TABLE 2
Univariate Analysis of Patients with Hepatocellular Carcinoma

Variable	No. of patients (%)	Survival rate (%) 3 Yrs	5 Yrs	P value
Age				
< 60 yrs	4097 (33.8)	67.3	53.7	0.01
≥ 60 yrs	8021 (66.2)	66.8	48.4	—
Gender				
Male	9550 (78.9)	67.1	50.3	0.8
Female	2561 (21.1)	66.6	50.9	—
History of blood transfusion				
Present	2595 (25.9)	67.1	49.4	0.25
Absent	7435 (74.1)	67.7	51.5	—
Hepatitis B surface antigen				
Positive	2295 (19.8)	64.9	53.0	0.57
Negative	9276 (80.2)	67.6	50.0	—
Hepatitis C virus antibody				
Positive	7577 (67.3)	68.4	49.8	0.25
Negative	3676 (32.7)	66.6	53.6	—
Degree of liver damage				
A	7362 (66.4)	72.3	56.1	0.0001
B	3299 (29.7)	60.9	42.9	—
C	429 (3.9)	44.2	26.7	—
α-Fetoprotein				
< 20 ng/mL	4980 (42.9)	77.1	61.5	0.0001
21–200 ng/mL	3296 (28.4)	67.2	47.0	—
201–1000 ng/mL	1504 (13.0)	58.7	41.5	—
1001–1000 ng/mL	1187 (10.2)	52.1	37.7	—
≥ 10,001 ng/mL	637 (5.5)	40.3	33.1	—
PIVKA-II				
< 100 mAU/mL	5352 (62.0)	75.3	57.3	0.0001
100–299 mAU/mL	839 (9.7)	67.8	53.9	—
300–499 mAU/mL	421 (4.9)	59.1	39.6	—
500–999 mAU/mL	502 (5.8)	53.1	36.5	—
> 1000 mAU/mL	1525 (17.7)	48.6	33.5	—
Maximal tumor dimension				
≤ 2.0 cm	2320 (21.0)	83.7	66.3	0.0001
2.1–5.0 cm	5956 (53.9)	70.4	52.9	—
5.1–10.0 cm	1946 (17.6)	53.0	37.5	—
> 10.0 cm	819 (7.4)	44.5	31.9	—
No. of tumors				
1	8412 (75.3)	73.0	56.5	0.0001
2	1655 (14.8)	68.2	45.0	—
≥ 3	1108 (9.9)	44.4	26.5	—
Intrahepatic extent of tumor*				
H0	1866 (17.1)	80.7	65.1	0.0001
Hs	3597 (33.0)	73.6	54.9	—
H1	2613 (24.0)	68.3	50.3	—
H2	2228 (20.4)	58.3	43.0	—
H3–H4	604 (5.5)	36.5	26.3	—
Extrahepatic metastasis				
Absent	11,644 (98.7)	67.7	50.9	0.0001
Present	150 (1.3)	20.5	14.9	—
Growth type				
Eg	9936 (92.6)	69.9	52.6	0.0001
Ig	798 (7.4)	54.8	41.8	—
Capsular formation				
Absence	2,670 (24.0)	70.0	55.5	0.037
Presence	8,433 (76.0)	68.1	50.7	—
Septum formation				
Absence	5249 (50.2)	71.3	54.7	0.0001
Presence	5202 (49.8)	66.7	49.7	—
Portal vein invasion				
Absence	8509 (76.5)	74.9	57.2	0.0001
Presence	2609 (23.5)	48.0	34.5	—
Hepatic vein invasion				
Absence	9951 (91.0)	71.3	54.1	0.0001
Presence	983 (9.0)	44.3	33.7	—
Bile duct invasion				
Absence	10,637 (96.6)	69.8	52.9	0.0001
Presence	370 (3.4)	41.8	31.6	—
Surgical curability				
Absolute curative	2239 (20.5)	81.2	65.7	0.0001
Relative curative	5434 (49.7)	72.5	55.6	—
Relative noncurative	2622 (24.0)	58.6	41.9	—
Absolute noncurative	649 (5.9)	35.8	19.7	—
Surgical free margin				
Presence	6349 (57.7)	72.0	56.0	0.0001
Absence	4652 (42.3)	64.3	46.7	—
Associated liver disease				
Normal	1064 (11.1)	69.5	58.2	0.0001
Chronic hepatitis	3359 (35.1)	74.9	61.0	—
Cirrhosis	5142 (53.8)	65.3	46.9	—

PIVKA-II: protein induced by vitamin K absence or antagonist-II; Eg: expansive growth (well demarcated border); Ig: infiltrative growth (poorly demarcated border).
* H0: a solitary tumor measuring ≤ 2.0 cm in greatest dimension with no vascular invasion; Hs: tumor(s) limited to 1 subsegment (Couinaud segment); H1: tumor(s) limited to 1 segment; H2: tumor(s) limited to 2 segments; H3: tumor(s) limited to 3 segments; H4: tumor(s) involving > 3 segments.

TABLE 3
Multivariate Analysis Using the Stratified Cox Proportional Hazard Model by Associated Liver Disease

Variable	HR	95% CI	P value
Age (≥ 60 yrs vs. < 60 yrs)	1.22	1.11–1.33	0.0001
Degree of liver damage (C, B, A)	1.26	1.17–1.35	0.0001
α-fetoprotein (ng/mL)			
21–200 vs. ≤ 20	1.35	122–1.50	0.0001
201–1000 vs. ≤ 20	1.53	1.34–1.74	0.0001
1001–10,000 vs. ≤ 20	1.57	1.36–1.80	0.0001
> 10,000 vs. ≤ 20	1.64	1.38–1.96	0.0001
Maximal tumor dimension (cm)			
2.1–5.0 vs. ≤ 2.0	1.38	1.22–1.56	0.0001
5.1–10.0 vs. ≤ 2.0	2.04	1.75–2.38	0.0001
> 10.0 vs. ≤ 2.0	2.53	2.07–3.09	0.0001
No. of tumors (multiple vs. solitary)	1.19	1.05–1.35	0.008
Intrahepatic extent of tumor*			
H2 vs. H1 or less	1.08	0.96–1.21	0.2
H3/H4 vs. H1 or less	1.03	1.07–1.57	0.007
Extrahepatic metastasis (present vs. absent)	2.19	1.55–3.09	0.0001
Growth type (Ig vs. Eg)	1.17	0.99–1.38	0.06
Capsular formation (present vs. absent)	1.08	0.97–1.38	0.17
Septum formation (present vs. absent)	0.97	0.89–1.06	0.53
Portal vein invasion (present vs. absent)	1.46	1.31–1.62	0.0001
Hepatic vein invasion (present vs. absent)	1.17	1.01–1.36	0.03
Bile duct invasion (present vs. absent)	1.0	0.79–1.27	0.98
Surgical curability (absolute noncurative vs. others)	1.4	1.18–1.65	0.0001
Surgical free margin (positive vs. negative)	1.1	1.01–1.20	0.03

HR: hazard ratio; 95% CI: 95% confidence interval; Eg: expansive growth (well demarcated border); Ig: infiltrative growth (poorly demarcated border).
* H0: a solitary tumor measuring ≤ 2.0 cm in greatest dimension with no vascular invasion; Hs: tumor(s) limited to 1 subsegment (Couinaud segment); H1: tumor(s) limited to 1 segment; H2: tumor(s) limited to 2 segments; H3: tumor(s) limited to 3 segments; H4: tumor(s) involving > 3 segments.

prognostic predictors for patients with HCC (Table 3). During the last decade, operative mortality was 2.3% in 1990–1991, 2.0% in 1992–1993, 1.4% in 1994–1995, 1.5% in 1996–1997, and 0.6% in 1998–1999.

DISCUSSION

The previous LCSGJ report analyzed predictive factors for prognosis of approximately 5800 patients who underwent liver resection for HCC between 1982 and 1989.[2] In the current study, we analyzed a large cohort of > 12,000 patients who underwent liver resection for HCC between 1990 and 1999 in a nationwide survey of primary hepatic cancer in Japan. The development of new diagnostic techniques, such as dynamic computed tomography and magnetic resonance imaging, has lead to an increase in patients who had negative HBs-Ag status with low AFP levels, small tumors, and portal vein invasion compared with our previous report.[2] The number of patients doubled during the last decade, and the patients' profiles have changed. Advances in therapeutic techniques and perioperative patient care have improved long-term outcomes after radical resection for HCC. In the current study, we analyzed patients with HCC in Japan in the 1990s,

during which the 5-year survival rate improved to 50.5% compared with < 40% in the 1980s.[2] The overall cumulative survival rate was better than the rates reported in series from Asian and Western countries.[9,10] Operative mortality also improved to < 1.0% in 1998–1999, compared with > 3.0% in 1982–1987.[2]Therefore, prognostic factors for patients with HCC should be reevaluated.

In our previous study, the univariate analysis showed significant differences for 13 of 14 factors; and multivariate analysis using the Cox proportional hazards model showed that 3 tumor factors (tumor size, number of tumors, portal vein invasion), 3 clinical factors (age, AFP level, and associated liver disease), and 1 operative factor (surgical curability) were independent predictors of long-term prognosis for patients with HCC. In the univariate analysis for the current study, 18 of 21 factors showed significant differences; and the multivariate analysis, which was stratified by associated liver disease, found that 6 tumor factors (tumor size, number of tumors, intrahepatic extent of tumor, extrahepatic metastasis, portal vein invasion, and hepatic vein invasion), 3 clinical factors (age, degree of liver damage, and AFP level), and 2 operative factors (surgical curability and free surgical margin) were independent prognostic factors for overall survival. Thus, tumor size, number of tumors, and portal vein invasion are well known prognostic factors after resection in patients with HCC.

With regard to tumor size, some reports have shown that patients with tumors that measured ≤ 5 cm in greatest dimension had a better prognosis compared with patients who had tumors > 5 cm. A new International Union Against Cancer (UICC) TNM classification system makes use of 5 cm as a tumor cut-off size.[11] However, establishing a screening program for HCC in patients who are at high risk for hepatitis virus infection will increase the diagnoses of small sized HCC tumors. In the current study, the percentage of patients with tumors that measured ≤ 2 cm increased to 21% of all patients, compared with 16% of all patients in our previous report. Three-fourths of patients had tumors that measured ≤ 5 cm. Patients who had tumors that measured ≤ 2 cm had a significantly better prognosis compared with patients who had tumors that measured 2–5 cm. Therefore, in the TNM classification system proposed by the LCSGJ, ≤ 2 cm is the tumor cut-off size.[12]

Hepatic vein invasion was not evaluated as a prognostic factor in the previous study,[2] because only a few patients had hepatic vein invasion. In this large-scale study, however, hepatic vein invasion was an independent prognostic factor, along with portal vein invasion, although the rate of hepatic vein invasion was only 9.0%. Conversely, bile duct invasion was not a predictor in the multivariate analysis, although there were significant differences in survival in the univariate analysis between patients with and without bile duct invasion. Bile duct invasion occurred along with vascular invasion in most patients, which may explain these findings.[13]

Most patients with HCC have HBV or HCV infection. The long-term survival of patients with HCC who have different hepatitis viral infections has been controversial.[14,15] In the current study, there was no significant difference in survival stratified by either hepatitis B or hepatitis C serology; although, in the previous report, patients who had negative HBsAg status had a better prognosis compared with patients who had positive HBsAg status. Conversely, associated liver disease was an important prognostic factor. Patients who had normal livers and chronic hepatitis had a better prognosis compared with patients who had cirrhosis, although there were no significant differences between patients with normal livers and patients with chronic hepatitis. To select adequate therapeutic options for patients with HCC, prognosis should be assessed at the time of preoperative clinical assessment. The degree of liver damage classification, defined by preoperative clinical data similar to the Child–Pugh classification system, was a significant predictor. The degree of liver damage classified by preoperative clinical data was more useful than histologic evaluation of associated liver disease, not only to estimate hepatic functional impairment for determining the appropriate surgical procedure but also to predict patient prognosis.

Serum levels of the tumor markers AFP and PIVKA-II were associated with significant differences in survival. Furthermore, AFP was an independent prognostic factor. We reported previously that AFP and PIVKA II were indicators of a poor prognosis in patients with HCC.[2,3] The Cancer of the Liver Italian Program investigators also reported that AFP was an independent prognostic factor.[16] Koike et al. reported that the PIVKA-II (des-γ-carboxy prothrombin) level was the most useful predisposing clinical parameter for the development of portal vein invasion.[17] However, PIVKA-II was recognized as a useful tumor marker for HCC only in the late 1990s, and it was not examined in 30% of patients in the current study. Therefore, PIVKA-II was not included in our multivariate analysis. Recently, some reports have shown that Lens culinaris agglutinin-reactive AFP is another useful predictor for HCC.[18] These three markers should be evaluated further to determine which tumor markers will be useful in the near future for clinical screening and for determining prognosis in patients with HCC.

Surgical curability was an important prognostic factor. Patients who underwent absolute noncurative resection in which residual tumor remained had a significantly worse survival compared with patients who underwent other types of surgical resection. Llovet et al. reported that the median survival of patients with unresectable HCC who were managed with systematic treatment was 17 months; and their 1-year, 2-year, and 3-year survival rates were 54%, 40%, and 28%, respectively.[19] Those results were similar to the survival results for patients in the current study who underwent absolute noncurative resection. Cytoreduction surgery for HCC does not contribute to improved outcome. Free surgical margin was another important prognostic factor. To avoid tumor recurrence, it is important to perform liver resection with adequate free margins, because microsatellite nodules and histologic venous permeation have been found in adjacent, apparently noncancerous liver.[20]

Overall, within 5 years after they underwent liver resection, 80% of patients developed recurrent disease, and the most common cause of postoperative death was HCC, either due to tumor recurrence or due to multicentric carcinogenesis in the remnant liver. Local ablation therapy and transcatheter arterial chemoembolization in the treatment of recurrent tumors have contributed to improvements in the prognosis for patients with HCC. Liver transplantation is another surgical modality for patients with HCC who have severely impaired liver function, although few Japanese patients underwent liver transplantation in the 1990s. New postoperative adjuvant therapies with interferon may be crucial in reducing the rate of recurrence, especially recurrent multicentric carcinogenesis.[21] However, it remains unknown whether this treatment improves survival.

There are some differences between the recent UICC[11] and LCSGJ TNM staging systems.[12] The major differences between these two TNM staging systems are the tumor cut-off size discussed above and the extent of vascular invasion. Some studies reported that microscopic vascular invasion reflected on prognosis after resection.[22,23] For patients with HCC, several effective, nonsurgical modalities were applied, such as transcatheter arterial chemoembolization, local ablation therapy, etc. Therefore, it is important that patients with HCC select adequate therapeutic options based on a reliable prognostic preoperative assessment using imaging studies and clinical data, and not based on histopathologic reviews of resected specimens. The results of the current study, in which we reevaluated prognostic factors for patients who underwent liver resection using a recent, large-scale data base, will provide useful information with which to evaluate these TNM staging systems. Recently, new staging systems for HCC reflecting tumor status and liver functional status also have been proposed by several groups, such as the Cancer of the Liver Italian Program score,[16] the Barcelona Clinic Liver Cancer stage,[24] and the Japan Integrating Staging score.[25] It will be necessary in the future to establish a common international staging system to guide discussions of treatment for patients with HCC.

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Case Report

Macrochimerism of Donor Type CD56+CD3+ T Cells in Donor Specific Transfusion via Portal Vein following Living Related Donor Liver Transplantation

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SUMMARY
Antigens given orally or through the portal vein are known to be less immunogenic and to induce immunologic unresponsiveness. The mechanisms responsible for graft enhancement are still unclear. Moreover, in actuality, it is difficult to perform transfer of donor antigens via the portal vein in clinical transplantation. We investigated the effect of transfer of donor blood via the portal vein intra- and postoperatively in living related donor liver transplantation for recurrent multiple hepatocellular carcinoma. A 62-year-old female, who suffered from recurrent multiple hepatocellular carcinoma with hepatitis C virus, underwent living related donor liver transplantation with the right lobe of her daughter. Eleven hepatocellular carcinomas were recognized in the resected specimen. Donor blood was administered via the portal vein using a catheter inserted in the middle colic vein intra- and postoperatively. Mononuclear cells were obtained by operative liver biopsy or postoperative biopsy using fine needle aspiration biopsy, and from peripheral blood. They were analyzed by two or three color-flow cytometry using several antibodies. The differentiation between donor and recipient was estimated by means of anti-HLA antibodies of donor and recipient. The postoperative course was uneventful. She did not suffer from acute cellular rejection and was discharged on day 30 the after operation. $CD^{56+}CD^{3+}$T cells in the liver increased notably from 20% to 50% after transplantation. One half of the $CD^{56+}CD^{3+}$T cells in the liver graft were of the donor type (donor anti-HLA A2 antibody) on day 8 after surgery. Donor type $CD^{56+}CD^{3+}$T cells occupied 17.4% of the total $CD^{56+}CD^{3+}$T cells even on day 42 after the operation. Stimulation index by mixed lymphocyte reaction continued at a low level (<2) from day 1 after the operation. Steroids were discontinued after 40 postoperative days. FK506 was also reduced to 0.5mg/day 4 months after the operation. There was no recurrence of hepatocellular carcinoma and hepatitis C virus for two years after the operation. Macrochimerism of donor type $CD^{56+}CD^{3+}$ T cells in a graft might be induced by the transfer of donor blood via the portal vein and may play an important role in transplantation tolerance. Inoculation of donor blood via the portal vein may also be very useful for rapid reduction of immunosuppression.

KEY WORDS:
Tolerance; Portal vein; Liver transplantation; NKT cell; $CD^{56+}CD^{3+}$ cell; Extrathymic T cell; Hepatic Immunity

ABBREVIATIONS:
Natural Killer (NK); Living Related Donor Liver Transplantation (LRDLT); Donor-Specific Transfusion (DST); Hepatocellular Carcinoma (HCC); Hepatitis C Virus (HCV); Fine Needle Aspiration Biopsy (FNAB)

INTRODUCTION

Pretreatment with donor leukocytes can prolong allograft survival in fully incompatible murine strain transplants. Graft survival is influenced by the source and amount of antigen administered, the time interval between pretreatment and subsequent transplantation, and the organ transplanted. It has been reported that donor-specific transfusion (DST) via other routes, such as the portal vein or gastrointestinal tract, induces donor specific tolerance and so-called portal tolerance (1) or oral tolerance (2). We reported that administration of donor leukocytes via the portal vein prolonged the survival of a cardiac allograft; however, systemic inoculation of donor leukocytes did not prolong graft survival in BN to Lewis combination (3,4). It is well known that the liver is a privileged organ in transplantation compared with others and also an extremely immunological organ (5). Thus, the potential for liver to survive transplantation is high. However, because of its clinical difficulty, there have been no reports until now about the portal venous inoculation of donor antigens in clinical liver transplantation. Recently, living related liver transplantation has been performed throughout the world, mostly in Japan (6). In this clinical study, we investigated the transfer of donor blood via the portal vein following living related donor liver transplantation (LRDLT) and tried the chemotherapy via the portal vein in multiple recur-

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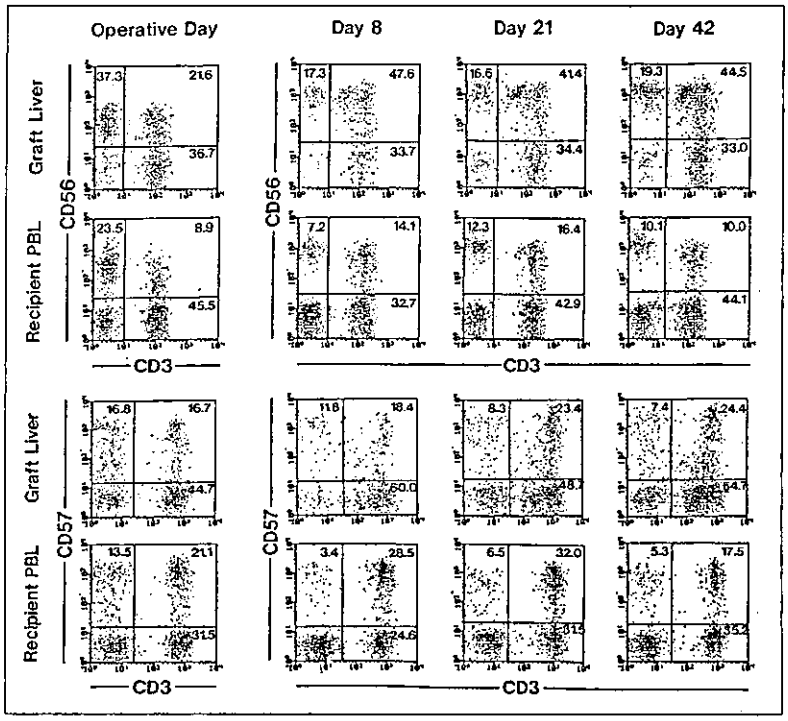

FIGURE 1
NKT Cells After LRDLT. Intrahepatic CD^{56+}T lymphocytes increased noticeably from 20% to 45% compared to those in the peripheral blood after LRDLT. The intensity of CD3 molecules was observed to be intermediate like intermediate TcR cells in the mouse.

rent hepatocellular carcinomas. Moreover, the intrahepatic leukocytes were investigated and particular attention was paid to the $CD^{56+}CD^{3+}$ cells, which are natural killer (NK) T cells in humans.

CASE REPORT

A 62-year-old woman presented for recurrent multiple hepatocellular carcinoma (HCC) with hepatitis C virus (HCV) to our department of surgery on April, 1999. In the past, she had been infected with HCV during a blood transfusion at her daughter's birth, in 1965. The patient had undergone lateral segmentectomy in 1994. Recurrent HCC was detected in 1996 and after that she had been treated several times with percutaneous ethanol injection and several times with microcoagulation therapy, but the HCC were uncontrollable. Her serum AFP level increased and six nodules were detected during the preoperative examination. Preoperative Child-Pugh was grade B. Systemic existence of cancer cells was suspected due to a positive teat for AFP mRNA in the peripheral blood. The patient was given 5-FU of 1000mg/day for 5 consecutive days preoperatively for neoadjuvant chemotherapy and also treated with interferon β nine times of six million to prevent reinfection with HCV after transplantation.

The patient underwent LRDLT using the right lobe of her daughter's liver. Graft volume/standard liver volume ratio was 45%. The postoperative course of the donor was uneventful, and she was discharged on day 14 after the operation. Adjuvant chemotherapy with systemic administration of 10mg of adriamycin once a week and intraportal treatment with 250mg of 5-FU for three consecutive days, which we called "Sandwich chemotherapy", was started as a single course on day 7 after LRDLT. In total, she had three courses of Sandwich chemotherapy. FK506 and prednisolone were used as immunosuppressant following LRDLT. Transfer of donor blood via the portal vein was started immediately after the reconstruction of hepatic artery. Transfusion of 50mL or 100mL of donor blood via the portal vein was performed almost once a week starting on day 1. Inoculation of donor blood via the portal vain was performed eight times after LRDLT.

Immunological Studies

Liver and blood samples: Liver specimens were obtained by open biopsy during LRDLT and by fine needle (18G or 21G) aspiration biopsy (FNAB) postoperatively.

FACS analysis: Mononuclear cells were examined by two or three color-flow cytometry with several monoclonal antibodies. Lymphocytes were gated and selected by staining of anti-CD45 antibody. A distinction between donor and recipient lymphocytes was made by means of anti-HLA serum (A2). Mixed lymphocyte reactions were estimated before and after the operation.

Results

NKT cells after LRDLT: Intrahepatic $CD^{56+}CD^{3+}$ T cells increased noticeably from 20% to 45% compared to those in the peripheral blood after LRDLT. The intensity of CD3 molecules was observed to be intermediate. Intrahepatic $CD^{57+}CD^{3+}$ T cells

increased gradually following transplantation **(Figure 1)**.

Analysis of macrochimerism in the grafts and the peripheral blood following LRDLT: 99.5% of both CD^{56+} T cells and CD^{56-} T cells in the donor PBL were of the donor type during operative days. Conversely, almost all of both CD^{56+} T cells and CD^{56-} T cells in the recipient PBL were occupied by the recipient type (above 99.5%). Surprisingly, one half of the CD^{56+} T cells were the donor type on day 8 after LRDLT. In contrast, CD^{56-} T cells in the graft and the lymphocytes of the peripheral blood were almost recipient type. 17.4% of CD^{56+} T cells were the donor type even on day 42 after LRDLT **(Figure 2)**.

Mixed lymphocytes reaction after LRDLT: Interestingly, the stimulation index of mixed lymphocytes reaction indicated a low level (<2) on day 1 after transplantation **(Figure 3)**.

The postoperative course of the recipient was also uneventful, and she was discharged on day 30 after LRDLT. The patient did not suffer from acute cellular rejection. Prednisolone was stopped 40 days after LRDLT. FK506 was also reduced rapidly to 0.5mg/day 4 months after transplantation. The serum AFP level decreased and continued to be within the normal limit for one year. There was no tumor recurrence. HCV-PCR also continued to be negative after LRDLT for one year.

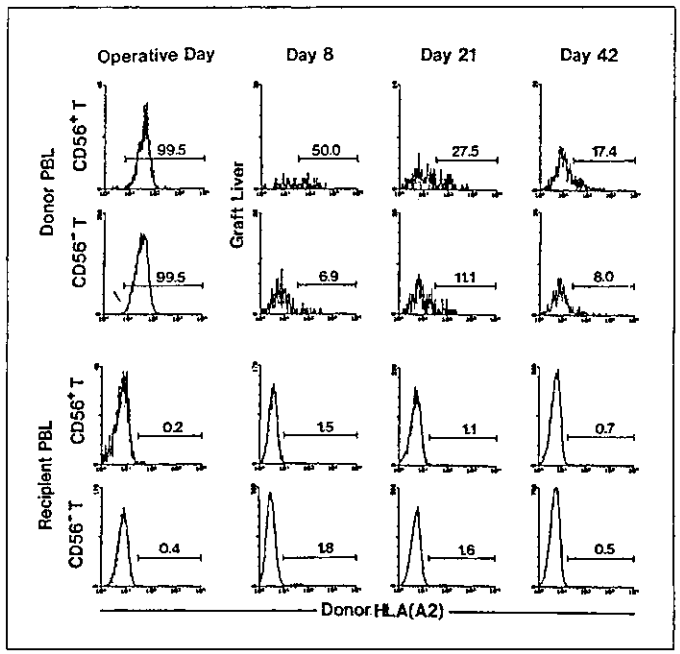

FIGURE 2 Analysis of macrochimerism in the grafts and the peripheral blood following LRDLT. 99.5% of both CD^{56+} T lymphocytes and CD^{56-} T lymphocytes in the donor PBL were of the donor type during operative days. Conversely, almost all of both CD^{56+} T lymphocytes and CD^{56-} T lymphocytes in the recipient PBL were occupied by the recipient type (above 99.5%). Surprisingly, one half of the CD^{56+} T lymphocytes were the donor type on day 8 after operation. In contrast, CD^{56-} T lymphocytes in the graft and the leukocytes of the peripheral blood were almost all the recipient type. 17.4% of CD^{56+} T lymphocytes were the donor type even on day 42 after LRDLT.

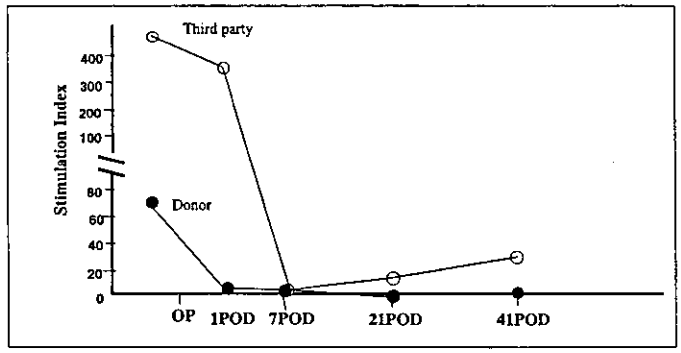

FIGURE 3 Mixed lymphocytes reaction after LRDLT. Closed circle is stimulation index (S.I.) of donor and open circle is S.I. of third party. Interestingly, the S.I. of mixed lymphocytes reaction indicated a low level (<2) on day 1 after operation.

DISCUSSION

The mechanism of the beneficial effect of blood transfusions remains elusive. Several mechanisms that have been suggested, include clonal deletion or inactivation, induction of suppressor cells, induction of blocking alloantibodies or anti-idiotypic antibodies, and maintenance of a chimeric state (7,8).

Routes of DST make it more complex to analyze the tolerance in transplantation immunity. Both thymic and portal tolerance are very interesting to investigate in terms of transplantation. The portal route for DST in particular may have much more potential for clinicians because of its practical utility (9). The liver is a highly immunologic organ (10) and consists of Kupffer cells, Natural Killer cells, and sinusoidal endothelial cells (11-13). Kupffer cells participate in tolerance by the administration of donor antigens via the portal vein (1,9). Liver-derived dendritic cell also may concern the tolerance (14,15). Recently, the liver has been found to be an organ in which extrathymic T cells can proliferate (16). We have previously reported that intermediate TCR cells and NKT cells were activated during liver regeneration following partial hepatectomy in mice and rats (17,18). In this study, we paid attention to the NKT cells in liver grafts after transplantation.

It became apparent that almost all of the donor leukocytes (>95%) in the graft faded after about one week in the patients without DST via the portal vein (19). It has been reported that donor-type Kupffer cells change to the recipient type after one month (20). Therefore, lymphocytes may decrease more easily compared with Kupffer cells which are resident leukocytes in the liver. NKT cells are thought to proliferate in local organs, such as the liver or small bowel. NKT cells may be resident lymphocytes (21,22). We have previously reported that both intrahepatic NKT cells and intermediate TCR cells were more likely to be resistant to shear stress by perfusion and were more likely to stay in the liver compared with conventional T cells in mice (23,24). We also recently confirmed that CD^{56+} T lymphocytes were likely to stay in the liver

graft after perfusion in human LRDLT (data not shown). We studied NKT cells, especially CD^{56+} T lymphocytes, following LRDLT based on these findings.

Because chimerism, the presence of donor cells in the recipient, was detected in human transplant patients with well-functioning grafts, there has been considerable interest in defining the role of donor-specific chimerism in the induction and maintenance of allograft tolerance (7,8). In contrast, it has been reported that despite donor-specific chimerism, liver and kidney transplant patients can undergo a rejection episode or allograft loss. Thus, chimerism has been associated with a possible beneficial effect, or with no effect, as well as with pathological conditions to the host harboring such cells (25-27). Tolerance due to donor-specific chimerism might be concerned by the number and the kind of chimeric donor cells. However, there were no reports about them until now. In this study, macrochimerism of CD^{56+}T lymphocytes, but not microchimarism, was demonstrated.

In the present case, the intrahepatic CD^{56+} T lymphocytes increased significantly compared with the intrahepatic CD^{56-} T lymphocytes or intrahepatic CD57+ T lymphocytes. Therefore the intrahepatic CD^{56+} T lymphocytes might be a different type of NKT cells compared with other types of NKT cells.

We recognized that many more CD^{56+} or CD161+ T lymphocytes (28,29) existed in the liver than in the spleen or peripheral blood (data not shown). Moreover, we found that a majority (70%) of the intrahepatic CD^{56+} T lymphocytes had costimulatory CD28 molecules as compared with CD^{56+} T lymphocytes in the PBL.

Donor-type intrahepatic CD^{56+} T lymphocytes with the specific characters mentioned above might induce clonal deletion or anergy of the recipient's reactive T cells such as Veto cells (30,31). It was demonstrated that the intrahepatic CD^{56+} T lymphocytes tended to increase in cases of acute cellular rejection (19). CD^{56+} T lymphocytes may be a two-edged sword. There are two mechanisms in transplantation tolerance: a central mechanism and a peripheral mechanism. The central mechanism, including thymic tolerance, is difficult to put to practical use. The peripheral mechanism may be practical for clinical transplantation, especially with a living donor. Ours may be the first case in the world in which this knowledge was applied to LRDLT.

It has been reported that neoadjuvant chemotherapy or adjuvant chemotherapy prolonged survival after liver transplantation for hepatocellular carcinoma except multiple (>3) or large tumor (>5cm) patients (32,33). The factors involved in the accelerated growth rate include the use of immunosuppressive drugs and the consequent suppression of host immunity against the growth of micrometastasis (33).

For this reason, we tried the postoperative "Sandwich chemotherapy" with neoadjuvant chemoimmunotherapy (5-FU+IFNβ). This regimen might reduce the preoperative circulating cancer cells and prevent the postoperative micrometastasis to the graft liver. Moreover, intra- and postoperative administration of DST via the portal vein brought rapid reduction of immunosupressants in the present clinical case. These results may be glad tidings in terms of expanding the indications for unresectable advanced HCC and may introduce an additional solution to transplantation immunology.

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FOOTNOTE

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インターフェロン抵抗性に関与する宿主免疫関連因子

山際 訓* 市田隆文**

索引用語：インターフェロン抵抗性，免疫関連因子，遺伝子多型，遺伝子発現解析

1 はじめに

C型慢性肝炎に対してはインターフェロン(IFN)とリバビリン(RBV)併用療法が現在最も有効な治療法である．しかし，これら併用療法が標準治療として普及するにつれて治療効果の改善がより期待されているものの，難治例である遺伝子型1b型かつ高ウイルス量の症例におけるウイルス学的著効(sustained virological response；SVR)率は50％強にすぎない．このようなIFN治療効果を規定する因子として，ウイルス側因子と使用薬剤側因子が重要であることが明らかとなっている．しかしながら，同じIFN＋RBV併用療法によりウイルス側因子からは難治例と予測される症例であってもSVRとなる症例があるのに対し，遺伝子型2a型かつ低ウイルス量であってもSVRに至らない症例など，治療効果を規定する因子が宿主側にも存在することが示唆される．したがって，現行以上の治療効果の改善には免疫応答を含めた宿主側因子の解析と理解が重要であることに間違いはないであろう．本稿では，IFN治療効果を規定する宿主側応答因子，特に免疫関連因子に関する最近の知見とともに，治療抵抗性機序の解明を目指した筆者らの研究成績を解説する．

2 HCV感染に対する免疫応答

ウイルス感染の初期免疫応答においてはnatural killer(NK)細胞，natural killer T(NKT)細胞が強力なエフェクター細胞として抗原非特異的な反応によりウイルス感染細胞の排除を行う．また，ウイルス感染細胞がIFN-α/βを，一方樹状細胞(dendritic cells；DC)がIFN-αをそれぞれ産生しウイルス増殖を抑制する．そして，早期にウイルス感染が制御できない場合には，引き続いて中和抗体および細胞傷害性T細胞(cytotoxic T lymphocytes；CTL)が誘導されてウイルス排除に働く．このような獲得免疫応答はDCやKupffer細胞などの抗原提示細胞によるウイ

Satoshi YAMAGIWA et al: Immunological factors associated with a poor response to interferon treatment for chronic hepatitis C
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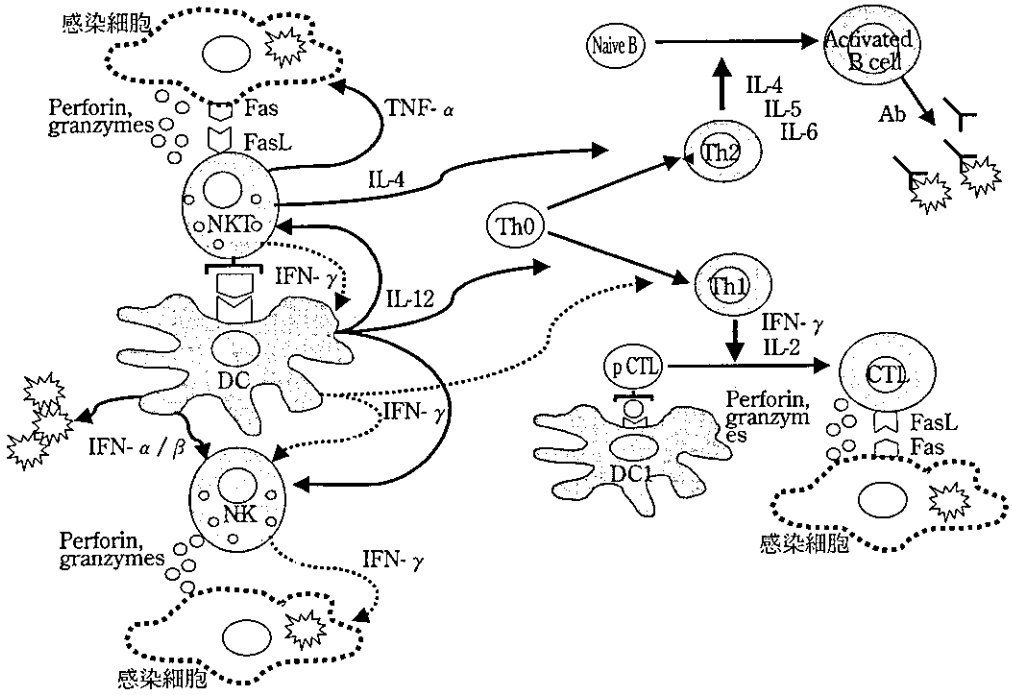

図1　HCV感染における免疫応答

ルス抗原提示時のT細胞活性化動態により規定されるが，特にNKT細胞はTh1やTh2サイトカインをともに産生し得るため，自然免疫系における初期刺激の質的差異により抗原特異的な獲得免疫応答の方向性が制御されている可能性が示唆されている（図1）[1,2]．

C型肝炎ウイルス（hepatitis C virus；HCV）感染においてもこの初期免疫応答の重要性が報告されており，HCV特異的Th1応答が強い場合は一過性感染で治癒するという報告[3]や，Th1応答は急性肝炎患者で強く反応する，あるいはTh2応答はむしろ慢性肝炎患者で強い[4]などの報告から，HCVの生体からの排除にはTh1応答が重要と考えられている．C型慢性肝炎においてはT細胞活性化に重要であるDCの機能不全が存在し，ウイルス排除に働くT細胞の活性化が不十分なため肝炎の慢性化につながっている可能性が示唆されている[5~7]．また，筆者らの検討を含めてC型慢性肝炎症例の肝臓内ではNK細胞やNKT細胞の関与する先天免疫応答の低下が示唆されており[8~12]，このような状況はウイルス感染細胞の排除の低下をきたす免疫環境にあると考えられる．そのような免疫環境下にあるC型慢性肝炎に対するIFN-α，RBVの治療効果の発現は，直接的な抗ウイルス作用に加えて，免疫系への修飾作用とくにTh1応答優位な免疫応答の誘導が関与していることが推測されている．

3　治療反応性に関与する免疫関連因子

1．リンパ球分画

IFN単独療法の奏効率が治療中のHCV特異的Th1細胞とCD8陽性細胞（CTL）の増加と関連することが報告されている[13,14]．同様にIFN＋RBV併用療法においてもSR例で治療中の末梢血中にHCV特異的なTh1細胞とCD8陽性細胞の有意な増加が認められたのに対し，NR例ではHCV特異的なTh2細胞が有意に増加することが報告されている

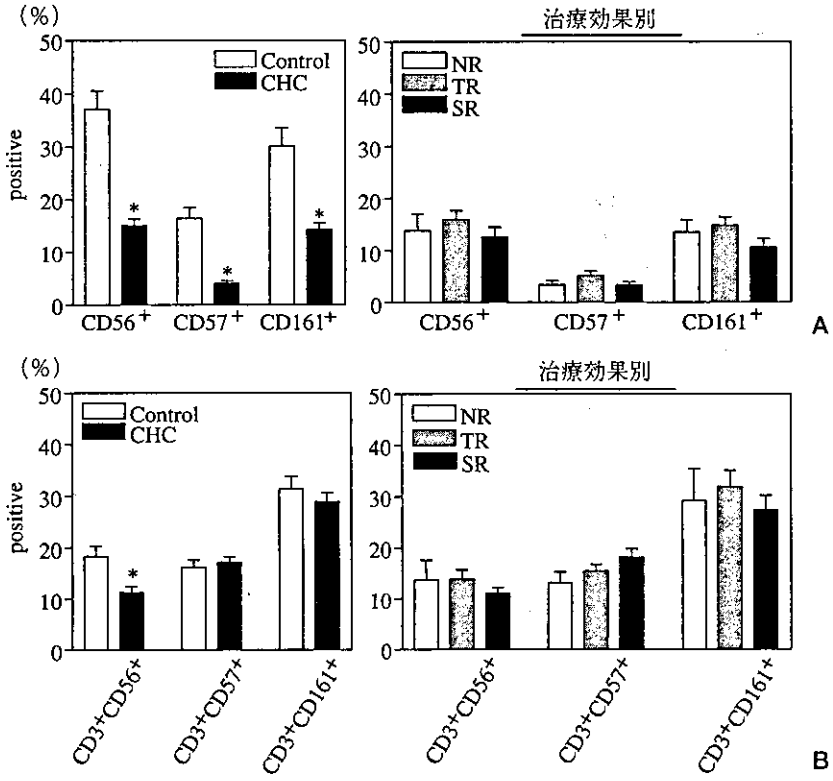

図2

A：正常肝およびC型慢性肝炎の肝内NK細胞陽性率
慢性肝炎の肝内ではNK細胞の有意な減少が認められたが，治療効果別に比較した場合，有意な変化は認められなかった．Mean ± SEを示す．*p < 0.01

B：正常肝およびC型慢性肝炎の肝内NKT細胞陽性率
慢性肝炎の肝内ではCD56陽性NKT細胞の有意な減少が認められたが，治療効果別に比較した場合，有意な変化は認められなかった．Mean ± SEを示す．*p < 0.05

[15,16]．Vrolijkらは治療前の肝組織中とくに門脈域におけるCD8陽性細胞数がIFN + RBV併用療法におけるSR例で有意に高値であったと報告しており[17]，治療前や治療中におけるCD8陽性細胞の増加が治療効果と関連することが示唆されている．また，HCV遺伝子型3a型の症例のみの検討ではあるものの，Kupffer細胞におけるCD8陽性細胞に抗原を提示するMHC class I分子の発現がIFN + RBV併用療法のSR例で有意に増加していたという報告もある[18]．

CTLとともにウイルス感染肝細胞の排除に重要と考えられるNK細胞，NKT細胞について，筆者らはIFN + RBV併用療法の治療前後における肝組織および末梢血について解析を行い治療効果との関連について検討した．図2に示すように，治療前の肝組織中では治療効果別に比較した場合に有意な変化は認められなかったが，NK細胞，NKT細胞比率ともSR例でNR例よりも減少傾向にあるものがあった．一方，治療前後での変化をみるとSR例では治療後の肝組織中のCD161陽性NK細胞とNKT細胞の有意な増加が認められ，治療前に認められた自然免疫担当細胞の減少が改善したことを示唆する変化が認められたのに対して，NR症例では変

化が認められなかった[12]．これらの結果はCD8陽性細胞と同様にIFN＋RBV併用療法によるTh1応答の増強によるNK細胞，NKT細胞の増加がウイルス排除に重要であることを示唆すると考えている．

2．サイトカイン

インターロイキン10(IL-10)のプロモーター遺伝子多型がIFN単独療法における初期反応性と関連していることが報告され[19]，またYeeらはそのようなIL-10プロモーター遺伝子多型がIFN＋RBV併用療法においても治療効果に関係すると報告している[20]．IL-10はTh2サイトカインであり，Th1サイトカイン産生を抑制するとともに肝臓におけるIFN-αによるsignal transducer and activator of transcription 1(STAT-1)活性化を抑制する[21]．IFN単独療法のNR例で血清中IL-10が高値であることや，SR例でIL-10産生がNR例と比べて低値であることが報告されていることから[22,23]，プロモーター遺伝子多型に由来するIL-10産生低下がIFN治療効果に関与している可能性が示唆される．Mangiaらは IL-10ハプロタイプの違いがHCV感染の持続化に関与し，急性感染後のウイルス排除の予測因子となるが治療効果との関連は認められなかったと報告している[24]．彼らはIFN-γやINF-αの遺伝子多型もIFN単独療法の治療効果に関連しなかったと報告している．

3．ケモカイン

HCV排除には細胞性免疫を賦活するTh1応答が重要であるが，Th1応答はIFN-γなどのサイトカイン産生とともにケモカイン受容体の一種であるCC-chemokine receptor 5(CCR5)のT細胞や単球における発現を増強する．CCR5はCCケモカインであるmacrophage-inflammatory protein(MIP)-1 α，MIP-1 βおよびregulated upon activation, normal T cell expressed and secreted(RANTES)のレセプターであり，活性化T細胞，特にTh1細胞とCD8陽性細胞や単球の遊走に関与している．C型慢性肝炎の肝組織中リンパ球にはCCR5の高発現が認められている[25]．これまでに，CCR5のプロモーターsingle nucleotide polymorphisms(SNPs)(59029-A allele)がIFN単独療法の奏功率に関与しているという報告[26,27]や，特に機能的なCCR5発現が欠損する32-base pair deletion(Δ32)をきたす変異がIFN単独治療の奏効率と関連していることが報告されている[28]．またWasmuthらはリガンドであるRANTESのSNPs(403 G/A, Int1.1 T/C, 3'222 T/C)を解析し，RANTESハプロタイプ(Int1.1 Cと3'222 C)がIFN＋RBV併用療法を施行された遺伝子型1および4型の症例の奏効率に関係すると報告している[29]．CCR5とそのリガンドの遺伝子変異はTh1細胞やCD8陽性細胞などの肝臓への遊走を低下させ，IFN治療によるTh1応答賦活作用の低下をきたし，その結果，治療抵抗性を示すという機序が示唆される．しかしながら，IFN＋RBV療法においてはCCR5-Δ32は治療効果に関与していないという報告[28,30]もあるなど，現在のところCCR5がINF＋RBV治療抵抗性に関与しているかどうかはコンセンサスが得られていない．RBVのTh1賦活作用がCCR5-Δ32によるTh1応答低下を埋め合わせている可能性もあるが，人種間の違いなどを含めて今後の検討が待たれる．

4．IFNレセプター

IFNはサイトカインであり，細胞表面のIFNレセプター(INF-αレセプターであるIFNAR1とIFN-α/βレセプターであるIFNAR2)と結合することによりその作用を

表1　IFN＋RBV治療前後における肝臓内IP-10遺伝子発現解析結果

Case		1	2	3	4	5	6	7
治療効果		NR	NR	TR	SR	SR	SR	SR
IP-10	治療前	26.52	136.8	14.21	14.87	24.79	16.47	10.63
	治療後	21.72	14.82	2.95	1.30	2.34	1.64	3.05

NR症例において治療後も肝臓内IP-10遺伝子発現の増加が持続していた．

表2　IFN＋RBV治療前の肝臓発現遺伝子解析に基づく治療効果予測

	正解数/総数	正解率
NR症例	5/6	83.33 %
SR/TR症例	27/29	93.10 %

IFN＋RBV治療前の肝臓発現遺伝子解析結果をもとにMahalanobis Distance（MD値）を用いたアルゴリズムにより治療効果を予測した．NR例，SR/TR例とも高率に予測可能であった．

発現する．IFN治療効果と肝組織内IFNレセプター発現量との関連についてはいくつかの報告があり，またIFNAR1遺伝子のプロモーター領域のマイクロサテライト多型がIFN効果予測因子になると報告されている[31]．Hijikataらは IFNにより誘導される蛋白であるMxAのプロモーターSNPsがIFN治療感受性と関連すると報告している[32]．IFNレセプターとIFN誘導遺伝子はIFN治療の反応性に関与する重要な因子であることが示唆されている[33]．

5．その他の因子

活性化T細胞に発現しT細胞機能の抑制に関与するco-stimulatory moleculeであるcytotoxic T lymphocyte antigen-4（CTLA-4）のSNPs（319 C/Tと 49 A/G）解析により，CTLA4ハプロタイプ（－318C-49G）がHCV遺伝子型1型のIFN＋RBV併用療法におけるSR例と関連していることが報告されている[34]．ハプロタイプの違いはCTLA-4の発現低下をきたしT細胞機能の活性化に関与するとともに，Th1/Th2バランスのシフトにも関与する可能性が示唆されている[34]．

4　肝臓発現遺伝子解析と治療効果予測

これまでに述べたように，筆者らはIFN＋RBV治療前後での肝臓内リンパ球の解析を行うとともに，治療前後の肝臓を用いた網羅的遺伝子発現解析を行い，治療による生体側因子の変動を解析するとともに，治療前の解析結果による治療効果予測を行った．方法は既報[35]のように肝組織よりRNAを抽出・増幅後，肝臓発現遺伝子から選択し機能別に分類した優位発現遺伝子を搭載したcDNAチップによる解析を行い，得られた発現データを用いてプロファイリング解析と診断アルゴリズムによる治療効果予測を試みた．

IFN＋RBV治療前後で解析可能であり，かつ治療効果が判定された遺伝子型1b型症例7例の，肝臓内リンパ球変動との関連が示唆される免疫応答関連遺伝子群の変化をみると，特に活性化T細胞やNK細胞に対する遊走活性をもつケモカインであるIFN-gamma inducible chemokine IP-10（IP-10）がNR症例で治療前の発現が特に高値で，治療後にも高値が持続していた（表1）．Narumiらは血清中のIP-10が低値の症例ほどIFN単独療法に対する反応性が高く，著効例では治療後に正常人のレベルまで低下するのに対し，無効例では治療後も高値が持続すること

Case	1	2	3	4	5	6	7
治療前 MD 値	24.75	113.00	6.10	1.49	0.52	4.12	2.11
効果予測	NR	NR	SR/TR	SR/TR	SR/TR	SR/TR	SR/TR
治療後 MD 値	14.27	13.52	6.63	0.48	1.33	0.60	0.29
治療結果	NR	NR	TR	SR	SR	SR	SR

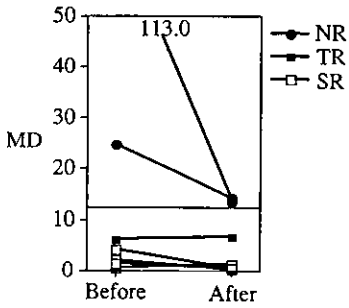

図3　IFN＋RBV 治療前後における肝臓発現遺伝子解析結果
IFN＋RBV 治療前後での肝臓発現遺伝子解析結果をもとに Mahalanobis Distance（MD 値）の変動を解析した．NR 例も治療後は MD 値の減少を認めた．

を報告しており[36]，筆者らの検討からも同様に IP-10 と IFN 治療抵抗性との関連が示唆された．また，治療反応性の違いに対する IFN 反応関連遺伝子群の関与も示唆された．

一方，治療効果予測は Mahalanobis Distance (MD 値) を用いたアルゴリズムにより行った．MD 値は既知の SR/TR 群データベースを基準データとして特性基準空間を作成し，これに対し対象となるサンプルのデータによる対象空間との乖離度を算出したもので MD 値が大きくなるほど基準となる SR/TR 群とは異なる特性を持つことになる．今回のアルゴリズムでは MD 値 13 以上を NR，それ以下を SR または TR と判定した．そのような診断アルゴリズムによる効果予測を基準空間作成には使われていない cross validation サンプル 35 検体で行ったところ，NR 予測は 6 検体中 5 例が的中し正解率は 83％に，SR または TR 予測の正解率は 93％と高率であった（表 2）．さらに，同一症例において治療前後で MD 値の変化をみると治療後に MD 値の減少している症例が多く，特に NR 症例においても治療後の MD 値は治療前と比べ明らかに減少しており，その遺伝子発現は SR/TR 症例の特性に近くなっていることが示唆された（図 3）．検討した症例数が少なくさらに検討が必要であるが，今回検討した遺伝子群のみの解析では NR 例の予測はある程度期待されるものの，治療後の再燃例の予測は困難であり，再燃例の予測にもつながる治療抵抗性を示す機序の解明を目指したさらなる検討が必要である．

5　おわりに

IFN とリバビリン併用療法によっても治療抵抗性を示す症例に関連する宿主側因子について，免疫関連因子を中心に概説した．遺伝子多型を含めてさまざまな因子が報告されているが，免疫関連因子をまとめると"IFN と RBV の併用によっても肝臓内に Th1 優位な細胞性免疫応答が十分に賦活されない" 症例が治療に抵抗性を示すことが示唆された．そのような細胞性免疫賦活が誘導されない要因としてゲノムレベルでの個体差の検討とともに，樹状細胞の機能不全のような HCV ウイルス自体による原因の解明が重要である．現時点では今後も IFN が治療の中心になると考えられ，更なる治療効果の改善のためには，そのような治療抵抗例においても Th1 応答を誘導しうる機序を解明し，新たな薬剤との併用や免疫賦活療法など新たな治療法の開発が急務であると考えられた．

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Genome-wide transcriptome mapping analysis identifies organ-specific gene expression patterns along human chromosomes

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Abstract

The Human Genome Project has revealed that there about 32,000 protein-encoding genes, which are distributed throughout the genome. It is unclear, however, whether genes are distributed on the chromosomes according to patterns linked to organ specificity. To explore the relationship between genes actively transcribed in normal tissues and their chromosomal locations, we analyzed serial analysis of gene expression libraries of normal human liver, brain, breast, and colon tissues. Transcriptome mapping analysis revealed that transcriptional activity in each tissue varied according to the chromosomal domains, and a weak positive correlation was observed between transcription density and gene density. We identified six liver-related and five colon-related chromosomal domains highly transcribed in each tissue, whereas no brain-related or breast-related chromosomal domains were identified. Representative genes located on these chromosomal domains were associated with the function of each organ and were highly conserved in both mouse and rat genomes. These data revealed that the transcriptional activities of normal human tissues are well orchestrated at chromosomal levels, suggesting that highly expressed genes may share physical proximity.

Keywords: Genomics; Gene expression profiling; Serial analysis of gene expression; Transcriptome mapping; Organ-related chromosomal domain

Introduction

Analysis of the human genome has shown that there are about 32,000 protein-coding genes distributed throughout the 46 chromosomes. Previous work, however, indicated that the highly expressed genes in all human tissues are clustered in several chromosomal domains [1]. In the nematode *Caenorhabditis elegans*, genes expressed in the muscle are clustered in small groups along the chromosomes [2], suggesting a relationship between muscle-specific functionality and chromosomal domains actively transcribed in *C. elegans*. Another report revealed that groups of adjacent genes were coregulated in the fly *Drosophila melanogaster*, suggesting that coregulated genes also share physical proximity in *D. melanogaster* [3]. Furthermore, a comparative analysis of the genomes of *D. melanogaster*, *C. elegans*, and *Saccharomyces cerevisiae* revealed the existence of a "core proteome" highly conserved in yeast, worm, and fly [4], suggesting the heredity of fundamental genomic or proteomic organization associated with the cellular processes or functions commonly observed in all eukaryotes. From these reports, we postulated a hypothesis that the distribution of genes along human chromosomes may exhibit a higher level of organization, which may be linked to organ-specific functionality and conserved in mammals.

To explore the relationship between genes actively transcribed in normal human tissues and chromosomal domains, we analyzed serial analysis of gene expression (SAGE) libraries derived from normal human liver, brain,

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breast, and colon tissues with human genome sequence information. We observed organ-specific gene expression patterns along the chromosomes in each tissue, identifying six liver-related chromosomal domains and five colon-related chromosomal domains. We found that transcriptional activity in normal human tissues is well orchestrated at the chromosomal level, suggesting that most of the highly expressed genes in each organ might be clustered. We further observed that representative genes located on these chromosomal domains were associated with organ-specific function, and the genes were also clustered on the *Rattus norvegicus* and *Mus musculus* genomes. These findings may be the results of binding of specific transcription factors onto specific chromosomal domains in each tissue or of chromosome rearrangements during mammalian evolution.

Results

Organ-specific gene expression patterns along the chromosome

An outline of the construction of the transcriptome map is shown in Fig. 1. We assigned 27,402 NCBI RefSeq genes (build 31) to 17,193 Locus ID clusters. All SAGE tags detected could be assigned to a total of 55,612 reliable UniGene clusters using the SAGEmap reliable tag-to-gene mapping table (http://www.sagenet.org/SAGEDatabases/unigene.htm). All SAGE tags assigned were related to the Locus ID clusters by UniGene ID, and the origins of the start sites in all genes were mapped to the SAGE tag.

When we investigated the correlation between the number of genes and the number of transcripts in each chromosome using a 5-Mb window moving along the chromosome at 1-Mb intervals, we found that Pearson's correlation coefficients ranged from 0.119 to 0.869 in the liver, 0.107 to 0.838 in the brain, 0.314 to 0.791 in the breast, 0.206 to 0.872 in the colon, and 0.304 to 0.883 in the reference library (Table 1). These data suggested that the transcriptional activity in each tissue varied and was not in accordance with the number of genes encoded by each chromosome. The strongest positive correlations, however, were observed in the reference libraries, indicating that gene expression levels in total tissues could be relatively correlated with the number of genes per chromosome.

To investigate the transcription density along the human chromosomes using digital gene expression data, we calculated transcription density factor (TDF), which would be well correlated with the abundance of transcripts and adjusted by gene density in each chromosomal domain, as described under Materials and methods. When we calculated the gene density and TDF in each tissue on whole chromosomal domains, we found that gene expression levels fluctuated along the chromosome, with most genes in most chromosomal domains expressed at a range of $0.5 < TDF < 1.5$ in each tissue (representative transcriptome maps are shown in Fig. 2). Gene expression patterns in each of

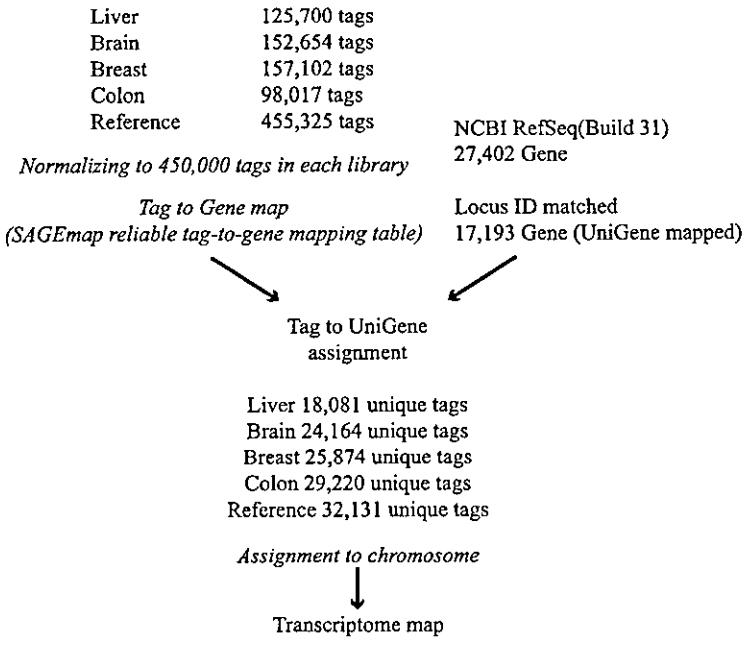

Fig. 1. Schematic outline of transcriptome map construction. All detected SAGE tags were assigned to a total of 55,612 reliable UniGene clusters and related to the Locus ID clusters by UniGene ID. Location of the SAGE tags on the chromosome was coordinated with physical distance information.

Table 1
Correlation between the number of transcripts and the number of genes in each chromosome

Chromosome	Liver	Brain	Breast	Colon	Reference
1	0.483	0.674	0.640	0.537	0.703
2	0.491	0.739	0.642	0.670	0.675
3	0.517	0.672	0.594	0.538	0.739
4	0.404	0.470	0.512	0.391	0.512
5	0.869	0.785	0.669	0.721	0.883
6	0.720	0.838	0.709	0.805	0.856
7	0.638	0.686	0.568	0.699	0.670
8	0.324	0.386	0.314	0.335	0.364
9	0.355	0.776	0.555	0.705	0.515
10	0.448	0.498	0.322	0.441	0.304
11	0.224	0.801	0.544	0.556	0.778
12	0.397	0.632	0.711	0.616	0.730
13	0.281	0.398	0.478	0.311	0.459
14	0.354	0.573	0.378	0.431	0.651
15	0.119	0.284	0.345	0.206	0.339
16	0.520	0.719	0.500	0.588	0.666
17	0.410	0.550	0.791	0.670	0.634
18	0.211	0.107	0.453	0.359	0.364
19	0.587	0.701	0.555	0.610	0.700
20	0.570	0.769	0.701	0.673	0.709
21	0.766	0.627	0.547	0.872	0.744
22	0.429	0.563	0.680	0.445	0.593
X	0.653	0.786	0.747	0.705	0.758
Average	0.468	0.610	0.563	0.560	0.624

these chromosomal domains were tissue specific, indicating the existence of distinct, organ-specific gene expression patterns along the chromosomes.

Identification of organ-related chromosomal domains

To determine whether each tissue type had distinct gene expression patterns along the chromosomes, we mathematically classified the chromosomal domains by TDF. For each tissue type, we determined if the TDF in a given SAGE library exceeded 1.8 and if the TDF in the other libraries was less than 1.0. The chromosomal domains identified by these criteria fulfilled the condition that gene expression levels in the given tissue were increased more than 6-fold compared with those of other tissues and that gene expression levels in the other tissues did not exceed the 2.7-fold of the reference levels. To diminish the effect of a gene highly expressed in a chromosomal domain of low gene density on a TDF score, we selected chromosomal domains more than 3 Mb long, containing more than two genes in a window of 1 Mb, with TDF >1.8 consecutively.

Using these criteria, we first compared the gene expression patterns of the liver. Using SAGE libraries derived from normal liver and a mixture of five normal livers, we investigated whether there were individual variations in hepatic gene expression patterns along the chromosome. The TDF derived from the normal liver SAGE library could be correlated with the TDF derived from the mixture of five normal liver SAGE libraries, with Pearson's correlation coefficient ranging from 0.618 to 0.960. In addition, the difference between the TDF in the normal liver library and that in the pooled liver library never exceeded 1.8 on all chromosomal domains (data not shown), indicating that there were no individual differences of more than sixfold along the chromosome. These data also suggested that the criteria used here could enable us to disregard the individual variations in gene expression patterns and could be useful for identifying chromosomal domains actively transcribed in each tissue type.

We investigated whether highly expressed genes clustered along the chromosome in each tissue and applied the criteria to the mapping data of the liver, brain, breast, and colon SAGE libraries. After filtering transcriptome mapping data of the liver, we found that genes highly expressed in the liver were clustered in six chromosomal domains, compared with the brain, breast, and colon libraries. Similarly, we identified five colon-related chromosomal domains, but no brain-related or breast-related chromosomal domains.

To rule out the possibility of chance observation of high variance in the selected domains, we performed a permutation test as described under Materials and methods. The identified liver-related chromosomal domains and colon-related chromosomal domains were fully significant (liver-related chromosomal domains $p < 0.0001$, colon-related chromosomal domains $p < 0.0001$), revealing the statistical validity of our criteria.

Representative transcriptome mapping data and chromosomal domains are shown in Fig. 2 (chromosomes 1 and 6). Cytogenetically, we found liver-related chromosomal domains at 1q23–q25, 4q21–q24, 6p12.1, 11q23–q24, 17q11, and 18q12; we found colon-related chromosomal domains at 2p21, 3q23, 8q21, 12q15, and 21q22.

The numbers of SAGE tags distributed in each organ-related chromosomal domain are shown in Table 2. In liver-related chromosomal domains, the average gene expression levels in the liver were increased 18.1-, 22.2-, and 22.5-fold compared with levels in the brain, breast, and colon, respectively. In colon-related chromosomal domains, the average gene expression levels in the colon were increased 15.0-, 13.3-, and 8.0-fold compared with levels in the liver, brain, and breast, respectively.

Conservation of the organ-related chromosomal domains in human and rodent genomes

To examine whether organ-related chromosomal domains were preserved in other mammalian genomes, we investigated the possible homologous genes and their locations on the *R. norvegicus* and *M. musculus* genomes using the HomoloGene database. Of 412 human genes comprising 11 organ-related chromosomal domains, we identified 213 rat homologous genes (51.7%) and 230 mouse homologous genes (55.8%). Almost all of the homologous genes on human chromosomes 1q23–q25, 2p21, 3q23, 4q21–q24, 11q23, 12q15, 17q11, 18q12.1, and Xq21.3–q22 were located on the same chromosomal