Osteopontin is an extracellular matrix protein with RGD motif mainly produced in the kidney and bone [11]. Previously we found that osteopontin was expressed in activated Kupffer cells and stellate cells in injured rat liver and contributed to migration of macrophages into the necrotic areas [12,13]. Osteopontin is shown to act also as a cytokine essential for the initiation of Th1 immune response in mice [14]. It is known that genetic polymorphisms exist in osteopontin gene (*OPN*) in mice, which determine the magnitude of immune response against bacterial infection [15]. Rickettsial infection subsides in mice with *OPN* of allele A, in which up-regulation of osteopontin expression develops in macrophages, but leads to death in mice with *OPN* of alleles B and C showing no up-regulation [15]. These observations prompted us to postulate whether similar polymorphisms in human *OPN* can determine hepatitis activity through immune response against HCV infection.

In the present study, we analyzed single nucleotide polymorphisms (SNPs) in the promoter region of *OPN* in chronic hepatitis C patients and assessed the relation of identified SNPs to hepatitis activity.

Patients and methods

Patients. Patients were 176 Japanese with chronic hepatitis C who had taken medical examination in the outpatient clinic in Saitama Medical School Hospital between 1st and 31st, August 2002, except for the following patients; (1) aged less than 40 years, (2) experienced interferon therapies with or without ribavirin administration, (3) had daily drinking habit, (4) with body mass index (BMI) greater than 25 kg/m^2. All patients were positive for HCV-RNA and negative for hepatitis B virus surface (HBs) antigen in the sera. The diagnosis of chronic hepatitis was made by histological findings on liver biopsy and/or serum biochemical tests and peripheral blood cell counts. Informed consent to gene analysis was obtained from all the patients.

Serum ALT levels were measured in all the patients until 30th, June 2003 at least for 2 years at intervals of 1–3 months, and they were classified into three groups based on the maximal levels of serum ALT as follows: lower than 30 IU/L (low-activity group), between 30 and 80 IU/L with no hepatoprotective treatment (medium-activity group), and higher than 80 IU/L irrespective of hepatoprotective treatment (high-activity group). Hepatoprotective agents included glycyrrhizin, ursodeoxycholic acid, and Japanese herbal medicine, Sho-saiko-to (TJ-9). The patients given hepatoprotective agents with the maximal levels lower than 80 IU/L were excluded from the assessment.

Measurement of serum HCV-RNA. Serum HCV-RNA levels were measured using a PCR kit (Amplicore HCV Monitor; Riche Diagnostica, Tokyo, Japan). HCV genotype was determined on the basis of sequence of the core region according to the method of Okamoto et al. [16].

SNPs analysis in the promoter region of osteopontin gene. Blood was collected from the patients and genomic DNA was extracted from peripheral blood mononuclear cells. SNPs in the promoter region of *OPN* were analyzed in 20 patients randomly selected from 176 patients by direct sequencing of DNA fragments amplified by polymerase chain reaction (PCR). Then, the identified SNPs were evaluated in 156 patients by Invader assay [17].

For direct sequencing, extracted genomic DNA was amplified in a 50 μl solution with Perkin–Elmer AmpliTag DNA polymerase (Roche Molecular System, Branchburg, NJ) and the oligonucleotide primers were determined from the sequence of the promoter region of human *OPN* [18] as shown in Table 1. The reaction mixture was kept at 94 °C for 3 min for the enzyme activation, followed by 35 amplification cycles. Each cycle consisted of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 30 s, and primer extension at 72 °C for 60 s. Then, the mixture was incubated at 72 °C for 7 min for final primer extension and the PCR products were checked by DNA agarose gel electrophoresis. Direct sequencing was performed with the use of Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin–Elmer Applied Biosystems) and 373A DNA sequencer (Applied Biosystems).

Invader assay was done according to the previous paper [19] with minor modifications. Primer probes and the Invader oligonucleotide for each SNP were designed with Invader Creator software to have theoretical annealing temperatures of 63 and 77 °C, respectively, shown in Table 2. The reactions were performed using 384-well plate Invader assay FRET detection plates (Third Wave Technologies, Maddison, WI), in which Cleavase XI enzyme, both F (FAM) dye and R (Redmond Red) dye (Epoch Biosciences, Redmond, WA), FRET cassettes, and reaction buffer were dried on each well. Three microliters of the mixtures consisting of appropriate primary probe, Invader oligonucleotide, and $MgCl_2$ was added to the wells, followed by addition of 3 μL of the heat-denatured genomic DNA (≥10 ng/μL) and overlaid with 6 μL of mineral oil (Sigma Chemical, St. Louis, MO). The plates were incubated at 63 °C for 4 h in the DNA thermocycler (RTC-200; MJ Research, Watertown, MA) and then kept at 4 °C until fluorescence measurements. The fluorescent intensities were measured on a Cytofluor 4000 fluorescence plate reader (Applied Biosystems, Foster City, CA) with excitation, 485/20 nm (Wavelength/Bandwidth) and emission, 530/25 nm for F dye detection; excitation, 560/20 nm and emission, 620/40 nm for R dye detection.

Statistical analysis. Univariate analysis was performed using StatView J-5.0 (SAS Institute, Cary, NC). Unpaired *t* test and Fisher's exact test were done for evaluation of the difference among three groups in age and sex of the patients, serum levels and genotypes of HCV-RNA, and the frequency of alleles in each SNP. Multivariate

Table 1
Primers for PCR amplification of the promoter region of osteopontin gene

Amplified regions		Primer sequences	nt
728 bp fragment	Forward	5′-TCA TCT CAA GAT GGC TGG GC-3′	−1880 to −1861
(−1880 to −1153)	Reverse	5′-GAA AAT TAC AGG GAA AGT CCG-3′	−1173 to −1153
609 bp fragment	Forward	5′-TCT GCT ATC CCT GAA TTC TGC-3′	−1203 to −1183
(−1203 to −595)	Reverse	5′-AAA GCA GTT TCT GAC TGA GAG-3′	−615 to −595
498 bp fragment	Forward	5′-GGG AAC AAG GAT AGG TAG GC-3′	−733 to −714
(−733 to −236)	Reverse	5′-GGC ATT CAG CAT CCA GGA AG-3′	−255 to −236
612 bp fragment	Forward	5′-AGC CCT CTC AAG CAG TCA TC-3′	−638 to −619
(−638 to −27)	Reverse	5′-ATG CTG CTG CAG ACA TCC TC-3′	−46 to −27

Table 2
Probes for invader assay of SNPs in the promoter region of osteopontin gene

SNPs	Types of probes	Probe sequences
nt −155	Primary probe 1	acggacgcggagCCAAAAACGCACACAC
	Primary probe 2	cgcgccgaggCAAAAACGCACACACAC
	Invader probe	CCACACTTCCCCCTCTGGTTTTGTGGTTAAAACAAAAAAAAAT
nt −443	Primary probe 1	cgcgccgaggAAACTTGCCTCTGTCC
	Primary probe 2	acggacgcggagGAACTTGCCTCTGTCC
	Invader probe	GAAGGCTATTGTTCAAGCCTGCAAGGAGTTCAGAT
nt −616	Primary probe 1	acggacgcggagAAGGATGACTGCTTGAG
	Primary probe 2	cgcgccgaggCAGGATGACTGCTTGAG
	Invader probe	GATGTTGCAGAAGTAAAGCAGTTTCTGACTGAGAGT
nt −1748	Primary probe 1	acggacgcggagGGACTTCCCTCCACTAA
	Primary probe 2	cgcgccgaggAGACTTCCCTCCACTAAA
	Invader probe	GGCACAGAGTAAACTACAGTAAATCCTGTGGAAATTTTGTTGTTTTTAGAATTT TCT

The small letter indicates the flap sequences of primary probes.

and univariate logistic regression analyses were performed using JMP 5.0.1a (SAS Institute) to select an independent factor that influences on serum ALT levels. Linkage disequilibrium among SNPs was calculated by D' and r^2 according to the method of Devlin and Risch [20].

Results

Demographic and clinical features of the patients

One hundred seventy-six patients were 83 men and 93 women aged 62.5 ± 10.8 (means ± SD) years. Genotypes of serum HCV-RNA were measured in 137 patients and showed 1b in 107 (78.1%), 2a in 21 (15.3%), and 2b in 9 (6.6%). Serum HCV-RNA levels analyzed in 155 patients were higher than 100 kIU/mL in 141 (91.0%).

Eighty-one patients were excluded from the analysis, because they received hepatoprotective treatment with the maximal ALT levels lower than 80 IU/L. There were 16 (16.8%), 19 (20%), and 60 patients (63.2%) in the low-, medium-, and high-activity groups, respectively. As shown in Table 3, men were included more frequently in the high-activity group than in the low-activity group. No differences were found in age of the patients, the genotypes, and serum levels of HCV-RNA among three groups.

SNPs in the promoter region of human osteopontin gene

Direct sequencing of DNA fragments between nt −1880 and −27 in 20 patients revealed 4 SNPs in the promoter region of *OPN*; locating at nt −155 [G/G homozygotes, G/– (deletion) heterozygotes, –/– homozygotes], −443 [C/C homozygotes, C/T heterozygotes, T/T homozygotes], −616 [T/T homozygotes, T/G heterozygotes, G/G homozygotes], and −1748 [A/A homozygotes, A/G heterozygotes, G/G homozygotes]. Prevalence of 4 SNPs including the data obtained from 156 patients by Invader assay is shown in Table 4.

Table 3
Demographic and clinical features of the patients with chronic hepatitis C classified on basis of serum ALT levels.

	Groups[a]		
	Low-activity	Medium-activity	High-activity
Number of patients:	16	19	60
Age	60.7 ± 12.1[d]	59.8 ± 11.4	61.4 ± 10.2
Male:female	4:12	9:10	34:26
Serum ALT levels (IU/L) medium (maximal–minimal)	24 (14–30)	44 (31–74)	173 (83–568)
Genotypes of HCV (1b:2a:2b:ND)[b]	10:2:1:3	12:3:1:3	35:8:6:11
HCV-RNA levels[c] (high:low:ND)[b]	15:0:1	15:3:1	51:5:4

[a] Maximal serum ALT levels were less than 30 IU/L in low-activity group, between 30 and 80 IU/L with no hepatoprotective treatment in medium-activity group, and higher than 80 IU/L irrespective of hepatoprotective treatment in the high-activity group.
[b] ND means not determined.
[c] HCV-RNA levels higher than 100 kIU/mL were defined as high, and the levels at 100 kIU/mL and lower than 100 kIU/mL as low.
[d] Mean ± SD.
* $p < 0.05$ vs low-activity group by Fisher's exact test.

Table 4
Prevalence of SNPs in the promoter region of osteopontin gene in 176 patients with chronic hepatitis C

SNPs	Number of patients (%)			
nt −155	G/G homozygotes 6 (3.5)	G/− heterozygotes[a] 70 (40.5)	−/− homozygotes[a] 97 (56.1)	ND[b] 3
nt −443	C/C homozygotes 29 (16.8)	C/T heterozygotes 88 (50.9)	T/T homozygotes 56 (32.4)	ND 3
nt −616	T/T homozygotes 6 (3.4)	T/G heterozygotes 70 (39.8)	G/G homozygotes 100 (56.8)	ND 0
nt −1748	G/G homozygotes 5 (2.8)	G/A heterozygotes 71 (40.3)	A/A homozygotes 100 (56.8)	ND 0

[a] − means deletion mutation.
[b] ND means not determined by Invader assay.

Table 5
Linkage disequilibrium coefficient (D' and r^2) among SNPs in the promoter region of osteopontin gene in patients with chronic hepatitis C

SNPs	D'			r^2		
	nt −155	nt −443	nt −616	nt −155	nt −443	nt −616
nt −443	−0.914	—	—	0.189	—	—
nt −616	0.983	−0.956	—	0.937	0.202	—
nt −1748	1.000	−0.955	0.984	0.953	0.198	0.953

Among 4 SNPs, SNPs at nt −155, −616, and 1748 showed linkage disequilibrium with D' and r^2 greater than 0.937 to each other (Table 5).

Table 6 shows the relation between hepatitis activity and 4 SNPs in the promoter region of *OPN*. SNP at nt −443 with T/T homozygotes was detected in 2 of 16 patients (12.5%) in the low-activity group. This prevalence was higher in the medium-activity group (8/19:42.1%) and in the high-activity group (25/57:43.9%) compared to the low-activity group ($p < 0.1$ and $p < 0.05$, respectively). In contrast, SNP at nt −443 with C/T heterozygotes was detected in 12 of 16 patients (75.0%) in the low-activity group, the prevalence was greater compared to the medium-activity group (8/19:42.1%) and the high-activity group (23/57:40.4%) ($p < 0.1$ and $p < 0.05$, respectively). However, there were no similar differences in SNPs at nt −155, −616, and −1748.

Multivariate and univariate logistic regression analyses were performed regarding the outcome variables between the low-activity group ($n = 16$) and the medium- and high-activity groups ($n = 69$). Age and sex of the patients, serum levels and genotypes of HCV-RNA, and alleles of SNPs at nt −155 and nt −443 were selected as predictor variables for univariate analysis. As shown in Table 7, sex of the patients and T/T homozygotes and C/T heterozygotes of SNP at nt −443 were significant variables on univariate analysis. G/− heterozygotes of SNP at nt −155 and C/T heterozygotes of SNP at −443 showing the smallest p values among alleles of each SNP on univariate analysis were selected as predictor variables, and multivariate analysis was done stepwise. Consequently, C/T heterozygotes of SNP at −443 were selected as an independent variable affecting hepatitis activity with odds ratio of 7.0876 ($p < 0.05$). Sex of patients also tended to affect hepatitis activity ($p < 0.1$).

Table 6
Maximal serum ALT levels and SNPs in the promoter region of osteopontin gene in patients with chronic hepatitis C

	Groups[a]		
	Low-activity	Medium-activity	High-activity
Number of patients:	16	19	60
SNP at nt −155 (G/G:G/−:−/−:ND)[b]	1:4:11:0	0:8:11:0	1:25:31:3
SNP at nt −443 (C/C:C/T:T/T:ND)	2:12:2:0	3:8:8:0*	9:23:25:3**
SNP at nt −616 (T/T:T/G:G/G:ND)	1:4:11:0	0:8:11:0	1:27:32:0
SNP at nt −1748 (G/G:G/A:A/A:ND)	1:4:11:0	0:8:11:0	1:27:32:0

[a] Maximal serum ALT levels were less than 30 IU/L in low-activity group, between 30 and 80 IU/L with no hepatoprotective treatment in medium-activity group, and higher than 80 IU/L irrespective of hepatoprotective treatment in the high-activity group.
[b] ND means not determined.
* $p < 0.1$ and ** $p < 0.05$ vs low-activity group by Fisher's exact test in the frequency of T/T homozygotes and C/T heterozygotes.

Table 7
Factors affecting serum ALT levels in patients with chronic hepatitis C

Variables	Parameter	SE	p value	Odds ratio (95% CI)	
Univariate logistic regression analysis					
Age (≥61:≤60)	−0.0634	0.2742	0.8172	0.8810	(0.2960–2.6200)
Sex (male:female)	0.6381	0.3100	0.0395	3.5832	(1.1377–13.6989)
HCV-RNA (low:high)[a]	−4.8606	47.8747	0.9191	0.00006	Cannot be calculated[b]
HCV genotype (2b:1b)	0.1852	0.4965	0.7092	1.4482	(0.2396–14.5213)
HCV genotype (2b:2a)	0.0280	0.6349	0.9648	1.0576	(0.0659–13.9989)
SNP −443 (C/C:others)	−0.1360	0.4094	0.7398	0.7619	(0.1107–3.2291)
SNP −443 (T/T:others)	−0.8406	0.3953	0.0335	0.1861	(0.0280–0.7276)
SNP −443 (C/T:others)	0.7356	0.3114	0.0182	4.3546	(1.3752–16.7276)
SNP −155 (−/−:others)	0.2886	0.2933	0.3252	1.7810	(0.5866–6.1075)
SNP −155 (G/−:others)	−0.4170	0.3110	0.1800	0.4343	(−1.0888–0.1595)
Multi variate logistic regression analysis (Stepwise method)					
Age (≥61:≤60)	−0.4210	0.3481	0.2265	0.4309	Cannot be calculated
Sex (male:female)	0.6603	0.3476	0.0575	3.4758	Cannot be calculated
HCV-RNA (low:high)[a]	−4.9114	42.6466	0.9083	0.0001	Cannot be calculated
SNP −443 (C/T:others)	0.9791	0.3889	0.0118	7.0876	Cannot be calculated
SNP −155 (G/−:others)	−0.3006	0.3460	0.3850	0.5481	Cannot be calculated

Outcome variable: the low-activity group ($n = 16$) vs the medium- and high-activity groups ($n = 79$).
[a] HCV-RNA levels higher than 100 kIU/mL were defined as high, and the levels at 100 kIU/mL and lower than 100 kIU/mL as low.
[b] 95% confidence interval (CI) cannot be calculated, because the parameter in serum HCV-RNA levels is unstable.

Discussion

In the present study, we analyzed the relation between polymorphisms of *OPN* and hepatitis activity in patients with chronic hepatitis C, because osteopontin is shown to be essential for initiation of Th1 immune response at the upstream of IL-18 and IL-12 in a cytokine network [14]. Genetic polymorphisms of *OPN* were determined in mice by restriction fragment length polymorphism (RFLP) survey using *Eco*RV and *Stu*I [15]. Considering that a restriction site for *Eco*RV is present within intron 5 of mouse *OPN* [21], polymorphisms in the intron may provoke diverse immunological response against rickettsial infection in mice through regulation of osteopontin expression in macrophages. In the present study, however, SNPs in the promoter region of *OPN* were analyzed, because nucleotide sequences in the introns are markedly different between human and mice [15], and the *cis*-acting enhancing elements are present in positions at nt −439 to −270, −124 to −80, and −55 to −39 in the promoter region in human [18].

To identify SNPs in the promoter region of human *OPN*, DNA fragments amplified by PCR were directly sequenced at the position between nt −1880 and −27 in 20 patients with chronic hepatitis C. As a result, 4 SNPs locating at nt −155, −443, −616, and −1748 were detected. Among them, SNPs at nt −155, −616, and −1748 had already been registered in a database of Japanese Single Nucleotide Polymorphisms (JSNP) and/or dbSNP (National Center for Biotechnology Information) as follows; SNPs at nt −155 as JST171776 and rs3841166, nt −616 as rs2853744, and nt −1748 as JST171775 and rs2728127 [22]. However, SNP at nt −443 locating at 13 bp-upstream of the *cis*-acting enhancing element was newly identified.

Four SNPs in 156 patients with chronic hepatitis C were evaluated by Invader assay, because DNA amplification by PCR is unnecessary, and thereby is convenient for SNP measurement of many samples [17]. As a result, SNPs at nt −155, −616, and −1747 showed linkage with disequilibrium with a coefficient greater than 0.9 to each other, but similar linkage was not found in the newly identified SNP at nt −443. As shown in Table 4, the frequencies of alleles in SNP at nt −443 were 16.8% for C/C homozygotes, 50.9% for C/T heterozygotes, and 32.4% for T/T homozygotes in Japanese patients with chronic hepatitis C.

In the evaluation of each SNP as a marker of hepatitis activity in chronic hepatitis C patients, hepatitis activity was based on serum ALT levels measured at intervals between 1 and 3 months at least for 2 years. The patients in whom the levels were constantly within the normal range (less than 30 IU/L) without hepatoprotective treatment were defined as the low-activity group. Although slight lymphocyte infiltration with fibrosis may exist in the liver even in HCV carriers with normal ALT levels [9,10], hepatitis activity would be less in the low-activity group than in the medium-activity or high-activity groups with abnormal ALT levels. The patients with maximal ALT levels less than 80 IU/L on hepatoprotective treatment were excluded from the evaluation, since hepatitis activity of such patients is not certain. Also, the patients with daily habit of drinking and with BMI greater than 25.0 kg/m^2 were excluded, because steatohepatitis can affect serum ALT levels. Considering that natural courses of HCV carriers differ

in age [23], the evaluation was done in patients older than 40 years.

As shown in Table 3, there were no differences in demographic and clinical features of the patients among three groups except for male prevalence in the high-activity group compared to the low-activity group. Also, multivariate logistic regression analysis revealed that sex of the patients was a variable having a tendency to affect hepatitis activity in chronic hepatitis C patients. These observations are in line with the report that HCV carriers with normal ALT levels were frequently found in women than in men [24]. It is noteworthy that the prevalence of SNP at nt −443 differed in patients between the low-activity group and the medium- and high-activity groups (Table 6). The frequency of T/T homozygotes in the medium- and high-activity groups (42.1% and 43.9%, respectively) was about 3.5 times higher than that in the low-activity group (12.5%). In contrast, C/T heterozygotes prevailed in the low-activity group; the frequency (75%) was significantly higher than that in the high-activity group (40.4%). Such difference was not found in SNPs at nt −155, −616, and −1748. On multivariate logistic regression analysis, SNP at nt −443 was selected as a significant variable reflecting hepatitis activity (Table 7). Since SNP at nt −443 is locating just at the upstream of the *cis*-acting enhancing element of human *OPN* [18], such SNP can affect the expression of osteopontin in the liver and may diverse immunological response against HCV infection. This matter should be investigated in future by promoter assay with each allele at nt −443.

The present study is the first observation of SNP in the promoter region of *OPN* associated with human diseases, as reported associations with susceptibility to multiple sclerosis [25] and lupus erythematosus [26] are shown in SNPs in the exons of *OPN*. Yee et al. [27] reported that TNF2 allele was more frequently seen in cirrhotic patients with HCV infection than in patients with less severe liver diseases. The significance of SNPs in the promoter region of *OPN* must be studied in relation to SNPs of proinflammatory cytokine genes.

In conclusion, SNP at nt −443 in the promoter region of human *OPN* may be a useful marker reflecting hepatitis activity in chronic hepatitis C patients.

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特集：九州大学病院統合1周年記念企画

移植医療の現状

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はじめに

移植の歴史は半世紀に及ぶ．末期臓器不全の究極的治療法としての臓器移植はいまや定着し，世界的には日常診療の一部になっていると言っても過言ではない．本邦でも遅々として進まなかった移植医療も生体肝移植の導入を契機としてようやく開花しつつある．

本稿では，現在の移植医療，特に我々が長年取り組んできた肝臓移植の現状と問題点について概説する．

1．世界における臓器移植の歴史と現状

臓器移植の研究及び実験は腎移植にその端緒を発する．1902年のウイーンの外科医ウルマンがイヌの腎臓を別のイヌの首に移植し報告したのが最初である．1954年に米国のメリルとマレーらが一卵性双生児間の腎臓移植に成功し，生物学的に等しい個体である一卵性双生児の間では移植臓器が拒絶されないことが実証された．1961年にイギリスのカーンらがイヌの腎移植でアザチオプリンの免疫抑制効果を確認，1963年にマレーがヒトの腎移植に応用した．1963年にはスターツルが世界初の肝臓移植を胆道閉鎖症の患児に行った．同様に1963年に肺移植，1966年に膵臓移植，1967年に心臓移植が次々と行われた．しかし，拒絶反応の制御は困難であり一時臓器移植は急速に退潮した．

臓器移植の決定的なブレークスルーは新しい免疫抑制剤サイクロスポリンの発見である．これは1970年にスイスのサンド社の社員ボレルがノルウェーの土壌から採取したカビから分離した物質であり，1972年にその強力な免疫抑制作用を発見した．カーンが1978年に腎移植に，1979年に肝移植に応用した．1980年にスターツルが肝移植に，シャムウェイが心臓移植に応用し，1年生存率が80％と驚異的な成績を報告し，以後飛躍的に症例数が増加し臓器移植が末期臓器不全に対する外科治療として確立されるに至った．UNOS（United Network for Organ Sharing）の最新のデータによれば，2003年には全米で6,457例の脳死（又は心停止）ドナーからの臓器提供が行われ，5,263例（肝），9,765例（腎），1,381例（膵），111例（小腸），2,084例（心）の移植がそれぞれ行われた．

一方で移植待機患者の急速な増加に対して臓器提供が追いつかず，臓器不足が深刻化し大きな問題となっている．その解決手段として，健康な肉親などが臓器の一部を提供する生体腎移植，生体肝移植，生体肺移植などの方法が開発され，脳死ドナーの望めない日本などを中心に爆発的に症例数が増加している．本邦における生体肝移植の施行数は現在，年間400例以上通算でも3000例を超え，成人間生体肝移植も積極的に施行されるようになってきた．

一方，本邦においては1997年臓器移植法が施行されて以来2004年4月までの脳死肝移植数は計24例に過ぎないのが現状であり，脳死ドナーからの提供がほとんど機能していないことが我が国の移植医療の最大の問題点であるといえる．

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2．九州大学における肝移植の現状

当教室においては，1985年に移植研究室が発足した．当初肝臓・血管・門亢症の各研究室からのメンバーで構成され，発足直後より臨床肝移植を目的とした大動物実験を開始し，同時に国内の他の施設よりいち早くピッツバーグ大学やマウントサイナイ医科大学に継続的に教室員を派遣し臨床トレーニングを重ねた．

一方国内における移植医療のシステム作りとして，1988年に西日本臓器移植ネットワークを設立し，西日本地区における臓器移植医療の確立と定着のための基盤整備も行ってきた．移植施設だけではなくドナー病院の登録を進め，脳死ドナー発生からの移植シミュレーションを行った．

1990年には肝移植の技術を応用した体外肝切除を我が国で初めて施行し，九州大学医学部倫理委員会が1991年に生体肝移植を，1993年には脳死肝移植を承認後，1993年10月22日に我が国では実に29年ぶりとなる心停止ドナーからの肝移植を施行した(血液型不適合症例)．1996年10月14日には1例目の生体肝移植を施行．1997年5月2日には初の成人間生体肝移植を施行，いずれも成功をおさめた．1999年7月26日には本邦2例目のドミノ生体肝移植を行った．1997年10月脳死移植法案が可決され，2000年には脳死肝移植認定施設に認定された．2001年には九州大学における脳死肝移植が高度先進医療として認可された．そして2003年10月7日，大分県在住の34才男性に対し，九州初，本邦23例目となる初の脳死肝移植が行われた．術後経過は極めて順調で特に合併症なく術後29日目に無事退院となり，現在も元気に外来通院中である（図1，2，3に経過を示す)．

生体肝移植については，1996年10月14日に胆道閉鎖症の7才男児に対し1例目を施行して以来，2004年9月までの8年間に小児23例，成人141例の合計164例の生体肝移植を施行した．図4に示すごとく，ここ数年の症例，特に成人間生体肝移植の延びは際立っており，コンスタントに年間30例以上を施行するようになり年間症例数では京都大学，東京大学につぎいまや本邦第3位を占めるようになった．九州大学での生体肝移植はいまや日常診療のひとつになったといえる．

手技的にはほぼ確立されたといえる生体肝移植であるが，今後の課題として，脳死肝移植のさらなる推進，成人間生体肝移植の成績向上，進行肝癌に対する適応拡大・再発予防，C型肝炎再発予防法の開発，過小グラフトに対する対策，血液型不適合移植，胆管合併症（特に長期胆管狭窄）に対する対策などがあげられる．そこで最近急速に症例数が増加しつつあるC型肝炎と肝癌に対する肝移植の現状を概説する．

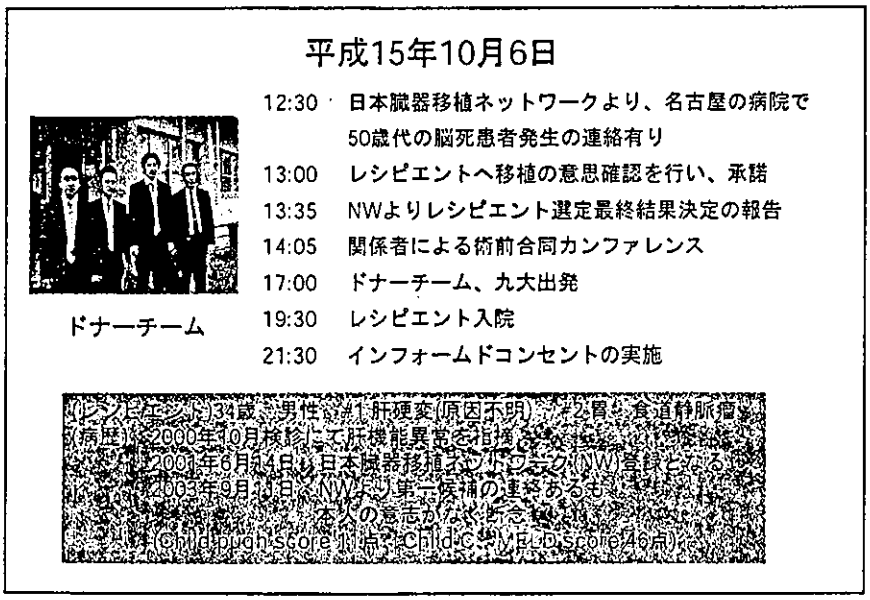

図1 九州大学初の脳死肝移植症例：第1日目

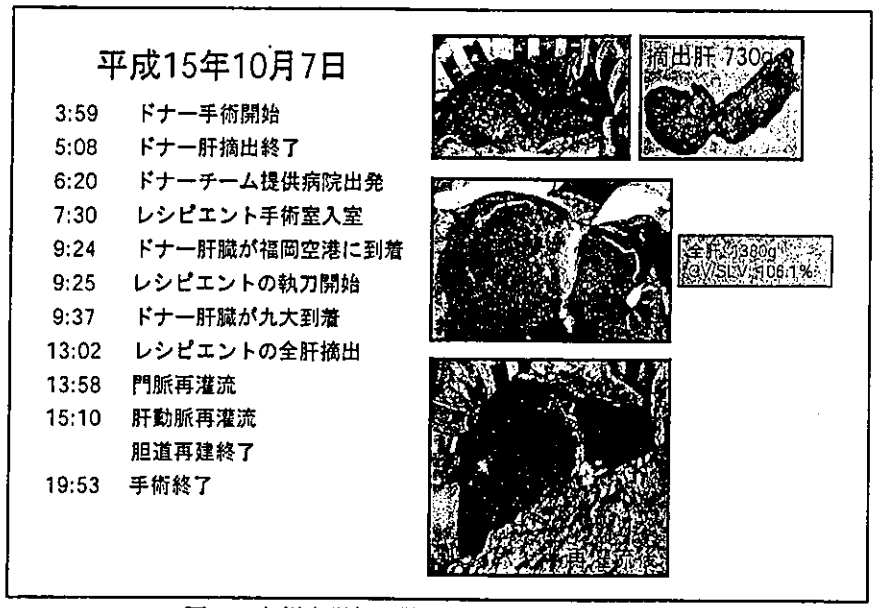

図 2 九州大学初の脳死肝移植症例：第 2 日目

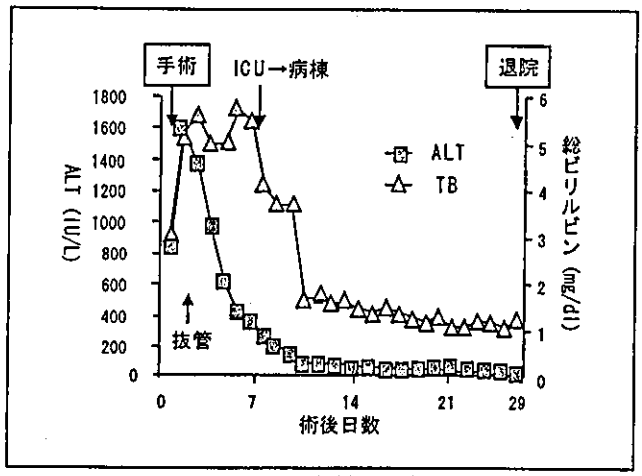

図 3 脳死肝移植の術後経過

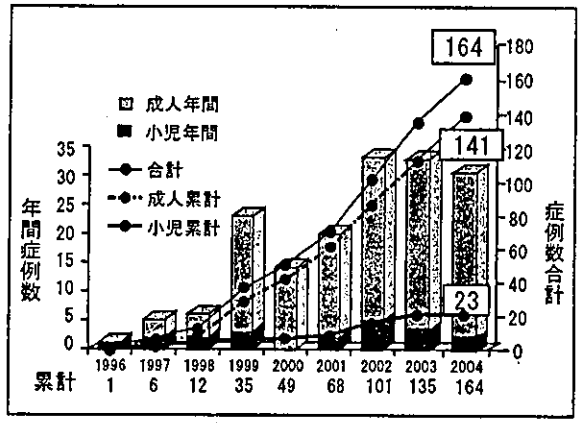

図 4 九州大学における肝移植症例数
(1996.10～2004.9)

（1）C型肝炎に対する肝移植

アメリカではC型肝炎はアルコール性肝硬変と並んで，脳死肝移植におけるもっとも頻度の高い適応症であり，本邦でも今後症例数の増加が予想される．C型肝硬変の移植ではほとんど全例で術後早期にHCV-RNAが検出され，ほぼ全例にウイルス学的再発は起こる[1]．その臨床経過はB型肝炎再発にくらべて比較的緩徐である．最近C型肝炎患者の移植成績が以前に比較して悪化していることが報告され[2]大きな話題をよんでいる．移植後10年以上を追跡観察した最近の報告によれば，1，5，10年の患者生存率は84，68，68％であった[1]．九州大学では2004年9月までに164例中54例（31.1％）のC型肝硬変症例（うち肝癌合併41例）に対して生体肝移植を施行している（図5）．肝移植後のC型肝炎再発予防にはIFNとRibavirinが一般的に用いられるが，ウイルス学的著効に至るのは12～30％に過ぎず，C型肝炎再発については未だ満足できる治療がないのが現状である．肝癌合併を含めC型肝硬変症例51例の生存率は1年79％，2年67％，3年67％であり，ウイルス学的には術前HCV RNA陰性であった2例を除き，49例に術後HCVRNAを検出した．組織学的再発は16例（32％）に認めた．累積再発率は6ヶ月29％，1年35％，3年39％であった．再発症例のうち12例では，インターフェロンα＋リバビリンにて治療を行い，ウイルス消失が持続したのは5例のみで，6例は副作用（白血球数減少，全身倦怠感，貧血等）により治療を中断した．無効症例が1例であった．このように現在のところC型肝炎再発に対する治療に関し

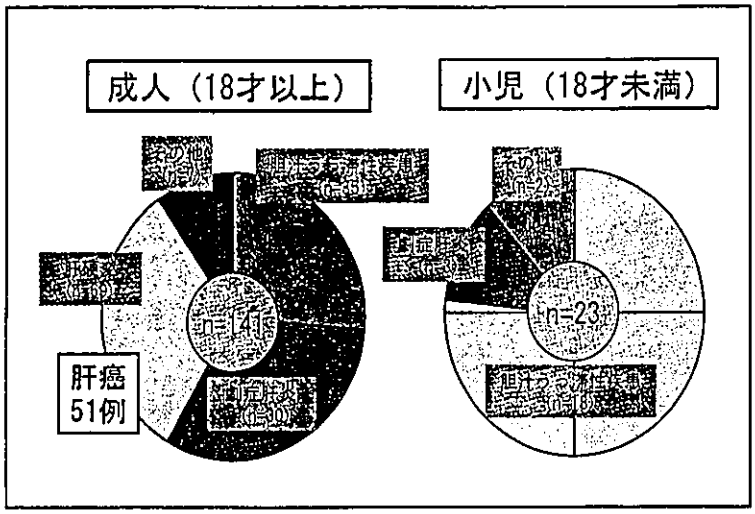

図5 九州大学における生体肝移植：適応疾患（164例：1996.10～2004.9）

ては満足な結果が得られていないのが現状である．

（2）肝癌に対する肝移植

肝癌に対する肝移植は，肝癌とともに肝癌の発生母地であるB型，C型肝硬変も同時に摘出し得ることからもっとも究極的な根治療法と考えられる．現在海外における肝癌に対する脳死肝移植登録基準は，(1)肝外，リンパ節転移がないこと，(2)血管浸潤がないこと，(3)単発：5 cm以下，多発：3 cm以下で3個以内（ミラノ基準[9]）を満足することである．

このような移植適応の厳格化により5年生存率は85％，無再発生存率は90％と良性肝疾患とほぼ同等の移植成績を示しており，我が国でも脳死登録の基準として，あるいは生体肝移植の保険適応基準として採用されている．

肝癌に対する生体肝移植においては健常ドナーからの臓器提供が必要なため適応基準をより厳格にすべきとの考えと，必然的に臓器提供は親族からに限定されるため双方がリスクを十分に理解した上でならより適応を拡大してもいいとの考えがある．現実的には我が国のほとんどの施設での適応は，肝移植以外の治療法で腫瘍がコントロール出来ず，肝外転移及び腫瘍血管への浸潤がない肝癌とされており，腫瘍径や腫瘍個数には制限を設けていない．

当科における肝癌への移植適応基準は肝外病巣がなく，肝内主要血管への明らかな腫瘍の侵襲を認めず，肝移植以外に有効な治療法が現存しない場合である．ただし，肝移植後の長期予後が他の治療法より明らかに上回ると予想される場合には個々の症例につき検討し適応を判断するとしている．

九州大学ではこれまで164例中50例（30.5％）の肝細胞癌に対する生体肝移植を施行した．背景にある肝硬変の原因としてはHCV 40例，HBV 6例，NonBNonC 4例であった．また術前のChild分類ではChildCが最多であったが，腫瘍が両葉，多発性に分布した症例等はChildAでも適応となる場合があった．このため，Stage IIIが大半を占めた．肝癌症例における1年，2年生存率は86.2％，68.4％であった．50例中7例に再発を認めた．

これまでは治療不能とされていた肝細胞癌も症例によっては肝移植によって根治させることが可能となってきた．今後はC型肝炎再発への対策等課題は多いものの保険適応となった現状を踏まえ，よりいっそう移植治療の必要性が高まるものと考えられる．

3．再生医療の現状と展望 —肝，肺について—

移植医療が急速に発展する中，細胞・臓器の供給不足，ドナー不足は深刻な問題であり，現在その新た

な細胞・臓器の供給源として，またそれに代替しうる治療として，幹細胞研究をはじめとする再生医療に大きな期待が寄せられている．

われわれ腹部外科・胸部外科での移植医療の領域においては，現実の医療となった肝移植や肺移植の見地からも，特に肝や肺の再生医学に注目するところである．胚性幹細胞（ES 細胞）や体性幹細胞からの肝や肺の細胞・組織への分化や，その発生におけるメカニズムに関する研究は近年飛躍的に進歩しているが，その成熟細胞への特異的な分化や組織の再構築に関しては未だ確立しておらず試験的なものも含めて臨床応用には至っていないのが現状である．

肝については，肝幹細胞とも考えられている oval cell や小型肝細胞の存在が示されており，これらから成熟肝細胞への分化が報告されている[4]．また ES 細胞から肝細胞への分化に関する研究も急速に進んでおり，肝の細胞レベルでの再生は可能となってきている．しかしながら，その特異的な分化誘導や組織の再構築，細胞数の獲得に関しては未だ確立しておらず，臨床応用までにはもう少し時間が必要と考えられる．

一方，肝のように求められる最低限の機能が細胞レベルでも良い臓器に比べ，肺は組織・臓器レベルでの高度な機能が要求されるため，その再生の実現は非常に困難と考えられる．この分野においては発生についての研究は多臓器同様，近年急速に発展しており，そのメカニズムの解明も進んでいるが，再生についてはその機能の複雑さもあってか，ES 細胞から分化した細胞集団に肺胞上皮特異的なサーファクタント C の産生を認めた報告[5]などはあるものの，具体的な再生の研究は始まったばかりであり今後の発展が切望される．

現在再生医療は，造血，血管，心臓などの一部の分野においてのみ臨床応用が実現しているが，再生医療が組織・臓器の機能障害や機能不全，欠損による疾患に及ぼすその恩恵は計り知れず，幅広い分野においてその実現に大きな期待が寄せられている．また同時にその研究，医療を進めていくうえでは，多くの社会的，倫理的な議論やコンセンサスが必須である．

おわりに

手技的な問題点はほぼクリアされたと思われる我が国の移植医療であるが，やはり脳死ドナーからの臓器提供が移植医療の本道であるのは疑いがない．今後，脳死法案の改訂などの基盤整備やさらなる啓蒙活動により，脳死ドナーの増加をはかることが，我が国の健全な移植医療の発展のためには必須であろう．

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症　例

C型肝硬変に対する生体肝移植後に急速な経過をたどり死亡したfibrosing cholestatic hepatitisの1例

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ウイルス性肝硬変に対する肝移植は近年増加傾向にある．C型肝硬変に対する移植の問題点は移植後のC型肝炎の再発であり，術後1年以内に50～60%が組織学的に慢性活動性肝炎を再発し，術後5年で約20%が肝硬変に進行するとされている．C型肝炎の場合，肝炎の再発はほとんどが軽度であり，緩徐な経過が特徴とされている．一方，fibrosing cholestatic hepatitis (FCH) は組織学的に胆汁うっ滞，門脈周囲の線維化，肝細胞腫大，軽度な炎症などの特徴を認め短期間で肝硬変に進行し，主にB型肝硬変における移植後の肝炎再発形式とされている．近年，C型肝硬変に対する移植後にもFCHが認められ，近年増加傾向にあるとされている．今回われわれはC型肝硬変に対する生体肝移植術後に高ビリルビン血症を認め組織学的に急速な線維化をきたし術後233日目にグラフト機能不全で死亡したFCH症例を経験したので報告する．

索引用語：生体肝移植，C型肝炎，fibrosing cholestatic hepatitis(FCH)

緒　言

C型肝炎に対する肝移植後の問題点は高率に肝炎が再発することである．一般に，C型肝炎の再発は軽度で経過が緩徐であるとされている[1)~3)]．一方，fibrosing cholestatic hepatitis (FCH) は急速に肝硬変へと進行する病態であり，主にB型肝炎に対する移植後に認められる．しかしながら稀ではあるが近年C型肝炎に対する移植後のFCHが報告されている[3)4)]．当科では，HCV陽性患者に対してこれまでに生体肝移植を51例施行しており，今回われわれは生体肝移植術後に，FCHを発症し急速な経過をたどった症例を経験したので報告する．

症　例

症例：40歳，男性．

主訴：全身倦怠感，腹部膨満．

既往歴，家族歴：特記すべき事項なし．

現病歴：1997年，腹部膨満を自覚し，近医を受診した際腹水を認めた．HCV陽性および肝硬変を指摘され，上部消化管内視鏡にて食道静脈瘤を認め内視鏡下静脈瘤結紮術を施行された．その後，利尿剤の内服にて経過観察されていた．2001年8月，再び腹部膨満感が出現．近医入院の上，腹水コントロールを行い3週間で退院した．2002年10月，腹水コントロール目的で再入院．精査にて著明な肝機能の低下を認めた．11月に感冒を契機に肝機能が悪化し，生体肝移植目的で当院を紹介され，12月10日，適応評価目的にて当科入院となった．

入院時現症：黄疸・腹水著明，脾腫あり，肝性脳症Ⅰ度．

入院時検査所見：血液型AB型Rh(+)，〈血算〉WBC 17,380/mm³，RBC 292/mm³，Hb 10.3g/dl，Ht 29.0%，Plt 13.3万/mm³，〈生化学〉TP 6.1g/dl，Alb 3.2g/dl，BUN 51mg/dl，Cr 1.81mg/dl，Ccr 29.0ml/min，TB 11.1mg/dl，DB 8.1mg/dl，TTT 6.8KU，ZTT 13.0KU，AST 94IU/l，ALT 49IU/l，LDH 242IU/l，ChE 15IU/l，Na 125mEq/l，K 5.1mEq/l，Cl 95mEq/l．T-chol 43mg/dl，TBA 241.5μmol/l，NH_3 42μg/dl．〈凝固系〉PT 16.8 (11.2) sec，PT% 46%，HPT 35%．〈ウイルスマーカー〉IgM-HA(−)，HBsAg(−)，HBsAb(−)，HBeAg(−)，HBeAb(−)，

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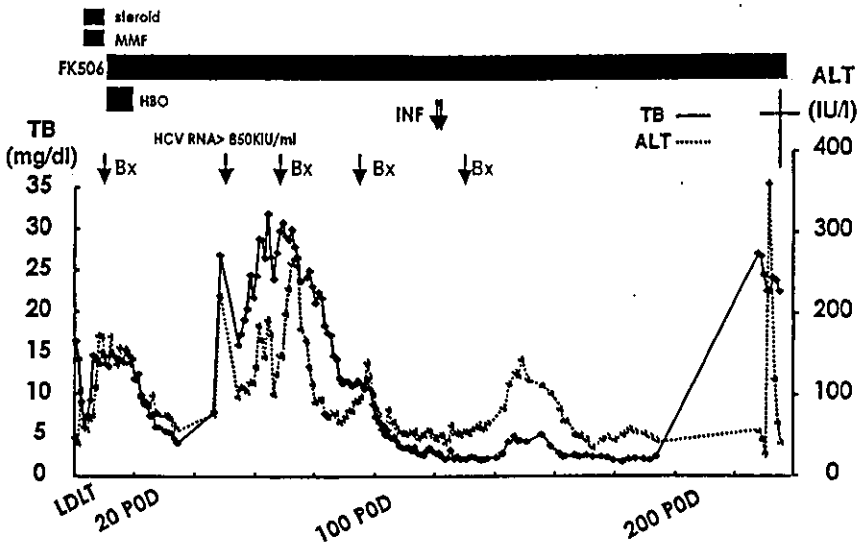

図1　肝移植後の経過(MMF：ミコフェノール酸モフェティル，HBO：高圧酸素療法，INF：インターフェロン，Bx：肝生検，TB：総ビリルビン，LDLT：生体肝移植，POD：術後日数)

HBcAb(−)，HBV DNA(−)，HCV Ab(+)，AMA(−)，ANA(−)．〈腫瘍マーカー〉AFP 6.2ng/ml，PIVKA 17mAU/ml．

入院時画像所見：肝萎縮，著明な脾腫，大量の腹水あり．腫瘍性病変やシャントは認めず．

手術：2003年1月7日，39歳の実弟(A型 Rh+，適合)をドナーとして生体肝移植術を施行．拡大左葉+尾状葉グラフト，グラフト重量370g，GV/SLV＝33.6%，GRWR＝0.73%，手術時間；12時間11分，出血量；11,486g．

術後経過：免疫抑制剤は，ステロイド，ミコフェノール酸モフェチル，バシリキシマブの3剤で導入．HCV 陽性のためステロイドは1週間で中止し，以後FK 506の投与のみとした(図1)．術後7日目よりビリルビン，トランスアミナーゼの上昇を認め臨床的にsmall-for-size graft syndrome と診断し，高圧酸素療法を開始した．ビリルビン(2～3 mg/dl)，トランスアミナーゼが正常化し術後34日目に退院となった．術後52日目より再度ビリルビン(15.9mg/dl)，トランスアミナーゼの再上昇を認めた．術後55日目の HCV RNA は検出限界の上限を超えており(HCV RNA> 850KIU/ml)，C型肝炎の再発が疑われた．術後76日目の肝生検(術後3回目)にて，門脈域周囲の偽胆管の増生と，軽度の炎症細胞浸潤，中心静脈周囲の胆汁うっ滞とうっ血および軽度の肝細胞の腫大を認め，軽度の肝炎の診断にて肝庇護剤の投与を施行(図2)．その後，ビリルビン，トランスアミナーゼは低下したが再上昇を認めたため術後97日目に肝生検(術後4回目)を施行した．門脈域周囲の偽胆管増生，炎症細胞浸潤，中心静脈周囲の肝細胞の腫大，脂肪化，好酸性小体を認め，C型肝炎の再発に伴う肝炎の所見であった(図3)．その後トランスアミナーゼは軽度上昇程度まで下がったが，白血球減少のため INF＋リバビリン療法が施行できず低容量 IFN 療法(300万 IU×1/day)を施行した．しかし，白血球減少が進行し，2回施行後に中止した．術後135日目(5回目)の肝生検では，門脈域周囲の偽胆管増生と炎症細胞浸潤を認め，マッソン・トリクローム染色では架橋線維化を認めた(図4)．その後，腹水の増加を認め肝不全となり術後233日目に死亡した．死亡時の肝組織は広範性壊死を伴った肝硬変の所見であった(図5)．

考　察

C型肝硬変症例に対する肝移植の問題点は，移植後の肝炎の再発である．術後1年以内に50～60%が組織学的に慢性活動性肝炎を呈し，術後5年で約20%が肝硬変に進行することが知られている．長期的には肝炎再発はほぼ全例に起こると考えられている[1)~3)]．一方，本症例のように，移植後急速に肝硬変へと進行する FCH は本来肝移植後のB型肝炎の再発形式と考えられ，急速に肝硬変へと進行し肝不全に至る肝炎として1991年に Davies ら[4)]によって報告されている．組織学的には胆汁うっ滞と門脈域周囲から小葉内へと進展する線維化と胆汁うっ滞を特徴とする肝炎であるが，炎症細胞浸潤は比較的軽度とされている[4)]．血清学的に

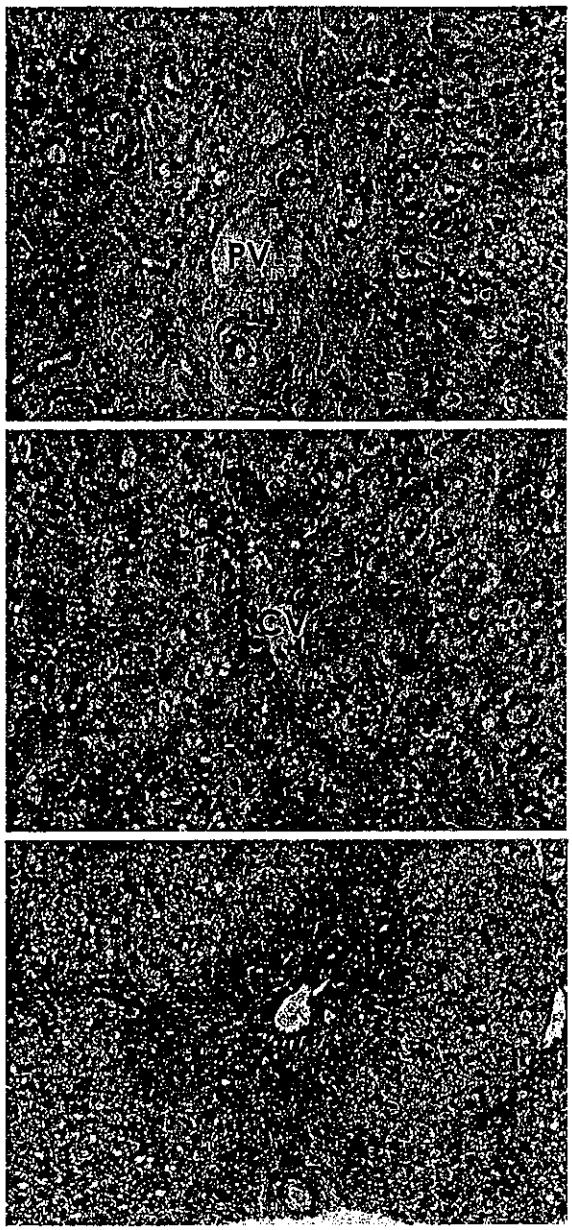

図2　肝生検病理組織標本(術後76日目)：a)門脈域辺縁部での偽胆管増生，炎症細胞浸潤は軽度(H-E染色，×100)．b)中心静脈周囲での胆汁うっ滞，うっ血，軽度の肝細胞の腫大(H-E染色，×100)．c)門脈域から小葉内へ進展する網目状の線維化(Masson's trichrome染色，×40)．

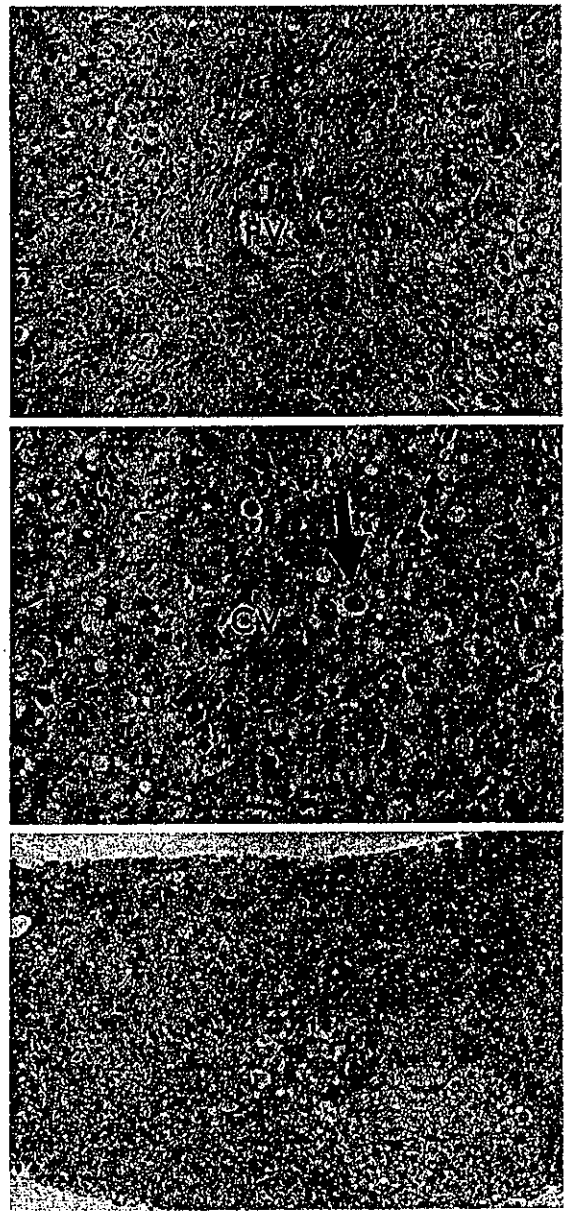

図3　肝生検病理組織標本(術後97日目)：a)門脈域周囲での偽胆管増生，炎症細胞浸潤は強い(H-E染色，×100)．b)中心静脈周囲での肝細胞の腫大，脂肪化，好酸性小体(H-E染色，×100)．c)門脈域から小葉内へ進展する線維化の増強(Masson's trichrome染色，×40)．

は肝機能低下に伴うプロトロンビン時間の延長，アルカリフォスファターゼの上昇，比較的低い値のALTである[6]．臨床的特徴は免疫抑制状態，つまり臓器移植後での免疫抑制剤投与下や，HIV陽性患者において発症しやすく，急速に肝の線維化が進行し肝硬変に至り肝不全に陥ることである[6)~9)]．FCHはHBV陽性患者における肝移植後の肝炎再発時の特徴とされてきたが，最近FCHが臓器移植後のHCVの再発の際に認められ注目されている[10)11)]．特にHCV陽性患者のFCHは，腎もしくは肝移植後の1～9％に認められその頻度は最近増加傾向にあるといわれている[3)12)]．HCVにおけるFCH発症にも免疫不全状態が関与し

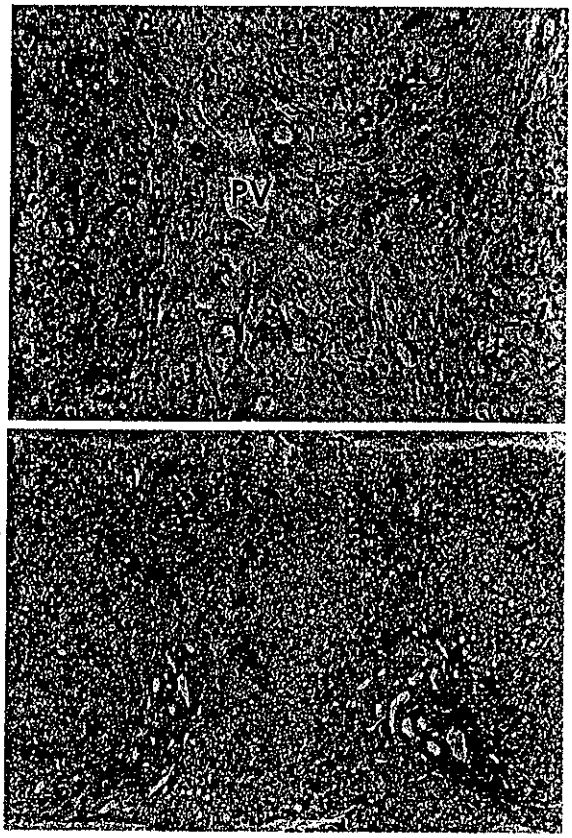

図4　肝生検病理組織標本（術後135日目）：a）門脈域周囲での偽胆管増生，炎症像（H-E染色，×100）．b）架橋線維化（Masson's trichrome染色，×40）．
a
b

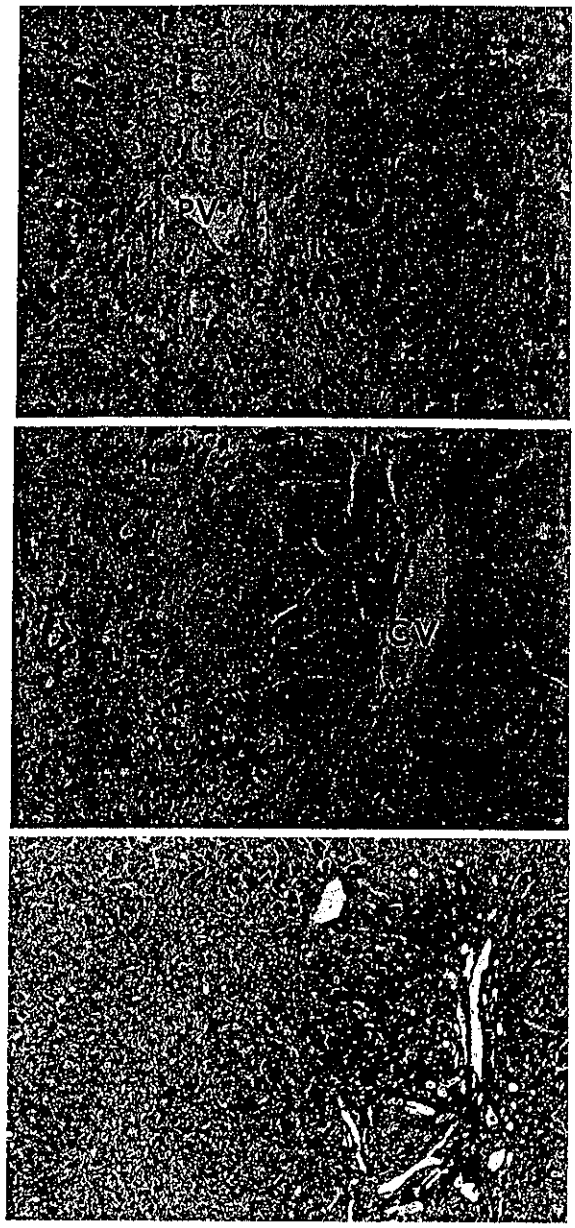

図5　死亡時肝病理組織標本（術後233日目）：a）門脈域周囲での偽胆管増生，軽度～中等度のリンパ球を主体とした炎症細胞浸潤（H-E染色，×100）．b）広範性肝壊死，高度の線維化（H-E染色，×100）．c）小葉構築を破壊する高度の線維化（Masson's trichrome染色，×40）．
a
b
c

ており，HBVと同様に臓器移植後，HIV陽性患者にも認められ，拒絶の治療をはじめ，CMV感染や胆汁鬱滞がリスクファクターである[13]．FCHの治療として再移植が挙げられるが，再移植後も再発することも多くドナーの有無の問題があり実際には行われないのが現状である[14]．したがって，早期発見が重要であり，肝機能が低下する前にIFN＋リバビリン療法を施行することでグラフト不全を回避できる可能性がある[15]．今回，われわれが経験した症例では，HCV RNA検査では術後早期（術後55日目）に高値となり，組織学的にも術後76日目の肝生検で既に，FCHを示唆する所見を認めた．しかし，肝機能不良のためIFN＋リバビリン療法が施行できず，低容量IFN療法を試みるも開始3日目で好中球減少をきたし治療継続困難であった．C型肝硬変に対する肝移植術後は肝炎の再発は必発であり，FCHのように急速にグラフト不全をきたす症例もあるため，早期診断には定期的な肝生検と予防的なIFN＋リバビリン療法の施行が安全かつ効果的に行えるプロトコールの作成が今後の課題である．肝炎再発の予防や再発後の効果的な治療法が切望される．

結　語

HCV陽性患者における生体肝移植術後にFCHを認めた場合，早期にグラフト不全をきたす可能性があることに十分留意すべきである．したがって，FCHに

対し，肝機能が低下する前にIFN＋リバビリン療法を行えるように肝移植後に高ビリルビン血症を認めた場合，肝生検を行い早期診断に努めるべきである。

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RAPIDLY PROGRESSED FIBROSING CHOLESTATIC HEPATITIS AFTER LIVING DONOR LIVER TRANSPLANTATION FOR HCV-RELATED CIRRHOSIS—REPORT OF A CASE—

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Liver transplantation (LTx) for liver cirrhosis caused by hepatitis C virus is increasing in recent years. The problem of LTx for cirrhosis due to hepatitis C is the reactivation of hepatitis C virus after transplantation. Approximately 50-60% of patients will develop chronic active hepatitis within one year after transplantation. In addition, 20% of those patients develop liver cirrhosis within five years. Unlike hepatitis B, recurrent hepatitis C is usually mild and is characterized by gradual progress. On the other hand, fibrosing cholestatic hepatitis (FCH), a specific histologic manifestation of hepatitis B virus infection, is characterized by periportal fibrosis, hepatocyte ballooning, cholestasis and relatively scant inflammation. FCH will rapidly progress to liver cirrhosis for a short period of time. In recent years, FCH has been increasingly reported to occur after LTx for hepatitis C cirrhosis. Herein, we report a case of FCH after living-donor LTx for hepatitis C, which led to a rapid graft loss on POD 233.

Alternatives to the Double Vena Cava Method in Partial Liver Transplantation

Yoji Kishi,[1] Yasuhiko Sugawara,[1] Yuichi Matsui,[1] Nobuhisa Akamatsu,[1] Noboru Motomura,[2] Shinichi Takamoto,[2] and Masatoshi Makuuchi[1]

Minimizing graft congestion in partial liver transplantation is important, especially when the graft weight is marginal for the recipient metabolic demand. We prefer the double vena cava technique for reconstructing middle hepatic vein tributaries with thick, short hepatic veins because the technique can reduce the warm ischemic time of the graft and make a wide anastomosis. This technique requires a cryopreserved superior or inferior vena cava. We devised an alternative double vena cava method using iliac or femoral vein grafts and applied it to two right liver transplantation patients. There was no postoperative hepatic venous outflow block in either patient. In conclusion, application of this technique, even in the absence of a suitable vena cava, can help to minimize graft congestion. (*Liver Transpl 2005;11:101–103.*)

Adequate outflow is indispensable for graft function. Hepatic vein reconstruction for adequate outflow, however, is technically demanding in partial liver transplantation because of eventual twisting or compression by the regenerated liver graft.[1] The double vena cava (VC) technique is indicated in a right liver graft if the graft includes major short hepatic veins.[2] Application of this technique, however, depends on the availability of a VC graft. Here we introduce two alternative methods to the double VC technique using venous grafts of smaller diameter.

Patients and Methods

From January 1996 to June 2004, 221 adult patients underwent living donor liver transplantation (LDLT) using a right liver graft at our hospital. The mean follow-up period was 850 days. The indications for LDLT in these patients included hepatitis C virus cirrhosis (n=54), primary biliary cirrhosis (n=50), hepatitis B virus cirrhosis (n=38), fulminant hepatic failure (n=25), cryptogenic cirrhosis (n=17), biliary atresia (n=14), metabolic disorder (n=6), primary sclerosing cholangitis (n=9), and autoimmune hepatitis (n=8). Details regarding the selection criteria and evaluation are described elsewhere.[3,4] All donors and patients provided written informed consent.

Homologous Vein Graft Preparation

Vein grafts were provided by the University of Tokyo Tissue Bank. The preservation and thawing methods have been previously described.[5] In brief, the vein grafts were obtained in a sterile manner from cadavers within 24 hours after cardiac arrest after obtaining informed consent. The specimens were frozen slowly in a programmable freezer at a rate of 1°C/min to -40°C and stored in liquid nitrogen until use. The cryopreservation medium consisted of Rosewell Park Memorial Institute 1640 solution (Whittaker Co., Sydney, Australia), 10% dimethylsulfoxide (Sigma, St. Louis, MO), and .5 g/L cefazolin sodium (Fujisawa, Tokyo, Japan).

For use, the packed vein grafts were placed at room temperature for 7 minutes and immersed gently in 37°C sterile saline for 30 minutes. Thereafter, the vein grafts were picked up from the bag and placed into the Alloflow (Lifenet, Virginia Beach, VA). Finally, they were rinsed with 1 liter of lactated ringer's solution (Lactec G, Ohtsuka Pharmaceutical, Tokyo, Japan).

Right Liver Harvesting

The right liver was harvested as described previously.[4] Briefly, in a basin, the graft was flushed with 1 liter of University of Wisconsin solution through a cannula inserted into the right portal vein. When the graft included major short hepatic veins, including inferior or middle right hepatic veins in the graft, the double VC technique was applied as described previously.[6] Briefly, a cryopreserved VC graft was prepared in a basin. A side hole was made in the wall of the VC, which was anastomosed with the hepatic veins in the graft. With this technique, all hepatic vein trunks of the recipient were sutured at their roots. Then, the inferior VC of the recipient was partially clamped and incised approximately 5 cm longi-

Abbreviations: LDLT, living donor liver transplantation; VC, vena cava.

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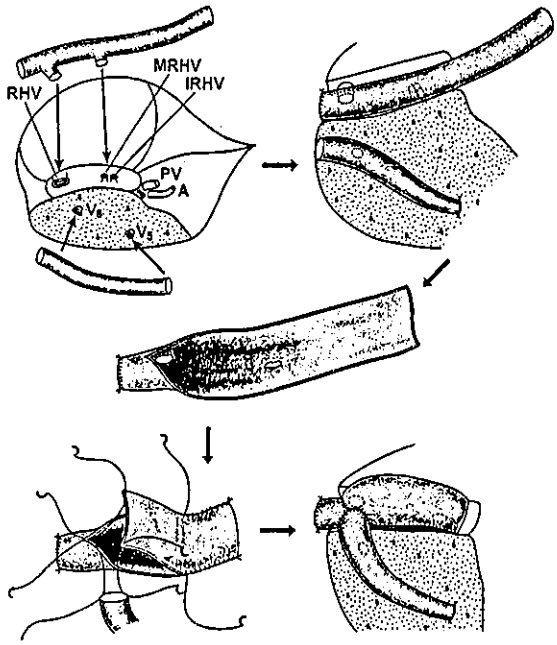

Figure 1. A Method Using Two Iliac Veins. One iliac vein was used to reconstruct a V8 and a V5. The other vein graft was folded at its caudal end. Abbreviations: RHV, right hepatic vein; MRHV, middle right hepatic vein; IRHV, inferior right hepatic vein; V5, drainage vein from segment V; V8, drainage vein from segment VIII; PV, portal vein; A, hepatic artery.

tudinally. The VC graft was similarly incised longitudinally, then anastomosed side-to-side with the inferior VC of the recipient. When a VC graft was not available, iliac or femoral vein grafts were used.

Results

Of 105 right liver grafts, 35 had major short hepatic veins. Of these 35 patients, 2 received two iliac veins and two femoral veins, respectively, as an alternative to VC grafts for the double VC technique.

Patient 1

The patient was a 61-year-old male with hepatitis C virus cirrhosis with a 2 cm diameter nodule of hepatocellular carcinoma. The right liver graft was harvested from his 56-year-old wife. The graft had a middle and inferior right hepatic vein. A VC graft was not available and two iliac veins (76 mm and 55 mm in length, respectively) were used for venous reconstruction at the bench (Figure 1).

One iliac vein was used for reconstruction of the middle hepatic vein tributaries (V5 and V8). The other iliac vein graft was used as an alternative to a VC graft. Its cranial end was closed. Two branches were anastomosed to the orifice of the right hepatic vein and orifices of the middle and inferior right hepatic veins together. The vein graft was 10 mm in diameter, which was too small for direct side-to-side anastomosis with the recipient's inferior VC. The iliac vein was incised longitudinally and the caudal side was folded. The two iliac veins were finally anastomosed side to end.

On the recipient side, all stumps of the hepatic veins were closed at their roots. The recipient inferior VC was semiclamped and incised longitudinally 5 cm in length. The folded iliac vein was incised similarly and sutured side-to-side. Cold and warm ischemic times were 140 minutes and 55 minutes, respectively. The postoperative course was uneventful. There were no vascular complications and Doppler ultrasonography 6 months after transplantation revealed well-maintained flow of all reconstructed veins.

Patient 2

A 44-year-old man underwent LDLT for alcoholic liver cirrhosis. The donor was his 42-year-old wife. A right liver graft with middle hepatic vein trunk was indicated.[7] The weight of the graft was 454 g, which corresponded to 36% of the recipient standard liver volume,[8] leading us to perform venous reconstruction at the bench. Two cryopreserved femoral vein grafts (each 100 mm long) were available.

Five hepatic vein orifices appeared on the inferior VC sulcus of the harvested graft (Figure 2). Two fem-

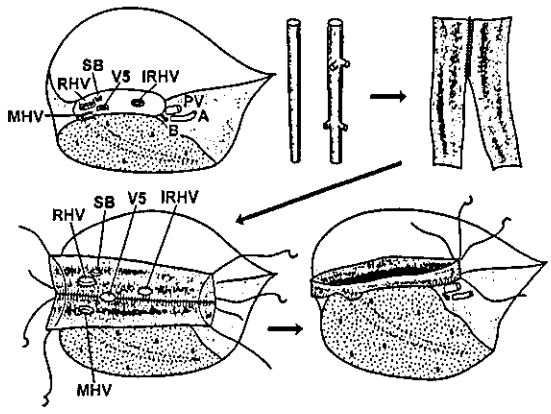

Figure 2. A Method Using Two Femoral Veins. One large rectangular sheet was made, which was sutured to five vein orifices. Abbreviations: MHV, middle hepatic vein; RHV, right hepatic vein; SB, superficial branch; IRHV, inferior right hepatic vein; V5, drainage vein from segment V; B, bile duct; PV, portal vein; A, hepatic artery.

oral veins were cut longitudinally and the two sheets were made into one sheet using interrupted sutures. The V5 orifice was sutured to the medial side of both sheets. Small holes were made on the sheet for anastomosis with the other four stumps of the short hepatic veins. The cranial and caudal ends of the sheet were then sutured to make a new "vena cava." On the recipient side, the inferior VC was cut longitudinally and sutured to the graft-side VC. Cold and warm ischemic times of the graft were 181 minutes and 85 minutes, respectively. Doppler ultrasonography revealed a well-maintained venous flow for 3 months after the operation. The postoperative course was uneventful except for surgical drainage of bile leakage on the fifteenth postoperative day.

Discussion

In adult-to-adult LDLT, right liver is frequently used. When the graft weight is marginal for recipient metabolic demand, the tributaries of the middle hepatic or short hepatic veins must be aggressively reconstructed. When the right liver graft has multiple short hepatic veins, the double VC method should be considered to decrease warm ischemic time. A large anastomosis should be made in a partial liver graft, which will regenerate and might compress the anastomotic site.[2] For this purpose, the double VC method is preferred, which secures a large outflow orifice. We describe two alternative techniques when VC grafts are not available.

The major concern in venous reconstruction using cryopreserved vein grafts is vein graft obstruction or the possibility of narrowing over the long term. We recently reported satisfying short-term results of hepatic vein reconstruction using cryopreserved grafts with a median follow-up of 9 months.[2] Millis et al.,[9] however, reported 51% complication rate after using a cryopreserved vascular graft. Kuang et al.[10] reported complications, including aneurysm, thrombosis, and stricture, in 8 of 9 cryopreserved vein grafts that were used for portal vein and hepatic arterial interposition. To date, we have not experienced any complications using cryopreserved vascular grafts, but the previous discouraging results indicate that long-term follow-up is necessary to confirm the practicality.

In right liver transplantation, the present techniques can be used to expand the chance for performing double VC reconstruction, which will contribute to satisfactory outflow.

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