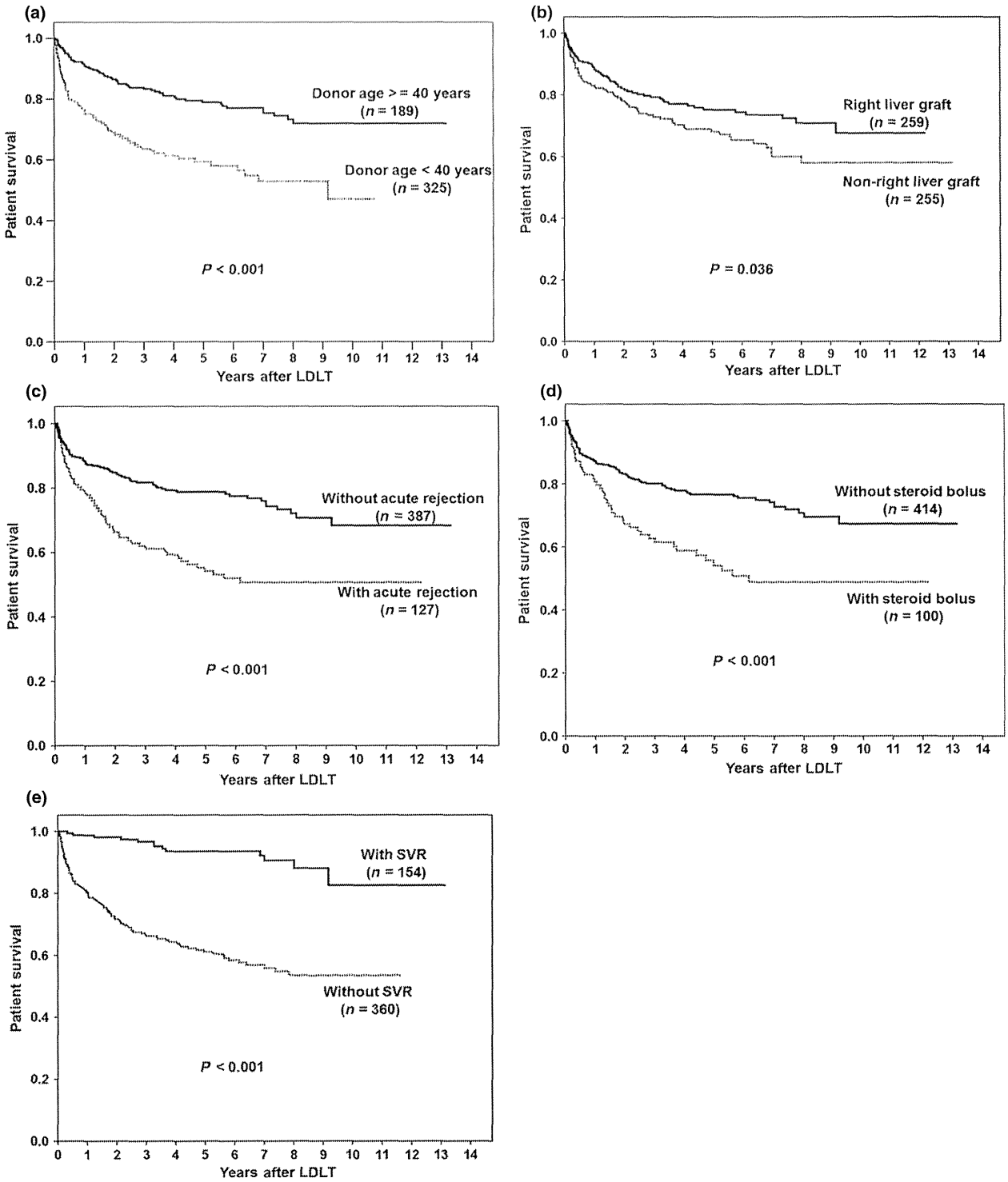

Figure 2 Kaplan–Meier curves stratified by each variable: (a) donor age, (b) graft type, (c) acute rejection, (d) steroid bolus, and (e) sustained virologic response. LDLT, living donor liver transplantation; SVR, sustained virologic response.

increased use of liver grafts from older donors. For HCV-positive recipients, two large retrospective reports from the Scientific Registry of Transplant Recipients and UNOS databases reported that donor age over 40 is an independent predictor of patient death [15,16]. Other accumulating reports [14,17,18] indicate that the grafts from older

Table 4. Factors associated with patient survival among those achieved SVR (n = 154).

Cox regression analysis	Hazard ratio (95% confidence interval)	P-value
Recipient age: ≥60 years (n = 43) vs. <60 years (n = 111)	1.424 (0.318–2.385)	0.644
Recipient gender: male (n = 100) versus female (n = 54)	4.709 (0.918–24.161)	0.063
Pretransplant antiviral treatment: yes (n = 66) versus no (n = 88)	1.666 (0.350–7.931)	0.522
HCV genotype: 1b (n = 112) versus other types (n = 42)	0.873 (0.203–3.747)	0.855
Co-existence of HCC: yes (n = 54) versus no (n = 100)	0.728 (0.179–2.694)	0.635
MELD score: ≥15 (n = 54) vs. <15 (n = 98)	1.354 (0.578–3.204)	0.785
LDLT cases per year: ≥20 (n = 82) vs. <20 (n = 72)	1.054 (0.458–1.254)	0.854
Calcineurin inhibitor: Tac (n = 94) versus CyA (n = 60)	3.580 (0.736–17.421)	0.114
Mycophenolate mofetil: yes (n = 78) versus no (n = 76)	0.932 (0.456–1.884)	0.781
Steroid withdrawal: yes (n = 40) versus no (n = 114)	0.449 (0.096–2.102)	0.31
Splenectomy: yes (n = 59) versus no (n = 95)	1.402 (0.335–5.873)	0.644
Episode of acute rejection: yes (n = 34) versus no (n = 120)	1.854 (0.216–15.914)	0.574
Steroid bolus injection: yes (n = 26) versus no (n = 128)	0.16 (0.019–1.386)	0.096
Donor age: ≥40 years (n = 43) vs. <40 years (n = 111)	1.18 (0.296–4.698)	0.815
Type of graft: right liver (n = 80) versus non-right liver (n = 74)	2.799 (0.818–9.573)	0.101

HCV, hepatitis C virus; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; MELD, model for end-stage liver disease; Tac, tacrolimus; CsA, cyclosporine; SVR, sustained virologic response.

donors are at greater risk for disease progression and impaired graft/patient survival compared with those from younger donors. Our results are definitely consistent with these reports.

Acute rejection in conjunction with treatment with a steroid bolus is one of the most critical factors to address with respect to HCV recurrence. Historical studies [19,20] have demonstrated that steroid bolus for acute rejection in HCV-positive recipients accelerates the recurrence of hepatitis and decreases patient survival. A recent study reported that HCV-positive recipients who receive high-dose steroid treatment for acute rejection are at increased risk of severe recurrent hepatitis, in which older donor age and an episode of rejection are the two most important predictors of developing fibrosing cholestatic hepatitis [21]. Similarly, our study also revealed that both older donor age and acute rejection are independent predictors for impaired patient outcome among LDLT recipients.

Table 5. Summary of antiviral treatment.

	Total (n = 361)	Treatment for established recurrent hepatitis C (n = 211)	Preemptive treatment (n = 150)
Time since LDLT (months)	3 (0–102)	4 (0.5–102)	1 (0–68)
Treatment duration (months)	15 (0.3–99)	14 (0.3–99)	17 (0.3–55)
Regimen: PEG-INF alfa-2a/RBV	45 (12%)	33 (16%)	12 (8%)
PEG-INF alfa-2b/ RBV	223 (62%)	146 (69%)	77 (51%)
INF alfa-2b	93 (26%)	32 (15%)	61 (41%)
Dose reduction	143 (40%)	85 (40%)	58 (39%)
Discontinuation	150 (42%)	66 (31%)	84 (56%)
Sustained virologic response	154 (43%)	89 (42%)	65 (43%)

LDLT, living donor liver transplantation; PEG-INF, pegylated-interferon; RBV, ribavirin; INF, interferon.

The association between achieving SVR and graft/patient survival after liver transplantation for HCV-positive recipients is a matter of debate [10]. Many studies with standard dual treatment of PEG-INF/RBV for 12 months in a DDLT setting have implied a survival benefit of achieving SVR [8,22], but there has been no evidence to support the recommendation of antiviral treatment for recurrent graft hepatitis C due to the lack of clinical benefit with sufficient long-term observation and the existence of frequent severe adverse effects, as concluded by a recent Cochrane meta-analysis [10]. Recent retrospective cohort studies with a long follow-up duration reported improved patient/graft survival in patients who obtained an SVR after antiviral treatment [23–25]. In accordance with those reports, our retrospective analysis indicated a positive effect of achieving SVR on patient survival. Caution should be taken in interpreting our results; however, as SVR was assessed among the whole cohort, including patients who were not indicated for antiviral treatment, the follow-up period after achieving SVR was rather short, and most importantly, a large variety of antiviral treatment regimens were used in Japan, which will be described later.

A noteworthy finding in the present retrospective analysis is the impaired patient survival in recipients who received a non-right liver graft (left liver in 239 cases and right lateral sector in 16 cases). Recent studies comparing outcomes between LDLT and DDLT in HCV-positive recipients have reported equal or even improved outcomes both in patient/graft survival and in fibrosis progression in the LDLT setting, which could be attributed to the younger donor age and shorter ischemic time of LDLT grafts [13,14,26–29].

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Based on these findings, LDLT for HCV-positive recipients is now widely accepted as an established alternative to DDLT, even in Western countries. On the contrary, however, the present finding may raise an alarm for reduced size grafts, as a left or posterior graft is clearly smaller than a right liver graft. Another point to be emphasized here is that all LDLTs investigated in the aforementioned studies comparing LDLT and DDLT were universally performed with right liver grafts. One possible explanation for the inferior outcome of the smaller graft is that the intense hepatocyte proliferation that occurs in smaller partial liver grafts may lead to increased viral translation and replication, as advocated by previous authors [30–32]. However, there are several limitations among these speculations. First, the data of the viral load, which is reported to reach a maximum level between the first and third post-transplant months [33], were not available in this study to demonstrate the higher viral replication in the smaller grafts during this period. Another is that the graft type selection is based on the ratio of the volume of the graft to recipient body weight or standard liver volume in our society, which will lead to the bias in the comparison of the right liver versus non-right liver graft. Despite these limitations, considering that comparable outcomes between left liver graft and right liver graft have been reported by us [34] and others [35] in LDLT recipients as a whole, caution should be taken in selecting the type of graft (left versus right) for HCV-positive recipients. Thus, future LDLT studies are required to investigate whether a smaller partial liver graft (left liver) is potentially inferior compared with a larger graft (right liver) in terms of graft/patient survival and recurrent hepatitis severity among HCV-positive recipients.

The antiviral treatment for recurrent hepatitis C after LDLT in Japan was also reviewed in the present study. As described elsewhere in detail [11], the antiviral treatment regimen in Japan differs widely from center to center; preemptive treatment versus treatment after confirmation of recurrent disease, starting dose and method of escalation, and the duration of treatment (usually longer than 12 months). Consequently, our data only present an overview of antiviral treatment in Japan, and no definite conclusion can be drawn regarding the actual efficacy of antiviral treatment after LDLT. Moreover, based on the recent prospective, multicenter, randomized study by Bzowej *et al.* [36], European and USA transplant societies do not support the routine use of preemptive antiviral therapy. A review of Western literature regarding the standard 12-month PEG-INF/RBV treatment for established recurrent hepatitis C after DDLT reveals that the median SVR rate is 33% (0–56%) with a dose reduction rate of 70% and a discontinuation rate of 30% [37]. The present result of an SVR rate of 43% with a dose reduction rate of 40% and a discontinuation rate of 42% seems not so different from those of previous literatures; however, as discussed above, the diversity in the methods, the doses, and the duration of treatment in Japan preclude the direct comparison with Western findings.

Conclusion

This retrospective analysis of the largest series of LDLT for HCV-positive recipients in Japan revealed 5- and 10-year survival rates of 72% and 63%, respectively, and that donor age (>40), non-right liver graft, an acute rejection episode, and the absence of SVR are independent predictors of patient survival. Based on the present result, caution should be made in the selection of the left liver graft for HCV-positive recipients; however, the development of more effective antiviral treatment in the near future may facilitate the application of the left liver graft.

Authorship

YM: designed the study. TI: collected data. NA, YS, NK, SE, TF, HO, HN, AT, YK, MS, YK, KY, KS, MM and MT: performed the study. NA and YS: analyzed and wrote the paper.

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Impact of alloimmune T cell responses on hepatitis C virus replication in liver transplant recipients

Yuka Tanaka [a,c], Masahiro Ohira [a,c], Hirotaka Tashiro [a,c], Michio Imamura [b,c], Kazuaki Chayama [b,c], Hideki Ohdan [a,c,*]

[a] Gastroenterological and Transplant Surgery, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, Japan
[b] Medicine and Molecular Science, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, Japan
[c] Liver Research Project Center, Hiroshima University, Japan

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ABSTRACT

We investigated the influence of alloimmune T cell responses on hepatitis C virus (HCV) replication in HCV-infected patients after liver transplantation (LT). To monitor the immune-status in 27 HCV-infected LT recipients, we routinely performed mixed lymphocyte reaction (MLR) assays within 4 weeks after LT. HCV RNA titers in most patients fluctuated in inverse proportion to the stimulation index (SI) of anti-donor reactive T cells early after LT. Two weeks after LT, recipients with high HCV RNA titers (>1000 KIU/mL) displayed a significantly lower SI for anti-donor reactive T cells than recipients with low HCV RNA titers did (<1000 KIU/mL). An *in vitro* transwell assay mimicking the anatomical features of the interaction between HCV-infected hepatocytes and alloreactive T cells in allograft livers demonstrated that interferon (IFN)-γ was necessary to suppress HCV replication. This study proves the significant impact of alloimmune T cell responses on HCV replication in HCV-infected LT recipients.

1. Introduction

Hepatitis C virus (HCV) infection is the most common indication for liver transplantation (LT) worldwide; however, recurrence of HCV post transplantation is almost universal and proceeds at an accelerated rate. Studies of HCV kinetics after LT have demonstrated that there is a sharp decrease in serum HCV RNA during the anhepatic phase and immediately after graft reperfusion, and this is followed by a steady increase in viral concentrations within days, suggesting a massive uptake of HCV virions and establishment of replication in the allograft [1–4]. Such studies have also revealed that HCV viral load reaches a plateau above the pre-transplantation level in most individuals, but markedly fluctuates in some individuals within a month after LT. Although it is generally believed that the resumption of viral replication is hastened by immunosuppression that accompanies organ transplantation, the mechanism underlying the instability of HCV RNA levels in some recipients remains entirely unknown.

We have previously shown that interleukin (IL)-2-stimulated $CD56^+$ cells derived from the liver exhibit inhibitory effects on HCV replication [5]. During the first month after LT, HCV RNA titers in the sera of recipients receiving immunotherapy with interferon (IFN)-γ-secreting natural killer (NK) cells derived from liver allografts were markedly lower than those in the sera of recipients who did not receive the immunotherapy [5]. Additionally, an *in vitro* study using genomic HCV replicon-containing hepatic cells revealed that the IFN-γ-secreting NK cells play a pivotal role in such anti-HCV responses [5,6]. Given these findings and the well-known capacity of T cells to actively produce IFN-γ as part of an alloimmune response after organ transplantation, we hypothesized that alloimmune T cell responses influence HCV replication in the recipients of allogeneic LT. This may explain the fluctuation of HCV viral load in some individuals after LT. To address this possibility, we monitored anti-donor alloreactivity at regular intervals by using a mixed lymphocyte reaction (MLR) assay employing an intracellular carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling technique [7]. This allowed us to examine the relationship between alloimmune responses and HCV replication.

Abbreviations: HCV, hepatitis C virus; LDLT, living donor liver transplantation; mAbs, monoclonal antibodies; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PBMC, peripheral blood mononuclear cell; CFSE, carboxyfluorescein diacetate succinimidyl ester; FCM, flow cytometry.

* Corresponding author at: Gastroenterological and Transplant Surgery, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Fax: +81 82 257 5224.
E-mail address: hohdan@hiroshima-u.ac.jp (H. Ohdan).

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2. Materials and methods

2.1. Patient population

This study was approved by the institutional review boards of Hiroshima University Hospital, Hiroshima, Japan (number 40017). Twenty-seven consecutive patients with HCV infection who underwent adult-to-adult living donor LT (LDLT) at Hiroshima University Hospital between 2004 and 2011 were enrolled in this study. The patients included 19 men and 8 women. Patient profiles are shown in Table 1.

2.2. CFSE-MLR assay

To monitor the immune status of the participants, an MLR assay using a CFSE-labeling technique was performed before LT as a baseline and again at 1, 2, and 4 weeks after LT, with the consent of the recipients and donors. In brief, peripheral blood mononuclear cells (PBMCs) were obtained from the recipients (autologous control), donors, and healthy volunteers (third-party control), prepared as stimulator cells, and irradiated with 30 Gy; responder cells from the recipients were labeled with 5-(and 6)-CFSE (Molecular Probes, Inc., Eugene, OR). Both the stimulator and responder cells were adjusted to 2×10^6 cells/mL of AIM-V medium (Invitrogen, NY, USA) and co-cultured in a total volume of 2 mL of the medium in 24-well flat-bottom plates (BD Labware, Franklin Lakes, NJ) incubated at 37 °C in a 5% CO_2 incubator in the dark for 5 days. After MLR culture, the non-adherent cells were harvested and stained using either phycoerythrin-conjugated anti-CD4 or CD8 mAb and allophycocyanin-conjugated anti-CD25 mAb. Four-color flow cytometry (FCM) was performed on a FACSCalibur® dual-laser cytometer (Becton Dickinson, Mountain View, CA) using standard Cell Quest™ acquisition/analysis, and fluorescence compensation was achieved using an appropriate single fluorochrome-labeled sample. Dead cells, identified by light scatter and propidium iodide staining, were excluded from the analysis.

2.3. Quantification of alloreactive T cell proliferation by FCM analysis

The precursor frequency (PF), mitotic index (MI), and stimulation index (SI) were quantitatively estimated using a previously described method [8]. In brief, the CFSE fluorescence intensity at peak cell division, which was divided once, shows the half-value of CFSE fluorescence intensity at the peak of nonreactive cell division. Divisions of reactive cells, which were identified and determined by their CFSE intensities, were labeled from 0–n to indicate the dividing time. A single cell dividing n times will generate 2^n daughter cells (Fig. 1A). Using this mathematical relationship, the number of division precursors was extrapolated from the number of daughter cells of each division and from proliferation events and PF in $CD4^+$ and $CD8^+$ T cell subsets. These values were used to evaluate mitotic events and calculate MIs. SIs were calculated by dividing MIs of allogeneic combinations by MIs of autologous controls.

Table 1
Characteristics of the study population ($n = 27$).

Median age recipient (year, range)	56.5 (49–68)
Median age donor (year, range)	35.9 (18–59)
Mean HCV RNA level pre-LT (KIU/ml, range)	3581.1 (19–69,000)
HCC (n)	16
Child-Pugh score (n): A/B/C	3/15/9
HCV genotype (n): 1b/2a/2b/1b and 2b	21/2/2/2
Donor relation (n)	
Children/siblings	19/3
Non-blood relations spouses/non-spouses	4/1
HLA-mismatch number (n): 1/2/3/4/5/6	3/12/8/3/0/1

We also analyzed CD25 expression in the proliferating $CD8^+$ T cell subset in response to anti-donor and anti-third party stimuli (Fig. 1B). We previously found that the remarkable elevation of CD25 expression in proliferating $CD8^+$ T cells reflects their cytotoxic activity toward donor cells [7,9]. In preliminary studies using PBMCs from healthy volunteers, the average $CD25^+$ cell proportion in proliferating $CD8^+$ T cells in response to human leukocyte antigen (HLA)-disparate donor cells without any immunosuppressive treatment was 41.01 ± 19.67% (ranging from 16.5–75.2%, $n = 10$). Thus, the positive threshold for this parameter was determined to be >50% [9].

2.4. Immunosuppressive protocol

The basic immunosuppressive regimen after LDLT was comprised of tacrolimus (TAC) and methylprednisolone, with gradual tapering of doses. Patients with renal insufficiency (RI) (eGFR < 60 mL/min/1.73 m^2) received a calcineurin inhibitor-sparing immunosuppressive regimen (CSR) comprising of a reduced dose of TAC, methylprednisolone, and MMF. In the regimen for patients without RI, trough whole blood levels of TAC were maintained between 8 and 15 ng/mL in the first few postoperative weeks and between 5 and 10 ng/mL thereafter. In the CSR, the trough whole blood levels of TAC were maintained between 5 and 10 ng/mL in the first few postoperative weeks and between 3 and 5 ng/mL thereafter. Optimal dosages of immunosuppressive drugs were determined based on immune monitoring by MLR assay. MLR assays were performed at 2–4-week intervals until 3 months after LT, and thereafter at intervals of 3–6 months.

Based on the proliferation analyses of $CD4^+$ and $CD8^+$ T cell subsets in response to the anti-donor and anti-third party stimuli in MLR, the immune status was categorized as hypo-, normo-, or hyper-response [7,9]. Therapeutic adjustments for immunosuppressants were determined by tapering the dosage in cases exhibiting anti-donor hypo-response in both T cell subsets or increasing the dosage in cases exhibiting anti-donor hyper-response in the $CD4^+$ or $CD8^+$ T cell subsets. SIs for $CD4^+$ T cells, which actively secrete IL-2 in proportion to their proliferation state, were used as an indicator for adjusting the doses of calcineurin inhibitors. Alternatively, SIs for $CD8^+$ T cells, which secrete IFN-γ in proportion to their proliferation state, were used as an indicator for adjusting the doses of methylprednisolone [7,9]. Patients who were diagnosed with acute rejection (by liver allograft biopsy and CFSE-MLR assay) received additional steroid-pulse therapy if necessary.

2.5. Trans-well assay

The Huh7/Rep-Feo cell line (HCV replicon cells) was gifted by Dr N. Sakamoto (Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan). A genotype 1b HCV subgenomic replicon plasmid, pRep-Feo, was derived from pRep-Neo (originally, pHCVIbneo-delS) [10]. pRep-Feo carries a fusion gene comprising firefly luciferase (Fluc) and neomycin phosphotransferase, as described elsewhere [11,12]. After culturing in the presence of G418 (Invitrogen, Carlsbad, CA), Huh7/pRep-Feo cell lines stably expressing the replicons were established. For co-culture experiments, trans-well tissue culture plates (pore size: 1 μm; Costar, Cambridge, MA) were used. HCV replicon-containing hepatic cells (10^5 cells) were incubated in the lower compartment with varying number of lymphocytes from healthy volunteers in the upper compartment. The hepatic cells in the lower compartments were collected for the luciferase assay, 5 days after co-culture. Luciferase activities were measured with a luminometer (Lumat LB9501; Berthold Technologies, Germany) using the Bright-Glo Luciferase Assay System (Promega, Madison, WI).

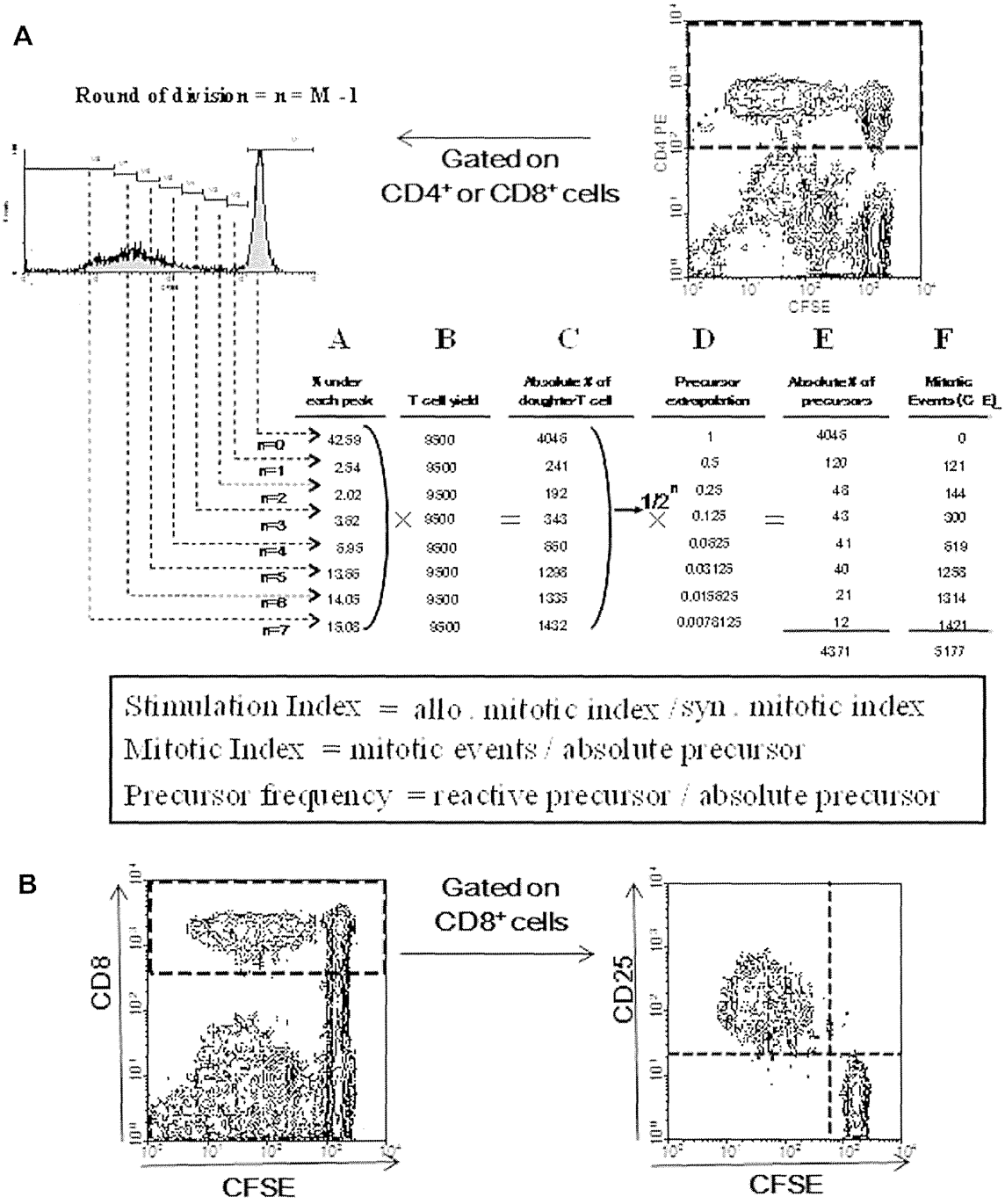

Fig. 1. Quantitative estimation of T cell proliferation in response to allostimulation by MLR assay using a CFSE-labeling technique. PBMCs obtained from recipients, donors, and third-party healthy volunteers were prepared as stimulator cells and irradiated, and responder cells from recipients were labeled with CFSE. Both cell types were co-cultured for 5 days. After MLR culture, cells were stained with either phycoerythrin-conjugated CD4 or CD8 mAb together with allophycocyanin-conjugated CD25 mAb, followed by four-color FCM. (A) PF and MI of alloreactive $CD4^+$ or $CD8^+$ T cells were calculated. The representative FCM profile for analyzing PF and MI of alloreactive $CD4^+$ is shown. (B) CD25 expression for the proliferating $CD8^+$ T cell subset in response to anti-donor or anti-third party stimuli was also determined. The representative FCM profile for analyzing the proportion of $CD25^+$ cells among alloreactive $CD8^+$ is shown.

When indicated, the assays were performed in the presence of varying doses of anti-human IFN-γ mAb or isotype-matched control rat IgG (R&D Systems, Minneapolis, MN). All the assays were performed in duplicate.

2.6. Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test or one-way analysis of variance (ANOVA). Differences with $P < 0.05$ were considered significant.

3. Results

3.1. HCV RNA titer fluctuations are inversely proportional to the SI of anti-donor reactive T cells

The MLR assay using a CFSE-labeling technique enables the determination of the SI of alloreactive $CD4^+$ and $CD8^+$ T cells, separately, as parameters of T cell responses to allogeneic stimulation [8]. Notably, the SI of anti-donor reactive T cells, as determined by MLR assay, was closely associated with the patient's HCV RNA

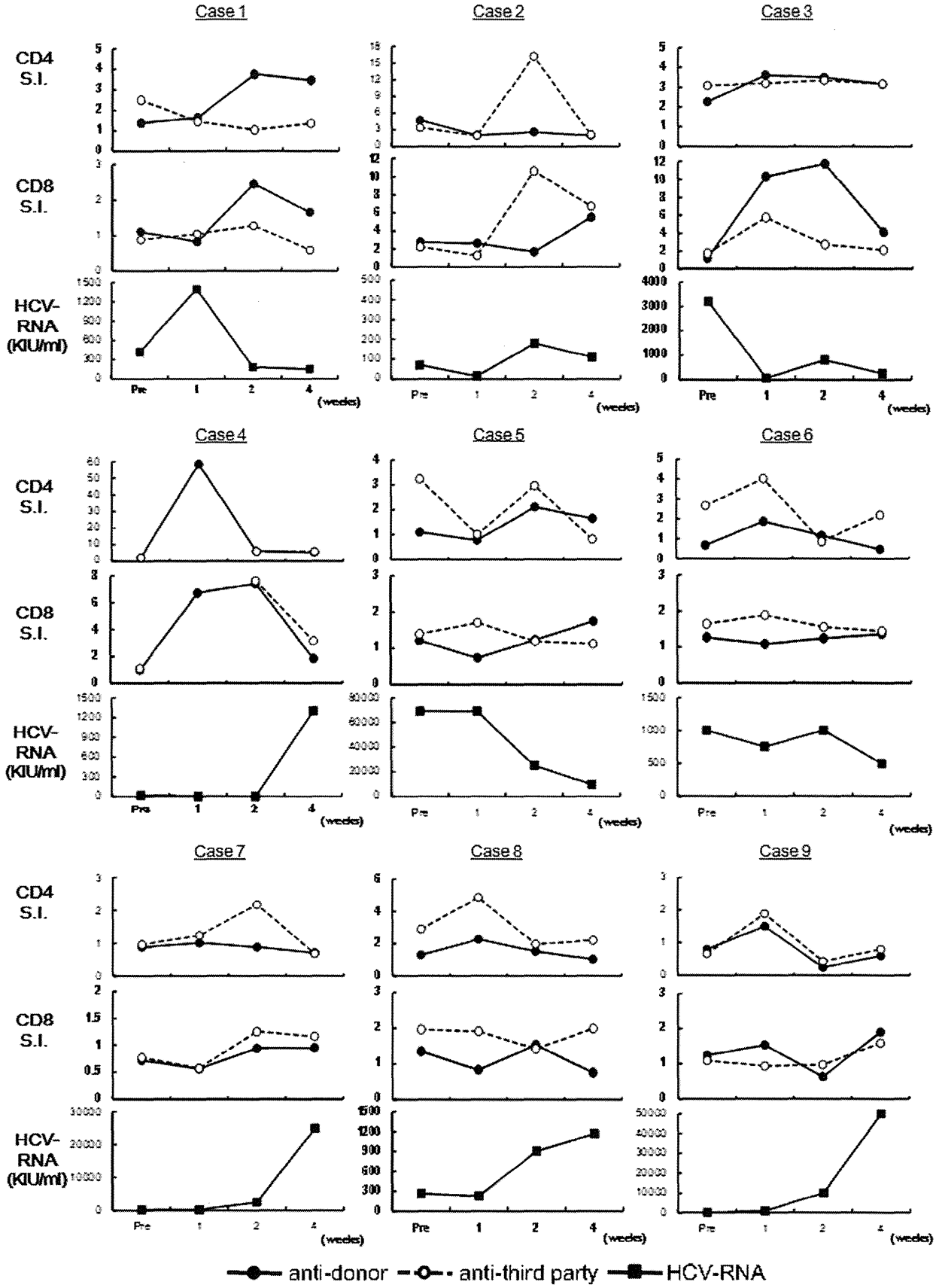

Fig. 2. Fluctuations of HCV RNA titers were inversely proportional to the SI of anti-donor reactive T cells in the majority of cases. Representative kinetics of SI of anti-donor or anti-third party reactive $CD4^+$ and $CD8^+$ T cells and HCV RNA titers in sera of patients within 4 weeks after LT. Representative nine patients were selected to demonstrate the relationship between SI for anti-donor T cells and serum HCV RNA titers. The data from the other 18 patients are shown in Supplementary Fig. 1.

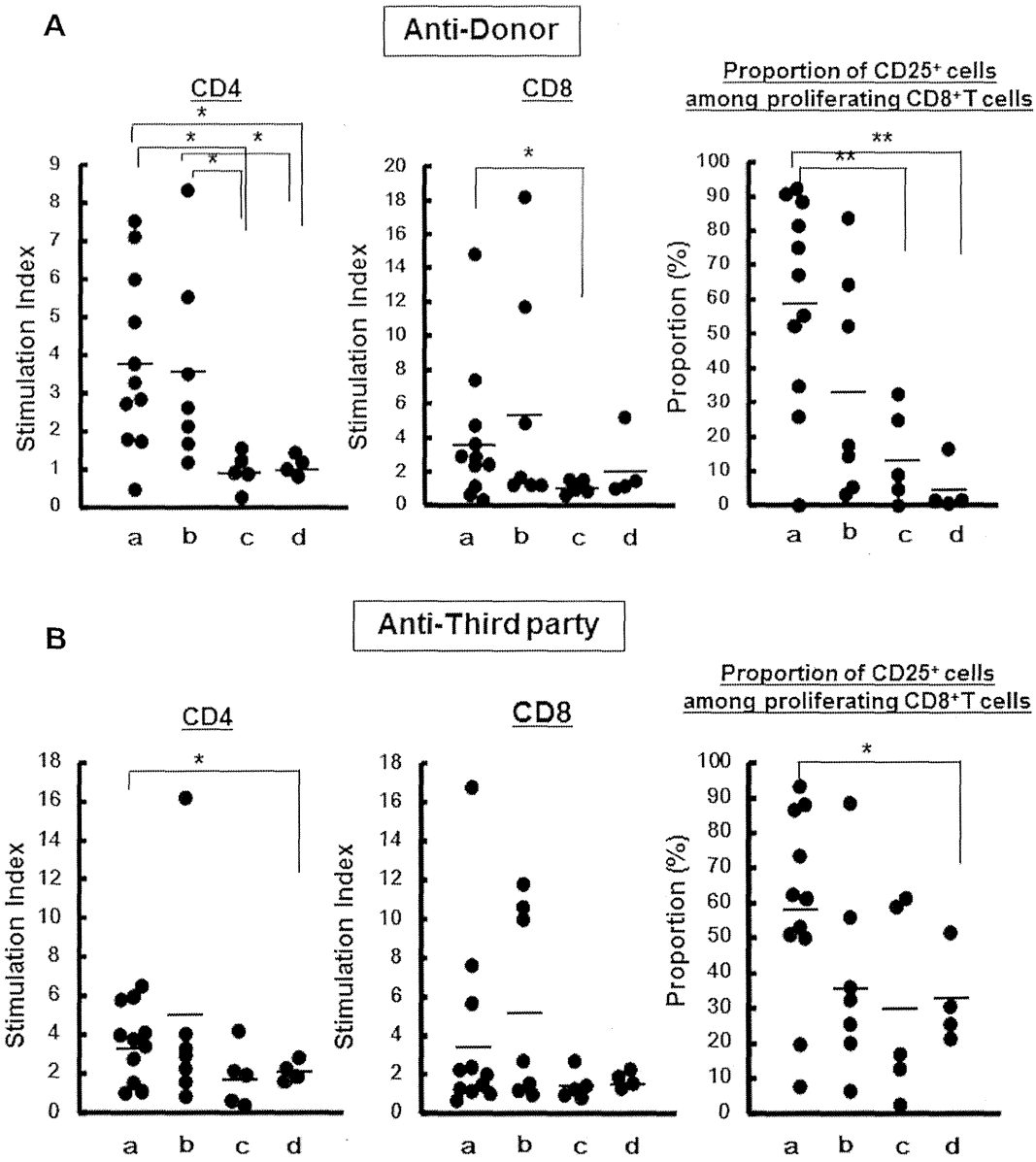

Fig. 3. Relationship between SI for anti-donor or anti-third party reactive T cells and HCV RNA titers in LT recipients 2 weeks after LT. HCV-infected patients were divided into 4 groups 2 weeks after LT: (a) HCV RNA, ⩽100 KIU/mL (n = 11); (b) 100–1000 KIU/mL (n = 7); (c) 1000–10,000 KIU/mL (n = 5); and (d) >10,000 KIU/mL (n = 4). SI for anti-donor (A) or anti-third party (B) reactive $CD4^+$ and $CD8^+$ T cells and proportion of $CD25^+$ cells among proliferating $CD8^+$ T cells. Each point represents an individual value. Lines indicate the average values of the group. Statistical analysis was performed using the Mann–Whitney U-test. *P < 0.05, **P < 0.01.

serum titer. In most patients, HCV RNA levels rapidly decreased after LT and subsequently began to increase, exceeding pre-transplantation levels within a few days after the procedure (data not shown). This observation is consistent with previous studies demonstrating HCV dynamics after LT [1–4]. Thereafter, HCV RNA titers characteristically fluctuated in inverse proportion to the SI of anti-donor reactive $CD4^+$ and $CD8^+$ T cells in representative patients (Fig. 2 and Supplementary Fig. 1).

In some MLR assays, transiently increased anti-third party response was observed (e.g., case 2). Since LT recipients frequently require blood transfusion during the perioperative period, allosensitization induced by blood transfusion might cause the unexpected anti-third party responses. Nevertheless, 2 weeks after LT, recipients with high HCV RNA titers (>1000 KIU/mL) displayed a significantly lower SI for anti-donor reactive $CD4^+$ T cells than recipients with low HCV RNA titers did (<1000 KIU/mL) (Fig. 3). A similar trend was observed relative to the SI for anti-donor reactive $CD8^+$ T cells. The proportion of $CD25^+$ cells among $CD8^+$ T cells responding to allostimulation, which actively produce IFN-γ [7,8], was lower in the recipients with high HCV RNA titers (>1000 KIU/mL) than in the recipients with low HCV RNA titers (<1000 KIU/mL). We also observed a similar trend in the anti-third party reactive T cell responses relative to the HCV RNA titers at this time point, although it was not as remarkable as the anti-donor reactive T cell responses.

Four weeks after LT, the similar relationship between HCV RNA titers in the recipients and SI for anti-donor reactive T cells was no longer remarkable (Fig. 4). At this time point, two patients received antiviral therapy with IFN-α and ribavirin, likely influencing the relationship between HCV replication and alloimmune responses. Thereafter, anti-donor responses determined by MLR assay lessened over time in many patients [9], likely reflecting adequate immunosuppressive therapy. This clinically preferable trend did not allow us to analyze the probable association of alloimmune

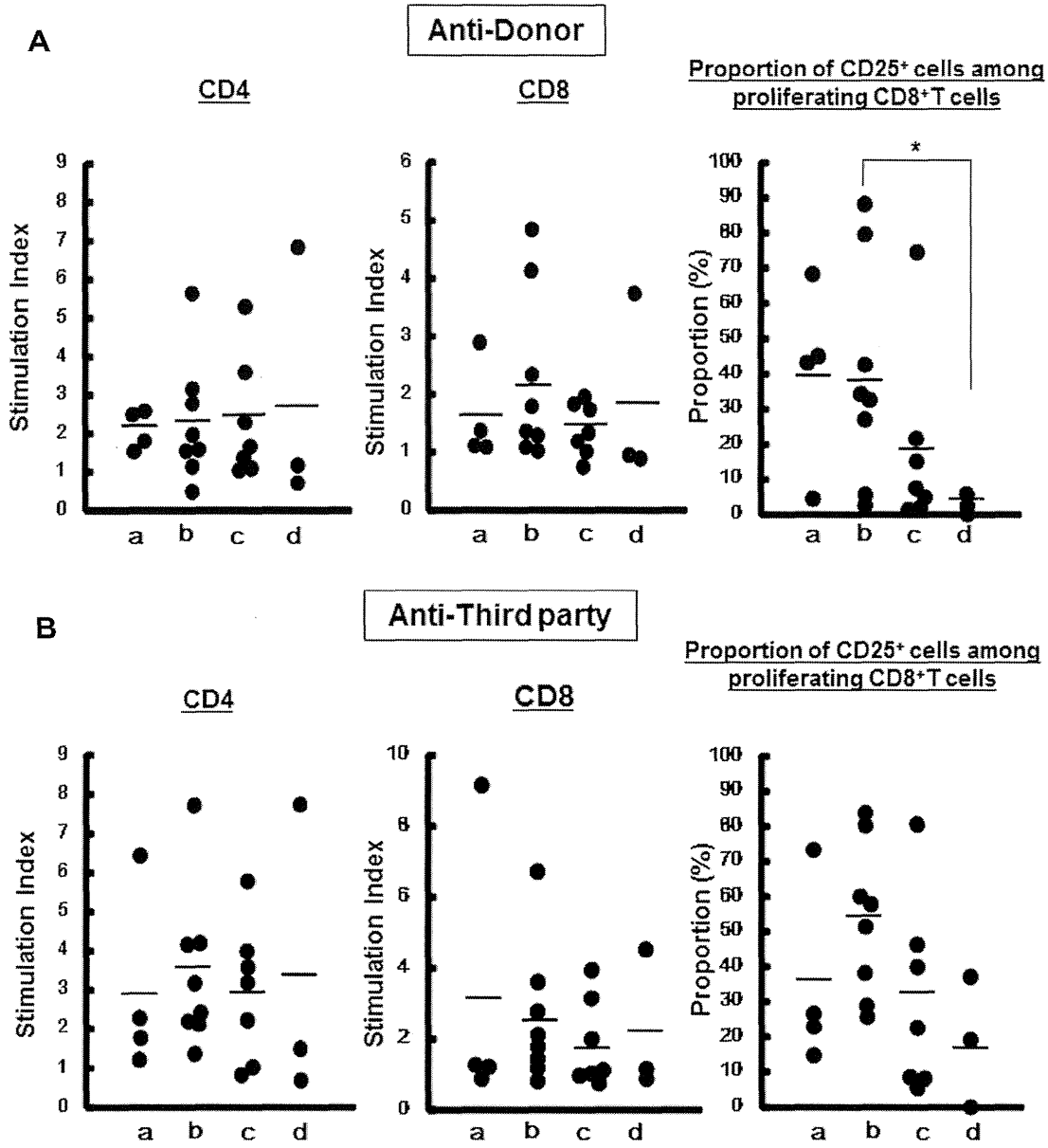

Fig. 4. Relationship between SI for anti-donor or anti-third party reactive T cells and HCV RNA titers in LT recipients 4 weeks after LT. HCV-infected patients were divided into 4 groups 4 weeks after LT: (a) HCV RNA, ≤100 KIU/mL ($n = 4$); (b) 100–1000 KIU/mL ($n = 8$); (c) 1000–10,000 KIU/mL ($n = 7$); and (d) >10,000 KIU/mL ($n = 3$). MLR was not performed in 5 patients at this time point because of a lack of donor consent. SI for anti-donor (A) or anti-third party (B) reactive $CD4^+$ and $CD8^+$ T cells and proportion of $CD25^+$ cells among anti-donor $CD8^+$ T cells. Each point represents an individual value. Lines indicate the average values for groups. Statistical analysis was performed using the Mann–Whitney *U*-test. $^*P < 0.05$.

responses with the progression of fibrosis and/or response to antiviral therapy.

Despite a significant influence of alloimmune responses on hepatitis C viral load in HCV-infected patients during the early phase after LT, we did not observe a significant relationship between the extent of HLA mismatch and HCV RNA titers in HCV-infected patients, both 2 and 4 weeks after LT (Fig. 5).

3.2. Allostimulated lymphocytes inhibited RNA replication of genomic HCV replicons in an HLA-dependent manner

The type and level of cytokines produced in response to allostimulation may affect HCV survival and replication in the host. Alloreactive T cells infiltrate the portal area in liver grafts, even in the early phase of alloimmune responses. Therefore, we hypothesized that cytokines produced from these activated anti-donor T cells inhibit HCV replication. To mimic the anatomical features of the interaction between HCV-infected hepatocytes and liver-infiltrated alloreactive T cells, we performed a trans-well culture assay. This consisted of a one-way MLR culture using PBMCs from healthy volunteers in upper chamber, resembling allostimulated T cells infiltrated in the sinusoid of liver allografts, and the genotype 1b Huh7/Rep-Feo subgenomic HCV replicon in lower chamber, resembling HCV-infected hepatocytes. These HCV replicon-containing cells were chosen because 23 of the 29 patients in the clinical study were infected with HCV genotype 1b.

An HLA haplo-identical 3-loci mismatched MLR combination significantly inhibited HCV replication; HCV replication was further suppressed by a fully allogeneic 6-loci mismatched MLR combination (Fig. 6A and B). Based on our previous finding that alloreactive $CD8^+$ T cells actively produce IFN-γ [8] and recombinant IFN-γ strongly inhibits HCV replication [5], we hypothesized

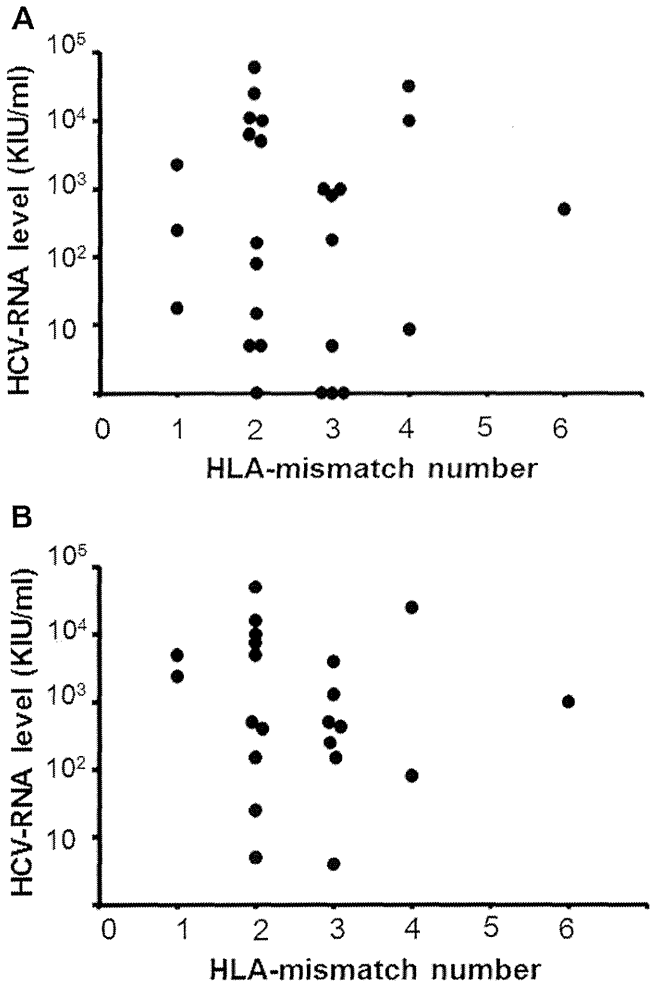

Fig. 5. Relationship between the number of HLA mismatches and HCV RNA titers in LT recipients 2 and 4 weeks after LT. The relationship between the number of donor–recipient HLA mismatches (HLA-A, -B, and -DR) and serum HCV RNA titer at (A) 2 and (B) 4 weeks after LT is shown. Each point represents an individual value.

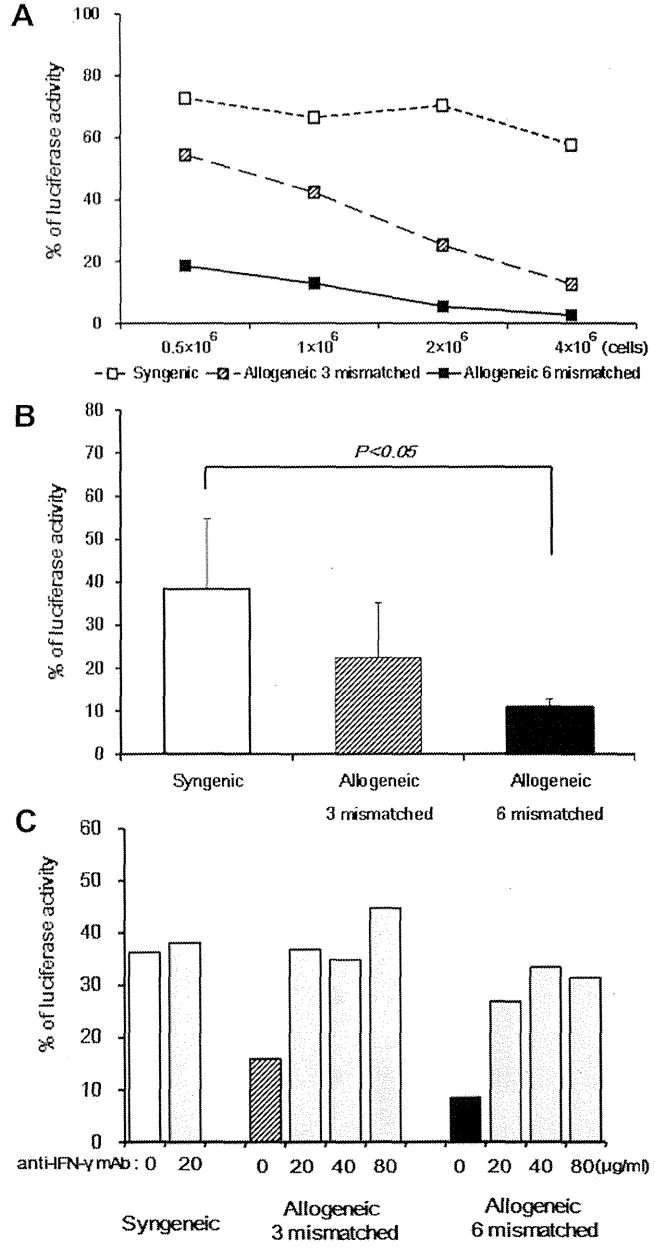

Fig. 6. Allostimulated lymphocytes inhibited RNA replication of genomic HCV replicons in an HLA-dependent manner. Trans-well co-culture of one-way MLR (upper chamber) using PBMCs from healthy volunteers and HCV-replicon-permissive hepatic cells (lower chamber) was performed. HCV replication was detected by luciferase activity encoded by an HCV replicon plasmid. (A) HCV-replicon-containing hepatic cells (10^5 cells/well) incubated with syngeneic, one-haplo-mismatched (3-mismatched), or fully allogeneic (6-mismatched) MLR combinations were collected for luciferase assay 5 days after co-culture. MLR assays were performed at doses of 0.5, 1, 2, and 4×10^6 lymphocytes of stimulator and the same dose of responder lymphocytes in a well. The lymphocyte doses are indicated on the *x*-axes. Results are representative of 2 similar experiments. (B) To statistically analyze the influence of allostimulated lymphocytes on HCV RNA replication, 5 similar experiments were performed using stimulator/responder lymphocytes at a dose of 1×10^6 cells/well. Results are presented as average values ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) ($^*P < 0.05$). (C) Various doses of neutralizing anti-IFN-γ were added to the abovementioned co-cultures. Results are representative of 2 similar experiments.

that the allostimulation-induced IFN-γ inhibits HCV replication after LT. Adding an anti-IFN-γ neutralizing monoclonal antibody (⩾20 μg/mL) to the co-cultures reversed the inhibitory effect of allostimulation on HCV replication (Fig. 6C). Thus, IFN-γ from activated anti-donor T cells is required for inhibiting HCV replication.

4. Discussion

Several risk factors such as genotype, donor age, and rejection treatment have been shown to affect the severity of HCV recurrence after LT, although the mechanisms remain unknown [13–16]. It has been speculated that HLA mismatches are also involved in the development and course of recurrent HCV [17]. However, the impact of HLA matching on HCV load in LT recipients remains controversial. It has been reported that the incidence of recurrent hepatitis B virus/HCV infection after LT is significantly higher for HLA-B-compatible liver transplant recipients [18,19]. In contrast, HLA-B14 and HLA-DRB1*04 matching has been demonstrated to provide beneficial effects on the outcome of patients receiving LTs for HCV hepatitis [20]. This might be explained by the fact that MHC-I restricted T cells are involved in the control of postoperative HCV spread [21,22].

It has also been shown that progression of fibrosis increases in patients with fewer HLA mismatches within the first year after LT, although HLA matching does not influence graft survival in patients after LT for end-stage HCV infection [23]. Thus, HLA matching has been thought to influence the outcome of LT in HCV-infected patients through 2 separate mechanisms: (1) the lower incidence of rejection in better-matched grafts leads to a

reduction in anti-rejection treatments, which has been shown to increase HCV-related graft loss, or (2) the lack of MHC I/II-restricted T cell response in patients with a complete HLA mismatch leads to less severe recurrent hepatitis. In this study, we could not observe a significant relationship between the extent of HLA mismatch and HCV RNA titers in HCV-infected patients during the first 4 weeks after LT (Fig. 5). Instead, we found a significant influence of alloimmune responses on hepatitis C viral load in HCV-infected patients during the early phase after LT.

Since liver transplant recipients generally share more HLA alleles with living donors than with deceased donors, anti-living donor T cell responses are likely weaker than anti-deceased donor T cell responses. Such variance may not significantly influence the function of allograft livers but could substantially impact HCV replication after LT, as HCV RNA levels rise more rapidly in patients receiving grafts from living donors than in those receiving grafts from deceased donors [24]. This interpretation seems consistent with our novel finding that alloimmune responses decrease HCV load in LT recipients (IFN-γ produced by anti-donor reactive T cells shows anti-HCV activity).

The influence of alloimmune responses on HCV load was apparent only during the early phase after LT (2 weeks) in this study. One possible explanation for this transient phenomenon is that alloimmune responses might interfere with viral resurgence in the uninfected liver allograft, but it did not significantly affect HCV replication after reinfection of the liver allograft. Nonetheless, it remains unclear whether alloimmune responses interfere with the progression of HCV (virus survival and progression of fibrosis) in LT recipients. Minimizing exposure of HCV-infected LT recipients to immunosuppressants by monitoring anti-donor T cell responses would help prevent excessive HCV viremia, at least during the early viral resumption phase after LT. However, the long-term benefit of this proposal for preventing hepatitis or fibrosis remains to be elucidated.

Our *in vitro* study demonstrated that IFN-γ produced by allostimulated lymphocytes inhibited HCV replication. This would consistently explain the instability of HCV RNA levels in some recipients in association with their T cell alloimmune response during the early phase after LT. Another explanation for these results is that the anti-donor immune-activity merely represents the recipient's immune status, including anti-HCV immunity. In cases where the LT recipients were excessively immunosuppressed, hypo-responses of T cells to both anti-donor and anti-third party stimuli in MLR assays may occur. Thus, unless patients are in donor-specific hyper- or hypo-responsive states (reflecting rejection or tolerance, respectively), anti-third party T cell responses potentially change in concert with their anti-donor responses. This might be a possible reason why we observed a similar trend in the anti-third party reactions relative to the HCV RNA titers as the anti-donor reactions in this study. A previous finding also suggests that LT patients with severe HCV recurrence are immunologically impaired compared with patients with mild HCV recurrence [25]. Thus, high HCV replication may lead to a state of general immune-impairment, leading to lower alloreactive activity. Further studies are needed to address this possibility.

One of the most difficult challenges in the care of HCV-positive LT recipients is the differentiation between acute cellular rejection and HCV recurrence, which can have considerable histologic overlap [26]. A method that can distinguish between HCV infection and HCV infection complicated by ACR has not yet been established. The findings in this study may indicate that the marked reduction of HCV RNA levels in sera of HCV-infected recipients of allograft livers could reflect acute rejection during at least the early phase after LT. A previous study also investigated whether *in vitro* donor-specific immune reactivity patterns could differentiate between patients undergoing a rejection episode or HCV recurrence [27]. In that study, no correlation was demonstrated between the frequency of IFN-γ-producing precursor T cells and rejection episodes. An intriguing correlation, however, was found between the frequency of donor-specific IFN-γ precursors and early recurrence of HCV in LT recipients; i.e., all of the patients who showed disease recurrence within the first year after transplant showed minimal frequency of IFN-γ-producing precursors. Both the results of the previous study and our study indicate that post-transplant immune monitoring of anti-donor T cells responses could identify patients at risk for early HCV recurrent.

In conclusion, this clinical study revealed that the instability of HCV RNA levels in some recipients is associated with the strength of T cell alloimmune response during the early phase after LT. This phenomenon can be explained by the *in vitro* study demonstrating that allostimulated lymphocytes inhibit RNA replication of genomic HCV replicons in an HLA-dependent manner.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2014.09.006.

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Hepatology Research

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Original Article

Sustained virological response to antiviral therapy improves survival rate in patients with recurrent hepatitis C virus infection after liver transplantation

Tomokazu Kawaoka,[1] Shoichi Takahashi,[1] Yoshiiku Kawakami,[1] Masataka Tsuge,[1] Akira Hiramatsu,[1] Michio Imamura,[1] Hideyuki Hyogo,[1] Hiroshi Aikata,[1] Kohei Ishiyama,[2] Hirotaka Tashiro,[2] Hideki Ohdan,[2] Junko Tanaka[3] and Kazuaki Chayama[1]

Departments of [1]Gastroenterology and Metabolism, and [2]Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, and [3]Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

Aim: Previous European and North American studies analyzed the relationship between survival rate and sustained virological response (SVR) to interferon (IFN) therapy in patients with recurrent hepatitis C viral (HCV) infection after liver transplantation (LT). The present study was designed to define the same relationship in Japanese patients who had undergone LT.

Methods: Forty-seven patients (genotype 1, 40; genotype 2, 7) with recurrent HCV after LT were treated with pegylated interferon (PEG IFN) or IFN/ribavirin (RBV). In possible, within 3 months after LT, patients started treatment with PEG IFN-α-2b or IFN-α-2b s.c. once weekly combined with RBV (200 mg/day).

Results: The SVR rate was 51% (24/47) for all patients, 42.5% (17/40) for genotype 1 and 100% (7/7) for genotype 2. The median follow-up period was 71 months (range, 24–152). The survival rate of 24 patients who achieved SVR was 95% at 5 years and 92% at 10 years. These rates were significantly better than those of 23 patients who did not achieve SVR (82% at 5 years, 58% at 10 years) ($P = 0.027$). Two patients of the SVR group died during follow up (due to hepatocellular carcinoma in one and chronic rejection in one), while six non-SVR patients died during the same period (three died due to liver failure by recurrent HCV).

Conclusion: SVR following IFN therapy contributes to improvement of survival rate in patients with recurrent post-LT HCV infection.

Key words: interferon therapy, HCV, LT, SVR

INTRODUCTION

THE HEPATITIS C virus (HCV) is estimated to have infected 170 million people worldwide, and such infection sometimes progresses to liver cirrhosis and/or hepatocellular carcinoma (HCC).[1] HCV-related end-stage liver disease is currently the main indication for liver transplantation (LT). However, the outcome of LT for patients with HCV-related liver disease has been less satisfactory than those with HCV negative liver disease.[2–8] HCV recurrence is universal after LT with accelerated progression to liver fibrosis. Approximately 20–25% of HCV positive patients develop cirrhosis within 5 years after LT, and approximately 50% within 10 years.[6,9,10] LT recipients with recurrent HCV are treated with a combination of pegylated interferon (PEG IFN)/ribavirin (RBV) for 48 weeks. However, eradication of post-LT HCV with IFN is often hampered by the use of immunosuppressants, anemia, frequent side-effects and the need to discontinue or reduce therapy. Unfortunately, the outcome of PEG IFN/RBV antiviral therapy after LT is often poor, with a sustained virological response (SVR) rate of only 10–30% in HCV-1-infected patients.[11–17]

Three European studies have reported that SVR improves patient survival.[18–20] Furthermore, two

Correspondence: Dr Yoshiiku Kawakami, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: kamy4419@hiroshima-u.ac.jp
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Canadian studies also confirmed the long-term benefits of SVR in LT recipients with HCV recurrence.[21,22] To our knowledge, however, there is only little information on the relationship between survival rate and virological response to IFN therapy in patients with post-LT recurrent hepatitis C in Japan.[23] The aim of this study was to determine the relationship between survival rate and virological response to IFN therapy in Japanese patients with post-LT recurrent hepatitis C.

METHODS

Patients

EIGHTY-TWO PATIENTS UNDERWENT living donor LT (LDLT) for HCV-related end-stage liver disease between 2000 to January 2013. Among them, 22 patients died before the start of antiviral therapy. Although patients started antiviral therapy, three patients who died within 2 years after the start of antiviral therapy were omitted. One did not become positive for HCV RNA after LDLT, and IFN therapy induced SVR before LT in four patients. Of the remaining 52 patients treated with IFN therapy at our institution, five remain currently on antiviral therapy. Thus, 47 patients were enrolled in the present retrospective study (Fig. 1). They included 40 patients with genotype 1 and seven with genotype 2. The median follow-up period was 71 months (range, 24–152).

Protocol of antiviral therapy

If possible, within 3 months after LDLT, patients started treatment with PEG IFN-α-2b or IFN-α-2b s.c. once

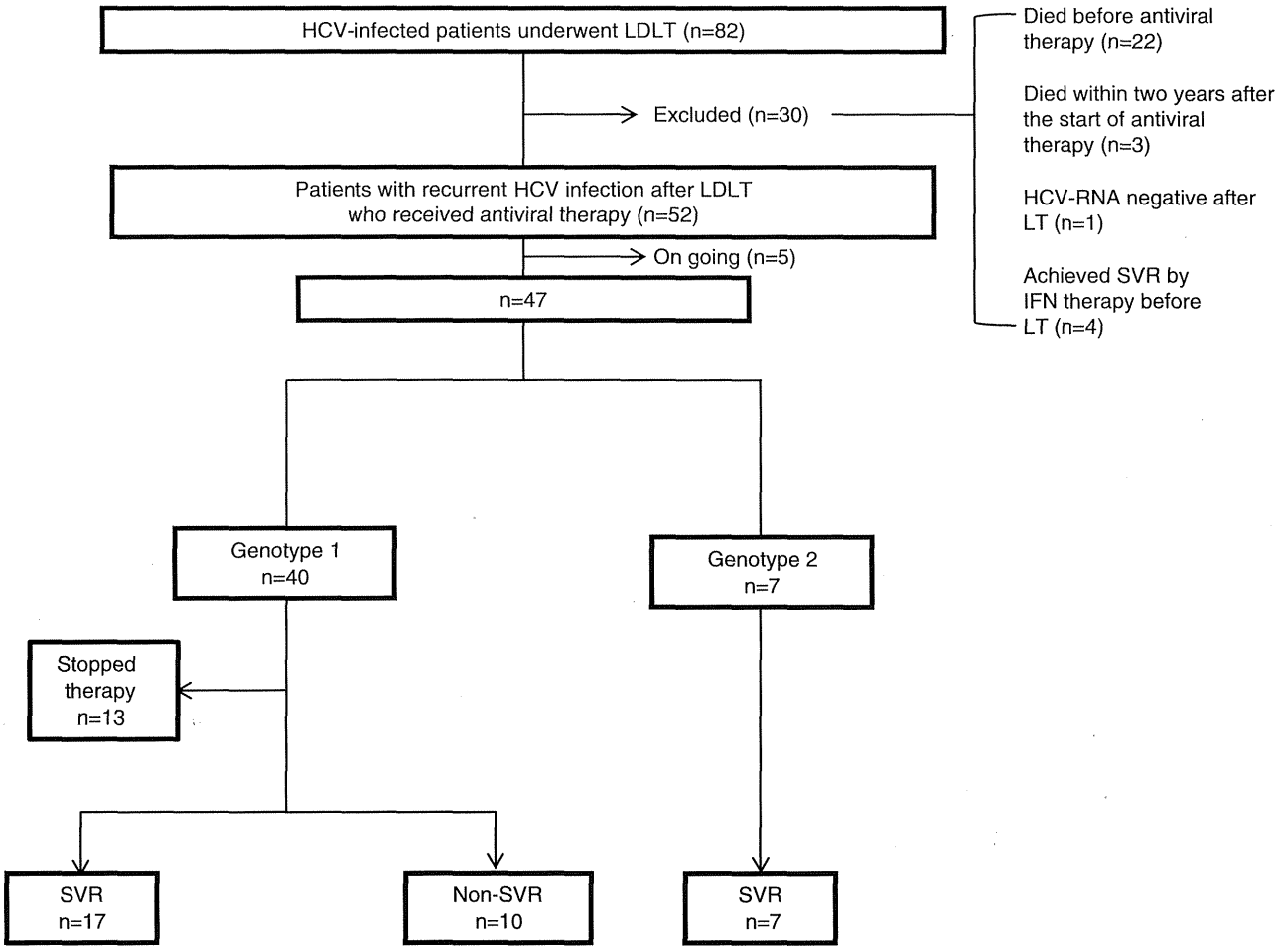

Figure 1 Effects of IFN therapy according to genotype. HCV, hepatitis C virus; IFN, interferon; LDLT, living donor liver transplantation; LT, liver transplantation; SVR, sustained virological response.

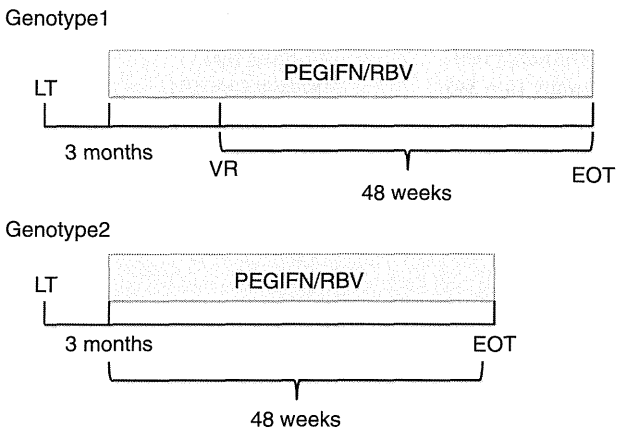

Figure 2 Our protocol of IFN therapy according to genotype after LT. EOT, end of treatment; LT, liver transplantation; PEG IFN, pegylated interferon; RBV, ribavirin; VR, viral response.

weekly combined with RBV (200 mg/day). The dose of the latter was increased to 800 mg/day in a stepwise manner according to individual tolerance within the first 12 weeks of therapy. The combination PEG IFN/RBV therapy was continued for more than 48 weeks in genotype 1 after the disappearance of serum HCV RNA. The combination PEG IFN/RBV therapy was continued for 48 weeks in genotype 2 (Fig. 2). At the end of the active treatment, patients were followed for another 24 weeks without treatment. In patients who remained positive for HCV RNA in spite of antiviral treatment for more than 48 weeks, PEG IFN was switched to PEG-IFN-α-2a and treatment was continued as described above.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committees of all participating centers.

Assessment of efficacy of therapy

Hepatitis C virus RNA levels were measured using one of several reverse transcription polymerase chain reaction-based methods (TaqMan RT-PCR test) at weeks 4, 8 and 12, and thereafter every 4 weeks of post-LT PEG IFN/RBV treatment, and at 24 weeks after the cessation of therapy.

Statistical analysis

Overall survival was calculated by Kaplan–Meier survival curves with log-rank survival comparisons and 95% confidence intervals (95% CI). Cox proportional hazards model was used to investigate the prognostic factors for overall survival. A *P*-value less than 0.05 denoted the presence of statistically significant difference. All statistical analyses were carried out with the Predictive Analytics Software version 21.0 (SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

TABLE 1 LISTS the baseline characteristics of the 47 patients with post-LT recurrent hepatitis C treated with PEG IFN or IFN/RBV. The median age of the patients (31 men and 16 women) was 57 years, and the median body mass index was 24.3 kg/m^2. The median latency between LDLT and start of antiviral therapy was 3 months. The median pretreatment serum HCV RNA viral load was 6.6 log IU/mL. LDLT-related immunosuppressive therapy included tacrolimus in 43 and

Table 1 Baseline characteristics of 47 patients who received IFN therapy for recurrent hepatitis C after liver transplantation

Recipient's age (years)†	57 (44–70)
Recipient's sex (male/female)	31/16
Donor's age (years)†	33 (18–60)
Donor's sex (male/female)	34/12/1
Graft volume† (mg)	698 (319.2–1256)
Hepatocellular carcinoma (yes/no)	29/18
Splenectomy (yes/no)	13/34
ABO incompatibility (yes/no)	2/45
Body mass index (kg/m^2)†	24.3 (18.8–42.2)
Living donor LT/orthotopic LT	46/1
Genotype (1/2)	40/7
White blood cells (/μL)†	4500 (1420–13720)
Hemoglobin (g/dL)†	10.8 (7.8–14.2)
Platelet count (×10^4/mm^3)†	12.4 (3.8–44.9)
Total bilirubin (mg/dL)†	1.0 (0.4–12)
Creatinine (mg/dL)†	0.83 (0.5–7.97)
Viral load at start of antiviral therapy (log IU/mL)†	6.6 (4.9–7.8)
Child–Pugh score	9 (5–13)
MELD score	13 (6–30)
Latency from transplantation to therapy (months)†	3 (1–80)
Immunosuppression (tacrolimus/cyclosporin)	43/4
IFN (PEG/RBV, IFN/RBV)	46/1
Completion of IFN therapy (yes/no)	34/13
SVR/non-SVR	24/23
Follow-up periods (months)†	71 (24–152)

Data are median (range)† or number or patients.
IFN, interferon; MELD, Model for End-Stage Liver Disease; PEG, pegylated; RBV, ribavirin; SVR, sustained virological response.

Table 2 Comparison baseline characteristics between patients completed of IFN therapy and stopped of IFN therapy

	Completed IFN therapy ($n = 34$)	Stopped IFN therapy ($n = 13$)	P
Recipient's age (<60 vs ≥60 years)	16/18	6/7	0.7
Recipient's sex (male vs female)	22/12	9/4	0.5
Donor's age (<35 vs ≥35 years)	15/19	6/7	0.7
Donor's sex (male vs female)	23/11	11/2	0.6
Graft volume (<700 vs ≥700 mg)	16/18	5/8	1.0
Hepatocellular carcinoma (yes/no)	20/14	9/4	0.7
Splenectomy (yes/no)	10/24	3/10	1.0
ABO incompatibility (yes/no)	0/34	2/11	0.07
Body mass index (kg/m^2)	22/12	8/5	1.0
Living donor LT/orthotopic LT	33/1	13/0	1.0
Genotype (1/2)	27/7	13/0	0.1
White blood cell (<4500 vs ≥4500/μL)	17/17	6/7	1.0
Hemoglobin (<10 vs ≥10 g/dL)	16/18	8/5	0.5
Platelet count (<12 vs ≥12 ×10^4/mm^3)	17/17	6/7	1.0
Total bilirubin (<1.0 vs ≥1.0 mg/dL)	15/19	8/5	0.3
Creatinine (<0.8 vs ≥0.8 mg/dL)	17/17	7/6	0.7
HCV viral load at start of therapy (<7 vs ≥7 log IU/mL)	7/27	4/9	0.4
Time from transplantation to therapy (<4 vs ≥4 months)	19/5	9/4	1.0
Child–Pugh score (<9 vs ≥9)	14/20	8/5	0.5
MELD score (<13 vs ≥13)	17/17	4/9	0.5
Immunosuppression (tacrolimus/cyclosporin)	31/3	12/1	1.0
IFN (PEG/RBV, IFN/RBV)	33/1	13/0	0.9

HCV, hepatitis C virus; IFN, interferon; MELD, Model for End-Stage Liver Disease; PEG, pegylated; RBV, ribavirin; SVR, sustained virological response.

cyclosporin in four patients. Table 2 lists comparison baseline characteristics between patients who had completed IFN therapy and stopped IFN therapy.

There was no statistical significant difference in all variables.

Efficacy and tolerance of IFN therapy and side-effects

Figure 1 shows the effects of IFN therapy according to genotype. At the end of the antiviral treatment phase, the SVR rate was 51% (24/47) for all patients, 42.5% (17/40) for those with genotype 1 and 100% (7/7) for those with genotype 2.

Thirty-four patients completed IFN therapy while 13 did not. The reason for stopping IFN therapy was liver failure in three patients, acute rejection in three patients, HCC recurrence in two patients, general fatigue in two patients, depression in two patients and cerebral infarction in one patient.

Relation between overall survival in patients with post-LT HCV infection

The survival rate of 24 patients who achieved SVR was 95% at 5 years and 92% at 10 years. These rates were significantly better than those of 23 patients who did not achieve SVR (82% at 5 years and 58% at 10 years, each $P = 0.027$; Fig. 3). Of the SVR group, two of 24 patients died during the follow-up period, and the cause

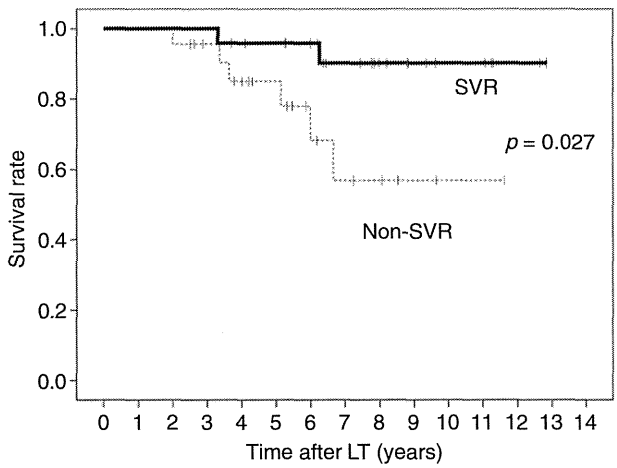

Figure 3 Relation between overall survival and viral response in patients with post-LT hepatitis C virus infection. LT, liver transplantation; SVR, sustained virological response.

Table 3 Results of univariate analyses for determinants of overall survival in patients treated with IFN for recurrent hepatitis C after liver transplantation

Variable	Hazard ratio	95% CI	*P*
Recipient's age (<60 vs ≥60 years)	1.6	0.5–6.4	0.3
Recipient's sex (male vs female)	1.1	0.3–5.4	0.5
Donor's age (<35 vs ≥35 years)	1.6	0.2–2.5	0.6
Donor's sex (male vs female)	2.0	0.1–2.6	0.5
Graft volume (<700 vs ≥700 mg)	5.4	0.3–4.7	0.6
Hepatocellular carcinoma (yes/no)	2.0	0.4–7.0	0.3
Splenectomy (yes/no)	3.3	0.1–2.9	0.5
ABO incompatibility (yes/no)	2.2	0.3–21.9	0.3
Body mass index (kg/m^2)	3.2	0.4–5.2	0.4
Living donor LT/orthotopic LT	20	0.6–2.4	0.4
Genotype (1/2)	1.2	0.1–12.3	0.6
White blood cell (<4500 vs ≥4500 /μL)	3.3	0.1–2.1	0.4
Hemoglobin (<10 vs ≥10 g/dL)	5	0.1–1.8	0.3
Platelet count (<12 vs ≥12 ×10^4/mm^3)	1.1	0.2–2.5	0.6
Total bilirubin (<1.0 vs ≥1.0 mg/dL)	2	0.3–3.5	0.8
Creatinine (<0.8 vs ≥0.8 mg/dL)	1.8	0.3–4.2	0.6
HCV viral load at start of therapy (<7 vs ≥7 log IU/mL)	4.0	0.03–0.5	0.06
Time from transplantation to therapy (<4 vs ≥4 months)	1.0	0.4–9.3	0.3
Child–Pugh score (<9 vs ≥9)	1.6	0.1–2.0	0.4
MELD score (<13 vs ≥13)	1.4	0.1–2.3	0.5
Immunosuppression (tacrolimus/cyclosporin)	25.3	0.1–12	0.6
IFN (PEG/RBV, IFN/RBV)	1.2	0.1–12	0.6
SVR vs non-SVR	5.2	0.03–0.6	0.027

CI, confidence interval; IFN, interferon; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; LT, liver transplantation; PEG, pegylated; RBV, ribavirin; SVR, sustained virological response.

of death was HCC in one and chronic rejection in one. Of the non-SVR group, six of 23 patients died during the follow-up period; three died of liver failure associated with recurrent hepatitis C, and one each died of HCC, infection and myelodysplastic syndromes.

Analysis of factors associated with overall survival in patients with post-LT HCV infection

Univariate analysis identified two parameters that correlated with overall survival either significantly or marginally: viral response (SVR; $P = 0.027$) and HCV viral load at start of antiviral therapy (<7 log IU/mL, $P = 0.06$; Table 3). The model of variable was acceptable ($P < 0.05$).

DISCUSSION

THE PRESENT RETROSPECTIVE study examined the effects of SVR induced by IFN therapy on survival rate in patients with recurrent post-LDLT HCV infection. The major finding of the study was a significantly higher survival rate in patients who achieved SVR compared with those who did not. This result suggest that SVR induced by IFN therapy contributes to the improvement in survival rate in patients with recurrent post-LDLT HCV infection. However, this study is retrospective and small, which would potentially bias the statistics. In the future, it prospectively needs to be studied at a larger scale.

We think the reasons why the SVR induced by IFN therapy contributes to the improvement in survival rate in patients with recurrent post-LDLT HCV infection are as follows.

First, SVR prevents the development of liver fibrosis. Approximately 20–25% of HCV positive patients develop cirrhosis within 5 years after LT, and approximately 50% within 10 years.[6,9,10] By avoiding development of cirrhosis with SVR, we think that SVR patients had improved the survival after LT.

Second, cholestatic hepatitis C is one of the most serious but still unaddressed disorders after LT. HCV

reinfection can result in very aggressive hepatitis in a small number of patients, and is characterized by rapid progression of cholestasis with fibrosis resulting in graft failure and death.[24,25] Ikegami *et al.* reported that higher HCV RNA titer at 2 weeks was the only significant factor for the development of cholestatic hepatitis C.[26] Therefore, we think that cholestatic hepatitis C may be avoided with SVR by a preemptive antiviral treatment approach.

Third, HCV is associated with various systemic diseases, for example, diabetes mellitus, malignant lymphoma, renal failure and intracerebral hemorrhagic stroke.[27–30] Kawamura *et al.* reported the annual incidence of malignant lymphoma among patients with HCV at 0.23%.[28]

Arase *et al.*[27] reported that SVR causes a two-thirds' reduction in the risk of type 2 diabetes development in HCV positive patients treated with IFN, the annual incidence for chronic kidney disease among cirrhotic patients with HCV was determined to be approximately 1.0–1.5% and HCV clearance reduced intracerebral hemorrhagic stroke to approximately one-fourth in cirrhotic patients. Therefore, we think that various systemic diseases may be avoided with SVR by preemptive antiviral treatment approach. As a result, SVR patients may improve survival after LT.

However, we should be careful of chronic rejection and plasma cell hepatitis associated with antiviral therapy for recurrent post-LDLT HCV infection. Ueda *et al.* reported that seven of 125 (6%) patients developed chronic rejection during or within 6 months after the end of antiviral therapy. In five patients, rejection progressed rapidly and resulted in death within 3 months after diagnosis.[31] On the other hand, plasma cell hepatitis induced by antiviral therapy for recurrent hepatitis C after LDLT.[32,33] Although it is important that SVR induced by IFN therapy improved survival rate in patients with recurrent post-LDLT HCV infection, IFN therapy may be recommended in these patients while we are careful of chronic rejection and plasma cell hepatitis.

The present study showed the higher SVR rate, compared with previous studies. We think that one reason for this is that prolonged IFN treatment is useful and can improve the SVR rate for transplanted patients. Tamura *et al.* and Ueda *et al.* reported a relapse rate of 14% and 3% under the same treatment, respectively. As a result, the SVR rate was 34% and 50%, respectively.[34,35] Another reason for this is that rapid induction of antiviral treatment with PEG IFN and RBV was attempted per protocol regardless of the clinical presentation of recurrent HCV (preemptive treatment approach). We started IFN therapy at 3 months (median) after LT. Tamura *et al.* suggested a preemptive treatment approach in his reports.[35]

The newly introduced triple therapy of protease inhibitors (telaprevir [TVR] plus PEG IFN/RBV) offers the prospect of better management of LT patients. Pungpapong *et al.* also compared the response to treatment with TVR plus cyclosporin with that of boceprevir in patients who had undergone LT.[36] We have also reported our results on overall survival in patients with genotype 1 recurrent HCV treated with TVR in combination with PEG IFN/RBV after LT.[37]

Simeprevir (SMV) is an investigational, single-pill, once-daily, oral HCV NS3/4A protease inhibitor currently in clinical development for the treatment of HCV infection with fewer reported side-effects than TVR, which is associated with anemia, renal failure and skin rash.[38] However, no study has evaluated SMV in patients with recurrent HCV infection after LT. In future, the study of SMV in patients with recurrent HCV infection after LT will be expected. On the other hand, for such hard-to-treat post-LT patients, combination therapy with IFN-free direct-acting antiviral agents (DAA) will likely be a standard therapy in the future because both efficacy and safety are higher in IFN-free DAA combination therapies compared with IFN-based triple therapy.[39,40]

We conclude that recurrent HCV infection following LT should be treated with IFN to eradicate HCV, achieve SVR and improve prognosis.

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