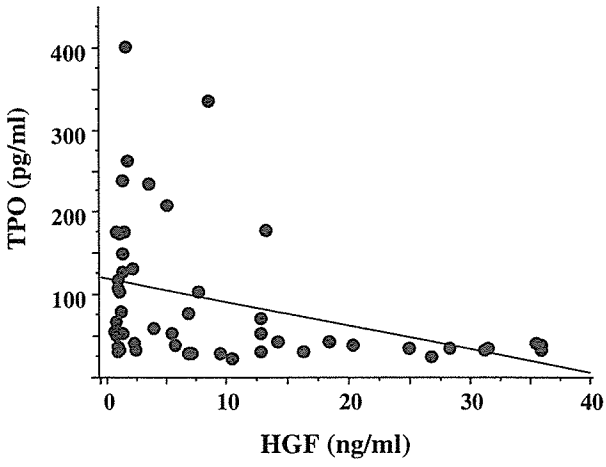

Figure 3 Relationship of the serum level of thrombopoietin (TPO) to that of hepatocyte growth factor (HGF) in patients with acute liver injury. A clear significant inverse correlation between the serum level of TPO and that of HGF was found. ($r = 0.134$, $P < 0.01$).

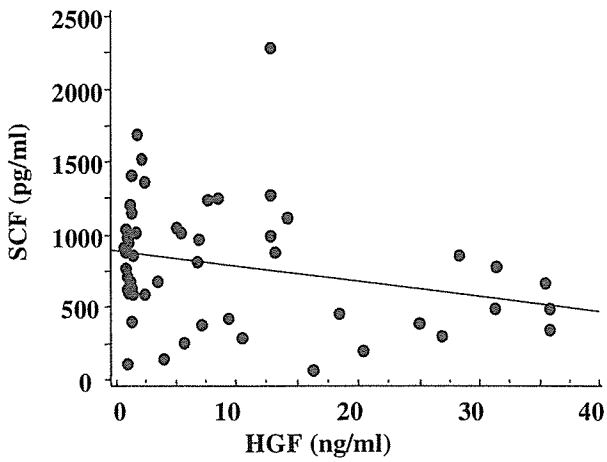

Figure 4 Relationship of the serum level of stem cell factor (SCF) to that of hepatocyte growth factor (HGF) in patients with acute liver injury. There was no significant correlation between the serum level of SCF and that of HGF, but there was a tendency for an inverse correlation ($r = 0.072$, $P = 0.057$).

factors in mobilizing hematopoietic stem cells of the bone marrow such that they move into the systemic circulation has not been investigated in the severely injured liver, in which bone marrow cells might be associated with liver regeneration. Therefore, in this study, we examined the serum levels of SCF, TPO and G-CSF in patients with acute liver injury.

Stem cell factor is mainly produced by bone marrow cells.[24] It is a hematopoietic stem cell factor that targets multipotent progenitor cells, mast cells and B cells.[25] SCF has a potential role in multiplication of stem cells and mast cells, in cooperation with interleukin (IL)-1 beta, IL-6, IL-3, interferon-gamma and erythropoietin.[26] Several pieces of evidence regarding the involvement of SCF in liver regeneration[27,28] suggest that it has an important role in recovery from severe liver injury. Hence, SCF has been reported to contribute to proliferation of both oval cells[18] and liver cells after 70% partial hepatectomy in IL-6-knockout mice.[29] Interestingly, in contrast to the high serum SCF level in AH patients with a favorable prognosis, the serum SCF level was markedly decreased in patients with fulminant hepatic failure, and especially in FH patients who subsequently died. The low serum level of SCF was associated with poor prognosis in FH patients. The mRNA expression level of c-kit, which is a receptor for SCF, has been reported to increase in the infant liver in fulminant hepatic failure,[27] and oval cells carrying the c-kit receptor may be induced to facilitate liver regeneration. Thus, impairment of SCF production in liver stem cells such as oval cells or bone marrow cells may be partially associated with poor regeneration of liver cells in patients with fulminant hepatic failure, leading to a poor prognosis for these patients.

Thrombopoietin is mainly produced by hepatocytes, as well as in bone marrow cells,[30] and is a hematopoietic factor that stimulates promegakaryocytes and multipotent stem cells to induce megakaryocytes, which in turn produce platelets in the presence of SCF.[16] As TPO is produced in hepatocytes and bone marrow cells, its relationship to liver disease has been investigated previously. The mRNA expression level of TPO has been shown to increase in acute inflammation[31] and, in fact, the serum level of TPO increases in patients with acute liver damage induced by acetaminophen.[32] However, the serum levels of TPO have been shown to be low in patients with chronic liver diseases, and especially in liver cirrhosis patients.[33–35] TPO is known to increase in response to thrombocytopenia when the level of circulating platelets is decreased. In this study, the platelet count of FH patients was lower than that of AH patients, and there was no significant difference in platelet count between FH patients who survived and those who died. A clear significant inverse correlation between the serum level of TPO and that of HGF was found in all the subjects with acute liver injury. Thus the decrease in serum TPO levels may be related to liver dysfunction rather than platelet count. TPO is inducible from liver cells in the acute phase of liver injury, but cannot be induced in end-stage liver diseases due to poor regeneration of the liver. The present study is the first to demonstrate that the serum TPO level can also be markedly decreased in acute liver injury, and particularly in FH patients with a poor prognosis. Furthermore, the TPO level showed a clear inverse correlation with the serum HGF level in all the subjects in the study. Thus, TPO production may be severely impaired due to the massive loss of hepatocytes caused by insufficient liver-cell regeneration in a severely injured liver in which the serum level of HGF is high. Such poor induction of TPO may result in impairment of the induction of hematopoietic bone marrow stem cells, thereby reducing platelet production and liver-cell regeneration in a severely injured liver, and this may have been particularly prevalent in FH patients who did not survive.

Granulocyte colony stimulating factor is produced by monocytes, macrophages, fibroblasts and endothelial cells, and is a hematopoietic factor that acts on neutrophils or multipotent bone marrow stem cells.[36] G-CSF supports cell growth and differentiation of preneutrophils and hematopoietic stem cells, and promotes mobilization of mature neutrophils and stem cells into the circulation.[37] It is an agent that is widely used clinically to support neutrophil induction or peripheral stem cell transplantation. Although G-CSF has been shown to induce liver-cell regeneration

in a rat liver-injury model[19,20] and to elevate the serum HGF level,[38] no differences in G-CSF levels were observed in any comparison made in the present study.

In summary, we showed for the first time that the serum levels of both SCF and TPO were markedly lower in FH patients who subsequently died, compared to those in surviving FH patients, and that these levels are lower in FH patients that in AH patients, who have a more favorable prognosis. Further studies are needed to clarify why the low serum levels of SCF and TPO are associated with a poor prognosis in patients with acute liver injury.

Acknowledgment

This study was supported in part by a Grant-in-Aid from the 21st century center of excellence (COE) program of the Japan Society for the Promotion of Science.

References

1 Bucher NL. Experimental aspects of hepatic regeneration. *N. Engl. J. Med.* 1967; **277**: 686–96.
2 Rubin EM, Martin AA, Thung SN, Gerber MA. Morphometric and immunohistochemical characterization of human liver regeneration. *Am. J. Pathol.* 1995; **147**: 397–404.
3 Thorgeirsson SS. Hepatic stem cells in liver regeneration. *FASEB J.* 1996; **10**: 1249–56.
4 Alison MR, Golding M, Sarraf CE, Edwards RJ, Lalani EN. Liver damage in the rat induces hepatocyte stem cells from biliary epithelial cells. *Gastroenterology* 1996; **110**: 1182–90.
5 Fausto N. Hepatocyte differentiation and progenitor cells. *Curr. Opin. Cell Biol.* 1990; **2**: 1036–42.
6 Pack R, Heck R, Dienes HP, Oesch F, Steinberg P. Isolation, biochemical characterization, long-term culture, and phenotype modulation of oval cells from carcinogen-fed rats. *Exp. Cell Res.* 1993; **204**: 198–209.
7 Lemire JM, Shiojiri N, Fausto N. Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. *Am. J. Pathol.* 1991; **139**: 535–52.
8 Yasui O, Miura N, Terada K, Kawarada Y, Koyama K, Sugiyama T. Isolation of oval cells from Long-Evans cinnamon rats and their transformation into hepatocytes *in vivo* in the rat liver. *Hepatology* 1997; **25**: 329–34.
9 Petersen BE, Bowen WC, Patrene KD *et al.* Bone marrow as a potential source of hepatic oval cells. *Science* 1999; **284**: 1168–70.
10 Lagasse E, Connors H, Al-Dhalimy M *et al.* Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat. Med.* 2000; **6**: 1229–34.
11 Okumoto K, Saito T, Hattori E *et al.* Differentiation of bone marrow cells into cells that express liver-specific genes *in vitro*: implication of the Notch signals in differentiation. *Biochem. Biophys. Res. Commun.* 2003; **304**: 691–5.
12 Okumoto K, Saito T, Hattori E *et al.* Expression of Notch signalling markers in bone marrow cells that differentiate into a liver cell lineage in a rat transplant model. *Hepatol. Res.* 2005; **31**: 7–12.
13 Okumoto K, Saito T, Hattori E *et al.* Differentiation of rat bone marrow cells cultured on artificial basement membrane containing extracellular matrix into a liver cell lineage. *J. Hepatol.* 2005; **43**: 110–16.
14 McNiece IK, Briddell RA, Hartley CA, Andrews RG. The role of stem cell factor in mobilization of peripheral blood progenitor cells: synergy with G-CSF. *Stem Cells* 1993; **11** (Suppl. 3): 83–8.
15 Uchida N, Dykstra B, Lyons KJ, Leung FY, Eaves CJ. Different *in vivo* repopulating activities of purified hematopoietic stem cells before and after being stimulated to divide *in vitro* with the same kinetics. *Exp. Hematol.* 2003; **31**: 1338–47.
16 Ramsfjell V, Borge OJ, Veiby OP *et al.* Thrombopoietin, but not erythropoietin, directly stimulates multilineage growth of primitive murine bone marrow progenitor cells in synergy with early acting cytokines: distinct interactions with the ligands for c-kit and FLT3. *Blood* 1996; **88**: 4481–92.
17 Fujio K, Evarts RP, Hu Z, Marsden ER, Thorgeirsson SS. Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat. *Lab. Invest.* 1994; **70**: 511–16.
18 Matsusaka S, Tsujimura T, Toyosaka A *et al.* Role of c-kit receptor tyrosine kinase in development of oval cells in the rat 2-acetylaminofluorene/partial hepatectomy model. *Hepatology* 1999; **29**: 670–6.
19 Theocharis SE, Margeli AP, Kittas CN. Effect of granulocyte colony-stimulating-factor administration on tissue regeneration due to thioacetamide-induced liver injury in rats. *Dig. Dis. Sci.* 1999; **44**: 1990–6.
20 Theocharis SE, Papadimitriou LJ, Retsou ZP, Margeli AP, Ninos SS, Papadimitriou JD. Granulocyte-colony stimulating factor administration ameliorates liver regeneration in animal model of fulminant hepatic failure and encephalopathy. *Dig. Dis. Sci.* 2003; **48**: 1797–803.
21 Usuki K, Tahara T, Iki S *et al.* Serum thrombopoietin level in various hematological diseases. *Stem Cells* 1996; **14**: 558–65.
22 Kato T, Matsumoto A, Ogami K, Tahara T, Morita H, Miyazaki H. Native thrombopoietin: structure and function. *Stem Cells* 1998; **16**: 11–19.
23 Sato S, Suzuki K, Takikawa Y, Endo R, Omata M, Japanese National Study Group of Fulminant Hepatitis. Clinical epidemiology of fulminant hepatitis in Japan before the substantial introduction of liver transplantation: an analysis of 1309 cases in a 15-year national survey. *Heptol. Res.* 2004; **30**: 135–61.
24 Kojima S, Matsuyama T, Kodera Y. Plasma levels and production of soluble stem cell factor by marrow stromal cells in patients with aplastic anaemia. *Br. J. Haematol.* 1997; **99**: 440–6.
25 Summerfield A, Horn MP, Lozano G, Carrasco CP, Atze K, McCullough K. C-kit positive porcine bone marrow progenitor cells identified and enriched using recombinant stem cell factor. *J. Immunol. Methods* 2003; **280**: 113–23.
26 Brugger W, Mocklin W, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L. *Ex vivo* expansion of enriched peripheral blood CD34+ progenitor cells by stem cell factor, interleukin-1 beta (IL-1 beta), IL-6, IL-3, interferon-gamma, and erythropoietin. *Blood* 1993; **81**: 2579–84.
27 Baumann U, Crosby HA, Ramani P, Kelly DA, Strain AJ. Expression of the stem cell factor receptor c-kit in normal and diseased pediatric liver: identification of a human hepatic progenitor cell? *Hepatology* 1999; **30**: 112–17.
28 Simpson K, Hogaboam CM, Kunkel SL, Harrison DJ, Bone-Larson C, Lukacs NW. Stem cell factor attenuates liver damage in a murine model of acetaminophen-induced hepatic injury. *Lab. Invest.* 2003; **83**: 199–206.
29 Ren X, Hogaboam C, Carpenter A, Colletti L. Stem cell factor restores hepatocyte proliferation in IL-6 knockout mice following 70% hepatectomy. *J. Clin. Invest.* 2003; **112**: 1407–18.
30 Nagahisa H, Nagata Y, Ohnuki T *et al.* Bone marrow stromal cells produce thrombopoietin and stimulate megakaryocyte growth and maturation but suppress proplatelet formation. *Blood* 1996; **87**: 1309–16.
31 Wolber EM, Fandrey J, Frackowski U, Jelkmann W. Hepatic thrombopoietin mRNA is increased in acute inflammation. *Thromb. Haemost.* 2001; **86**: 1421–4.
32 Schiodt FV, Balko J, Schilsky M, Harrison ME, Thornton A, Lee WM. Thrombopoietin in acute liver failure. Acute Liver Failure Study Group. *Hepatology* 2003; **37**: 558–61.
33 Giannini E, Borro P, Botta F *et al.* Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. *J. Hepatol.* 2002; **37**: 572–7.

34 Freni MA, Spadaro A, Ajello A *et al.* Serum thrombopoietin in chronic liver disease: relation to severity of the disease and spleen size. *Hepatogastroenterology* 2002; **49**: 1382–5.
35 Koruk M, Onuk MD, Akcay F, Savas MC. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis, and its relationship with circulating thrombocyte counts. *Hepatogastroenterology* 2002; **49**: 1645–8.
36 Dokter WH, Dijkstra AJ, Koopmans SB *et al.* G (AnH) MTetra, a naturally occurring 1,6-anhydro muramyl dipeptide, induces granulocyte colony-stimulating factor expression in human monocytes: a molecular analysis. *Infect. Immun.* 1994; **62**: 2953–7.
37 Hematti P, Tuchman S, Larochelle A, Metzger ME, Donahue RE, Tisdale JF. Comparison of retroviral transduction efficiency in CD34+ cells derived from bone marrow versus G-CSF-mobilized or G-CSF plus stem cell factor-mobilized peripheral blood in nonhuman primates. *Stem Cells* 2004; **22**: 1062–9.
38 Fujii K, Ishimaru F, Kozuka T *et al.* Elevation of serum hepatocyte growth factor during granulocyte colony-stimulating factor-induced peripheral blood steG-CSFm cell mobilization. *Br. J. Haematol.* 2004; **124**: 190–4.

Association of Transforming Growth Factor–β1 Functional Polymorphisms with Natural Clearance of Hepatitis C Virus

Tomomi Kimura,[1,a] Takafumi Saito,[3,a] Mika Yoshimura,[1] Song Yixuan,[1] Masanori Baba,[1,2] Guijin Ji,[2] Masaaki Muramatsu,[1,2] and Sumio Kawata[3]

[1]Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, and [2]HuBit Genomix, Tokyo, and [3]Department of Gastroenterology, Faculty of Medicine, Yamagata University, Yamagata, Japan

Transforming growth factor (TGF)–β1 suppresses the proliferation and cytotoxicity of natural killer (NK) cells, which play critical roles in resolving hepatitis C virus (HCV) infection, especially during the acute phase. We examined 230 anti–HCV antibody–positive subjects for HCV RNA and the −509T/C genotype in the TGF-β1 gene promoter. The −509CC genotype and the −509C allele were significantly associated with higher HCV clearance rates ($P = .01$) and with lower transcriptional activity. The genetic effect remained significant even after adjustment for a history of transfusion. Low TGF-β1 producers might have less suppression of NK cells and be more likely to resolve HCV infection.

Viral infection triggers a series of host immune responses. Among hepatitis C virus (HCV)–infected patients, ~15%–20% experience natural clearance, most likely during the acute infectious stage, during which increased an level of interferon (IFN)–γ is a positive marker of the resolving infection [1]. NK cells produce IFN-γ, and their proliferation and cytotoxicity are critical for viral clearance [2]. Because NK cells are suppressed by default, an activation signal should overcome inhibitory regulation. Recently, a weaker inhibitory combination of the NK cell receptor killer cell immunoglobulin-like receptor (KIR)–2DL3 and HLA-C1 was reported to indicate a higher rate of resolution of HCV infection [3]. Moreover, the interleukin (IL)–10 −1082GG genotype, which prompts the production of a higher amount of IL-10, was associated with a lower clearance rate [4]. Considering that IL-10 inhibits the development and activation of NK cells with an IFN-γ–secreting phenotype [5], oversuppression of NK cell cytotoxicity may result in persistent HCV infection.

Transforming growth factor (TGF)–β is another well-known suppressor of NK cells that inhibits IFN-γ and IL-12 production and blocks the proliferation and cytotoxicity of NK cells [6]. The −509T allele is associated with a higher plasma concentration of TGF-β1 [7], and TGF-β1 dysregulation has been shown to be involved in the progression of liver cirrhosis and hepatocellular carcinoma [8]. However, TGF-β1 gene variants have not been studied in association with the natural clearance of HCV. Here, we demonstrate that the −509C/T mutation in the TGF-β1 gene is associated with the natural clearance of HCV and with promoter activity.

Subjects and methods. Study subjects were from northern Japan, where there is a very high prevalence (~40% of inhabitants) of anti-HCV antibodies among the population [9]. The study was approved by the ethical review committee of Yamagata University, and written informed consent was obtained from all subjects recruited. Subjects with a history of antiviral IFN therapy or who were positive for hepatitis B surface antigen were excluded; 230 anti-HCV antibody–positive subjects were enrolled in the study. There were no users of illicit drugs in the study population, and the routes of HCV transmission are still obscure in this community except in those with a history of receiving blood transfusion. Reuse of syringes and needles and the use of folk remedies, such as acupuncture, may be factors in community-acquired HCV infection, but the routes of HCV transmission remain unknown in most cases.

All subjects were tested for their serum HCV RNA status using an Amplicor HCV RNA detection kit (Amplicor HCV version 2.0; Roche Diagnostics). The persistence of resolution was confirmed over a minimum of 12 months by independent sampling.

The −509T/C (rs1800469) single-nucleotide polymorphism (SNP) was determined using the TaqMan assay (Hubit Genomix), and genotypes for the 2904T/G (rs2241715) and 5738G/T (rs2241717) SNPs were obtained from an earlier study [10]. The 869C/T (rs1982073) genotype was determined by direct sequencing, and the IL-10 genotypes, −592A/C (rs1800872) and −1082G/A (rs1800896), were determined using melting-

Received 27 September 2005; accepted 15 December 2005; electronically published 5 April 2006.

Potential conflicts of interest: none reported.

[a] T.K. and T.S. contributed equally to the work.

Reprints or correspondence: Masaaki Muramatsu, Dept. of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan (muramatsu.epi@mri.tmd.ac.jp).

The Journal of Infectious Diseases 2006; 193:1371–4

0022-1899/2006/19310-0006$15.00

curve analysis. The detailed protocol is available from the authors on request.

A TGF-β1 reporter construct, pHTG-luc(−845), which contains an 845-bp genomic fragment of the human TGF-β1 gene, and an expression vector for HCV core protein, PCXN2/HCV-core [11], were provided by Dr. Hiroyoshi Taniguchi (Tokyo University, Japan). Because pHTG-luc(−845) contains C at position −509, it was designated pTGFB(−509C) in the study. Site-directed mutagenesis was used to obtain pTGFB(−509T), using a QuikChange multisite-directed mutagenesis kit (Stratagene) with the following primer pair: forward, 5′-CAACAGGACACCTGAAGGATGGAAGGGTCAG-3′, and reverse, 5′-CTGACCCTTCCATCCTTCAGGTGTCCTGTTG-3′.

Human hepatoma cell line HepG2 cells were seeded at a density of 2.5×10^5 cells/well on 6-well cell culture plates on the day before transfection. We transfected 600 ng of pTGFB(−509C), pTGFB(−509T), or pGL3-basic luciferase reporter vector and 10 ng of pRL-TK (Promega), with or without 100 ng of pCXN2 or pCXN2/HCV-core, using Lipofectamine 2000 and Opti-MEM (Invitrogen); then we replaced the medium 4 h after transfection. The cells were harvested 48 h after transfection. Luciferase activity was measured and normalized with *Renilla* luciferase activity, to compensate for transfection efficiency. The 20-μg lysate of the transfected cells was subjected to SDS-PAGE, and Western blotting was performed using an HCV core–specific mouse monoclonal antibody with horseradish peroxidase–conjugated goat anti–mouse IgG (Santa Cruz Biotechnology).

Allele and genotype frequencies of SNPs between the subjects with persistent infection and those whose infection resolved were tested with Fisher's exact test. Odds ratios (ORs) were computed using logistic-regression models. Promoter activities were standardized to a negative control (pGL3-basic vector) and compared using Student's t test. The D' and r^2 were calculated for all pairwise combinations of the 4 SNPs, using the LDSUPPORT program (available for academic use from N. Kamatani, Tokyo Women's Medical University, Tokyo, Japan) based on the EM algorithm. All other analyses were performed with SAS software (version 9.1.3; SAS Institute). Differences of $P < .05$ were considered to be significant.

Results. HCV antibody–positive subjects (84 men and 144 women) were a mean of 66.8 years old (range, 32–88 years old) and were tested for serum HCV RNA levels. In 46 subjects, HCV RNA was not detected, whereas the other 184 tested positive. One half of subjects with persistent HCV infection (92/184 [50%]) had genotype 2b infection, 77 (41.9%) had genotype 1b infection, and 12 (6.5%) had genotype 2a infection. The frequency distribution was not significantly different between subjects with and without a history of blood transfusion (data not shown). The mean age and sex ratios were not significantly different between the groups with resolved versus persistent infection. Twenty-two percent of subjects with persistent infection (33/151) had a history of blood transfusion, compared with only 9.5% of the subjects whose infection resolved (4/42).

Every pair of −509T/C, 869C/T, 904T/G, and 5738G/T SNPs were in tight linkage disequilibrium ($D' > 0.9$; $r^2 > 0.8$). The results for these genotypes and their haplotypes showed essentially the same as those of −509T/C; thus, we set this genotype as a representative. The genotype frequency of the −509T/C variant did not deviate from Hardy-Weinberg equilibrium. The

Table 1. Association between the transforming growth factor–β1 509T/C single-nucleotide polymorphism and hepatitis C virus clearance.

Variable	Subjects, no.	Type of infection, no. (%)		OR (95% CI)	*P*
		Persistent	Self-limiting		
Genotype	230	184 (91)	46 (23)		
TT	64	56 (30.4)	8 (17.4)	1.0 (Referent)	
TC	109	89 (48.4)	20 (43.5)		
CC	57	39 (21.2)	18 (39.1)	2.4 (1.2–4.8)	.0133[a]
Allele					
Total	460	368	92		
T	237	201 (54.6)	36 (39.1)	1.0 (Referent)	
C	223	167 (45.4)	56 (60.9)	1.9 (1.2–3.0)	.0084
No transfusion	386	302	84		
T	200	165 (54.6)	35 (41.7)	1.0 (Referent)	
C	186	137 (45.4)	49 (58.3)	1.7 (1.0–2.8)	.0364
Transfusion	74	66	8		
T	37	36 (54.5)	1 (12.5)	1.0 (Referent)	
C	37	30 (45.5)	7 (87.5)	8.4 (0.98–72.1)	.0524

NOTE. Odds ratios (ORs) were computed using logistic regression analyses. A positive OR indicates a protective association with resolution of infection. CI, confidence interval.

[a] TT and TC vs. CC.

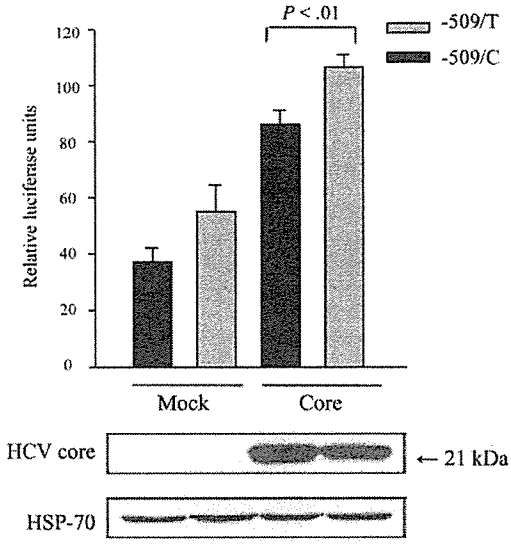

Figure 1. Promoter activity and the −509T/C single-nucleotide polymorphism in hepatocytes with and without hepatitis C virus (HCV) core protein coexpression: transforming growth factor–β1 gene promoter activity. Data are shown as means and SDs of 3 measurements of luciferase activity standardized to corresponding *Renilla* luciferase activity. Results are representative of 3 experiments, each using at least 3 wells for each condition. Promoter activity under the condition of HCV core protein coexpression was significantly higher in subjects with the −509T allele than in those with the −509C allele ($P = .0067$, Student's t test). HCV core protein expression was confirmed using Western blotting. Heat-shock protein (HSP)–70 is shown as a control for the total amount of protein.

frequencies of the −509CC genotype and −509C allele were significantly higher in subjects whose infection resolved (table 1). Multivariate logistic-regression analysis that included a history of blood transfusion, which was a significant negative predictor (OR, 0.44; $P = .0351$), showed that the −509C allele was an independent predictor of HCV clearance (OR, 1.89; $P = .0076$).

The IL-10 genotypes, −592A/C (rs1800872) and −1082G/A (rs1800896), were not associated with HCV clearance (data not shown). The allele frequencies did not deviate from those of a previously reported healthy Japanese population [4], but no subjects carried the −1082GG genotype, which corresponds to a higher IL-10 level and a lower clearance rate, and only a few subjects possessed the −1082AG genotype ($n = 14$). In subjects with the −592AA genotype, the lower production of IL-10 and higher rate of resolution of infection [4] were not associated with clearance in our study population (OR, 0.96; $P = .89$). A multivariate logistic-regression analysis that included the IL-10 genotypes did not show any effect of the association of −509T/C with HCV resolution. Therefore, we did not include the IL-10 genotypes in the final model.

When we stratified the subjects into 2 groups according to their transfusion history, the frequency of the −509C allele was significantly higher in subjects with self-limiting infection, compared with subjects who had persistent infection, in those with no history of blood transfusion (table 1). Among subjects who had received blood transfusions, this tendency was more apparent, although the significance did not reach statistical significance. Seven (87.5%) of 8 alleles in subjects whose infection resolved were the C allele, compared with 30 (45.5%) of 66 alleles in subjects with persistent infection. Age and sex were not associated with the clearance of HCV and were not included in the final model.

To investigate whether the −509T/C polymorphism is involved in the promoter activity of the TGF-β1 gene, we performed an in vitro reporter assay. The promoter reporter construct possessing −509C had ~30% lower promoter activity, compared with that of the −509T reporter construct. Given that the frequency of the −509C allele was significantly higher in subjects whose infection resolved ($P = .01$), there was an inverse relationship between the TGF-β1 gene promoter activity and natural clearance of HCV.

Because hepatocytes infected with HCV can also secrete TGF-β1 and the HCV core protein can enhance its production [11], we examined TGF-β1 gene-promoter activity of the −509T or −509C alleles under the coexpression of HCV core protein. The in vitro promoter assay showed that the −509C allele had significantly lower promoter activity than the −509T allele (~20%; $P < .01$) under the coexpression of HCV core protein (figure 1). The magnitude of the activation induced by HCV core protein coexpression did not differ between the −509T and −509C alleles ($P = .29$).

Discussion. In the present study, we investigated the association of TGF-β1 gene polymorphisms with the natural clearance of HCV. Our results clearly showed that the −509C allele is associated with a higher clearance rate of HCV as well as with lower promoter activity.

Recently, several candidate loci for the natural clearance of HCV have been reported, including HLA, KIR [3], and IL-10 [4]. In whites in the United Kingdom, subjects with the IL-10 −592AA genotype were more likely to clear their infection (13.3% vs. 7.0% of subjects with self-limiting and persistent infection, respectively), whereas subjects with the −1082GG genotype had a higher risk of persistent infection (13.8% vs. 25.0%) [4]. However, we did not find any association with IL-10 promoter genotypes. The discrepancy may arise as a result of differences in genotype frequency between the populations. In the Japanese population, the frequency of the −1082G allele is much lower than that in whites (7% vs. 54%) (see http://www.hapmap.org/cgi-perl/snp_details?name=rs1800896), and there were no −1082GG homozygous and few AG heterozygous subjects in our study population.

TGF-β is a potent immunosuppressive cytokine that inhibits

the function of NK cells. Recently, it has been reported that TGF-β down-regulates NKG2D receptor expression at the transcriptional level and that RNA interference that targets the TGF-β gene enhances NK cell activity [12, 13]. Thus, it is tempting to speculate that high TGF-β1 producers might oversuppress their immune system, particularly during the early stages of infection. In the present study, we have demonstrated that promoter activity was significantly lower in subjects with the −509C allele than in subjects with the −509T allele ($P < .01$) in both the presence and absence of the HCV core protein (figure 1). Although TGF-β1 might suppress the in vitro replication of the HCV replicon [14], our results reflect, to some extent, the in vivo condition of HCV-infected hepatocytes. However, whether the different levels of TGF-β1 gene expression and/or secretion between alleles actually contribute to differing NK activity or IFN-γ secretion in vivo remain unclear. Because the autocrine effect of the TGF-β1 produced by NK cells might not be high enough to impede the activation of NK cells by various cytokines [15], the in vivo interaction of the immune cells, including NK cells and hepatocytes, is an interesting issue.

Transfusion seems to be one of the inhibitory factors for natural clearance [3]. In our study population, a protective tendency of the −509C allele was found even in subjects with a history of blood transfusion, although the difference was not statistically significant (table 1), and the OR remained significant even after adjustment for a history of transfusion. Further study with a larger population is necessary to elucidate this interaction more clearly. The HCV genotype may also contribute to the natural clearance rate. Although the HCV genotype of subjects whose infection resolved was not available for the present study, the frequency of genotype 2b infection was relatively higher in subjects with persistent infection (50%), compared with the average in Japan (~10%–15%). We do not have any data to support the correlation between HCV genotype and the natural clearance rate. A prospective study of newly infected patients may help to clarify the association.

Our results suggest that a lower production of TGF-β1 is correlated with a higher incidence of HCV clearance. TGF-β1 might play an important role in the clearance of HCV viremia during the acute phase of infection. The role of TGF-β1 in HCV clearance warrants further investigation.

References

1. Su AI, Pezacki JP, Wodicka L, et al. Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci USA **2002**;99: 15669–74.
2. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol **1999**; 17:189–220.
3. Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science **2004**; 305:872–4.
4. Knapp S, Hennig BJ, Frodsham AJ, et al. Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. Immunogenetics **2003**; 55:362–9.
5. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol **1993**; 11:165–90.
6. Rook AH, Kehrl JH, Wakefield LM, et al. Effects of transforming growth factor β on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. J Immunol **1986**; 136:3916–20.
7. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type β1. Hum Mol Genet **1999**; 8:93–7.
8. Okumoto K, Hattori E, Tamura K, et al. Possible contribution of circulating transforming growth factor-β1 to immunity and prognosis in unresectable hepatocellular carcinoma. Liver Int **2004**; 24:21–8.
9. Ishibashi M, Shinzawa H, Kuboki M, Tsuchida H, Takahashi T. Prevalence of inhabitants with anti-hepatitis C virus antibody in an area following an acute hepatitis C epidemic: age- and area-related features. J Epidemiol **1996**; 6:1–7.
10. Saito T, Ji G, Shinzawa H, et al. Genetic variations in humans associated with differences in the course of hepatitis C. Biochem Biophys Res Commun **2004**; 317:335–41.
11. Taniguchi H, Kato N, Otsuka M, et al. Hepatitis C virus core protein upregulates transforming growth factor-β 1 transcription. J Med Virol **2004**; 72:52–9.
12. Castriconi R, Cantoni C, Della Chiesa M, et al. Transforming growth factor β 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. Proc Natl Acad Sci USA **2003**; 100:4120–5.
13. Friese MA, Wischhusen J, Wick W, et al. RNA interference targeting transforming growth factor-β enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. Cancer Res **2004**; 64:7596–603.
14. Murata T, Ohshima T, Yamaji M, et al. Suppression of hepatitis C virus replicon by TGF-β. Virology **2005**; 331:407–17.
15. Bellone G, Aste-Amezaga M, Trinchieri G, Rodeck U. Regulation of NK cell functions by TGF-β 1. J Immunol **1995**; 155:1066–73.

＜速　報＞

C型慢性肝炎患者における脂肪酸β酸化障害—絶食試験を用いた検討—

三沢　慶子　斎藤　貴史*　菅原　一彦
斉藤　孝治　冨樫　整　河田　純男

緒言：HCV感染時に見られる肝細胞脂肪化は，HCVコア蛋白によるミトコンドリア脂肪酸β酸化の障害が一因と考えられている[1]．C型慢性肝炎患者における脂肪酸β酸化障害について，患者の絶食下における経時的な総ケトン体産生能を測定し検証した．

対象と方法：対象は，メタボリックシンドロームを有さないC型慢性肝炎（CH）6例および健常者3名である．夕食後，水分のみ経口摂取可能として，午後9時より12時間後，15時間後，18時間後に採血し，総ケトン体(3-ヒドロキシ酪酸，アセト酢酸)，中性脂肪を測定した．また12時間後採血時にHCVコア抗原量とHOMA-IRを測定した．

結果：絶食15時間後の総ケトン体は，CH5例で健常者より低値を示し，CH群は健常者群に比し有意に低値であった（170.6±158.4 vs. 370.6±70.2μmol/L：$p<0.05$，t-test）．絶食18時間後の総ケトン体は両群間で有意差はなかったが，コア抗原量が10,500(fmol/L)と高値であったCase 1においては，総ケトン体の増加はほとんど見られなかった．健常者の総ケトン体は絶食後の時間経過に伴い著明な増加を示した．中性脂肪は，

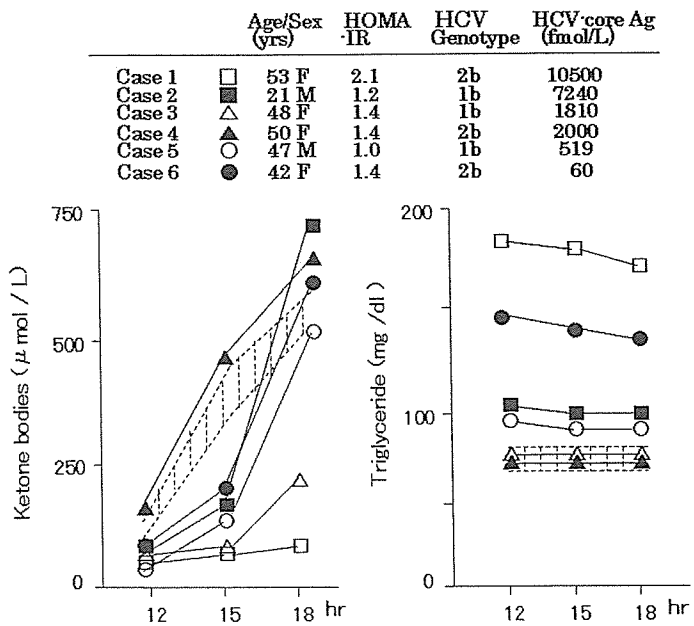

Fig. 1 Levels of blood ketone bodies (3-hydroxybutyrate and acetoacetate) and triglyceride during the 18-hour fasting in subjects. Dashed area indicates mean ± SD for healthy controls.

山形大学消化器病態制御内科学
*Corresponding author: tasaitoh@med.id.yamagata-u.ac.jp
＜受付日2006年10月3日＞＜採択日2007年1月23日＞

いずれの例も，ほぼ不変ないし若干の減少を示した．HOMA-IRは，コア抗原量が高値のCase 1は2.1であり，他の5例は1.4以下であった（Fig. 1）．

考案：絶食時，血中ケトン体は，ミトコンドリアにおける脂肪酸β酸化の亢進により増加するが，脂肪酸β酸化障害下では抑制される．この現象は，成人型のシトルリン血症患者における肝脂肪蓄積時にも観察される[2]．今回，5例のCH症例における脂肪酸β酸化障害が確認された．HCVコア蛋白は脂肪酸β酸化調節に拘わるPPARα発現を抑制する[3]．また脂肪酸β酸化障害時には，酸化されない脂肪酸は中性脂肪の合成源となり得るが，中性脂肪の増加が見られなかった．HCVコア蛋白によりMTP活性の抑制がもたらされている可能性がある[4]．C型慢性肝炎患者において，HCVコア蛋白により脂肪酸β酸化が障害される例があることが絶食時の血中ケトン体測定により始めて実証された．今後，多数例でのさらなる検討を要す．

索引用語：HCV，脂肪酸β酸化，ケトン体

文献：1）Koike K, Moriya K. J Gastroenterol 2005; 40: 329—336　2）Inui Y, Kuwajima M, Kawata S, et al. Gastroenterology 1994; 107: 1154—1161　3）Dharancy S, Malapel M, Perlemuter G, et al. Gastroenterology 2005; 128: 334—342　4）Perlemuter G, Sabile A, Letteron P, et al. FASEB J 2002; 16: 185—194

英文要旨

Impaired mitochondrial β-oxidation in chronic hepatitis C patients, evidenced by fasting test

Keiko Misawa, Takafumi Saito*,
Kazuhiko Sugahara, Koji Saito,
Hitoshi Togashi, Sumio Kawata

Impaired mitochondrial β-oxidation by HCV core protein causes hepatic steatosis in hepatitis C patients. We assessed mitochondrial β-oxidation and HCV core antigenemia in six patients with chronic hepatitis C by detecting serum concentrations of total ketone bodies, triglyceride, and HCV core protein. All but one (5/6) patients showed significantly lower level of total ketone bodies than normal controls at 15 hours after the start of fasting test (170.6 ± 158.4 vs 370.6 ± 70.2 μmol/L , $p < 0.05$). The result suggests that impaired mitochondrial β-oxidation exists in some or most of hepatitis C patients.

Kanzo 2007; 48: 85—86

Department of Gastroenterology, Yamagata University Faculty of Medicine
*Corresponding author: tasaitoh@med.id.yamagata-u.ac.jp