Isolation of lymphocytes from liver allograft perfusate. Donor hepatectomy and the transplantation procedure were performed as described previously (32). After hepatectomy, ex vivo perfusion of the liver allograft was performed through the portal vein. Liver allograft-derived lymphocytes were isolated by gradient centrifugation with Ficoll-Paque (GE Healthcare Bio-Sciences AB).

Adoptive transfer of IL-2/OKT3–treated liver lymphocytes. Liver lymphocytes were cultured with human recombinant IL-2 (100 Japanese reference units/ml [JRU/ml]; Takeda) in complete medium at 37°C in a 5% CO_2 incubator for 3 days. One day before the infusion, 1 μg/ml of OKT3 (Janssen-Kyowa) was added in order to opsonize the $CD3^+$ fraction. On the day of infusion, the cells were washed twice with 0.9% sodium chloride and resuspended with 5% human serum albumin in 0.9% sodium chloride for injection (Figure 1). The viability of the cells was assessed by the dye-exclusion test, and the cells were checked twice for possible contamination by bacteria, fungi, and endotoxins.

Cytotoxicity assay. A ^{51}Cr-release assay was done as previously described (5), using HepG2 tumor cells (Japanese Cancer Research Resources Bank) as targets. Briefly, ^{51}Cr-labeled target tumor cells were added for 4 hours at 37°C to effector cells in round-bottomed 96-well microtiter plates (BD Biosciences – Discovery Labware). The percentage of specific ^{51}Cr release was calculated as follows: % cytotoxicity = [(cpm of experimental release - cpm of spontaneous release)/(cpm of maximum release - cpm of spontaneous release)] × 100. All the assays were performed in triplicate.

Flow cytometry. Flow cytometric analyses were performed using a FACSCalibur dual-laser cytometer (BD Biosciences). The following mAbs were used for the surface staining of the lymphocytes: FITC-conjugated anti-CD3 mAb (clone HIT3a; BD Biosciences – Pharmingen); PE-conjugated anti-CD56 mAb (clone B159; BD Biosciences – Pharmingen); and biotinylated anti-TRAIL (biotin-conjugated anti-TRAIL) mAb (clone RIK-2; eBioscience). The biotinylated mAb was visualized using APC-streptavidin (BD Biosciences – Pharmingen). Dead cells identified by light scatter and propidium iodide staining were excluded from the analysis. IFN-γ production in the lymphocytes was measured by a combination of cell surface and cytoplasmic mAb staining and subsequent flow cytometric analysis, as described previously (33).

Isolation of $CD56^+$ and CD56 fractions and that of NK and NKT cells. Liver allograft-derived lymphocytes were separated into a $CD56^+$ fraction – including NK and NKT cells – and a $CD56^-$ fraction by using auto MACS (Miltenyi Biotec) with anti-human CD56 microbeads (Miltenyi Biotec) according to the manufacturer's instructions. The NK and NKT cells were also isolated by magnetic cell sorting, using the human NK cell isolation kit or human $CD3^+CD56^+$ NKT cell isolation kit (Miltenyi Biotec). The purity of the isolated fractions was assessed by flow cytometric analysis, and only the fractions with purities greater than 90% were used for functional studies.

Coculture with HCV replicon-containing hepatic cells. An HCV subgenomic replicon plasmid, pRep-Feo, was derived from pRep-Neo (originally, pHCVIbneo-delS; ref. 34). The pRep-Feo carries a fusion gene comprising firefly luciferase (*Fluc*) and neomycin phosphotransferase, as described elsewhere (35, 36). After culture in the presence of G418 (Invitrogen), pRep-Feo cell lines stably expressing the replicons were established. For coculture experiments, transwell tissue culture plates (pore size, 1 μm; Costar) were used. HCV replicon-containing hepatic cells (10^5 cells) were incubated in the lower compartment with different numbers of lymphocytes in the upper compartment. The hepatic cells in the lower compartments were collected 48 hours after coculture for the luciferase assay. Luciferase activities were measured with a luminometer (Lumat LB9501; Promega), using the Bright-Glo Luciferase Assay System (Promega).

Cytometric bead array. Cytokine (IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10) levels in the coculture assay supernatants were measured with the FACSCalibur dual-laser cytometer (BD Biosciences), using a BD Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) Kit according to the manufacturer's instructions.

Generation of human hepatocyte–chimeric mice. Generation of the *uPA*$^{+/+}$ *SCID*$^{+/+}$ mice and transplantation of human hepatocytes were performed as described recently by our group (20, 37). Mouse serum concentrations of human serum albumin correlated with the repopulation index (20), and these were measured as described previously (37).

In vivo studies using human hepatocyte–chimeric mice. Human hepatocyte-chimeric mice were intravenously injected with 50 μl of the human serum samples positive for HCV genotype 1b. The serum HCV RNA titer in human hepatocyte-chimeric mice was detected by nested PCR, as previously described (38, 39). All animal protocols described in this study were performed in accordance with the guidelines and with approval of the Ethics Review Committee of Animal Experimentation of the Graduate School of Biomedical Sciences, Hiroshima University. Either 2 or 4 weeks after injecting the infected serum, the mice were intraperitoneally inoculated with IL-2/OKT3–treated liver lymphocytes (20×10^6 cells/mouse) for adoptive immunotherapy. When indicated, anti-human IFN-γ mAb (R&D Systems) (1.5 mg/mouse) was injected intraperitoneally 1 day before the immunotherapy. In a separate experiment, intraperitoneal injection of recombinant human IFN-γ (Imunomax-γ; Shionogi & Co. Ltd.) was commenced at either 2 or 4 weeks after injecting the infected serum. IFN-γ was administered as follows: 1×10^5 IU on the first day and thereafter 2×10^4 IU/day for 13 days.

Statistics. Data are presented as mean ± SEM. The statistical differences of the results were analyzed by 2-tailed, paired Student's *t* test, Mann-Whitney *U* test, and Mann-Whitney *U* test with Bonferroni correction after the Kruskal-Wallis *H* test, using the Stat View program. *P* values of 0.05 or less were considered statistically significant.

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1. Petrovic, L.M. 2006. Early recurrence of hepatitis C virus infection after liver transplantation. *Liver Transpl.* **12**:S32–S37.
2. Brown, R.S. 2005. Hepatitis C and liver transplantation. *Nature.* **436**:973–978.
3. Garcia-Retortillo, M., et al. 2002. Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology.* **35**:680–687.
4. Berenguer, M. 2002. Natural history of recurrent hepatitis C. *Liver Transpl.* **8**:S14–S18.
5. Ishiyama, K., et al. 2006. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. *Hepatology.* **43**:362–372.
6. Ohira, M., et al. 2006. Adoptive transfer of TRAIL-

expressing natural killer cells prevents recurrence of hepatocellular carcinoma after partial hepatectomy. *Transplantation.* **82**:1712–1719.
7. Miller, J.S., et al. 2005. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood.* **105**:3051–3057.
8. Hirata, M., et al. 1998. Increase in natural killer cell activity following living-related liver transplantation. *Transpl. Int.* **11**(Suppl. 1):S185–S188.
9. Harada, N., et al. 2004. IL-12 gene therapy is an effective therapeutic strategy for hepatocellular carcinoma in immunosuppressed mice. *J. Immunol.* **173**:6635–6644.
10. Golden-Mason, L., and Rosen, H.R. 2006. Natural killer cells: primary target for hepatitis C virus immune evasion strategies? *Liver Transpl.* **12**:363–372.
11. Li, Y., et al. 2004. Natural killer cells inhibit hepatitis C virus expression. *J. Leukoc. Biol.* **76**:1171–1179.
12. Deignan, T., et al. 2002. Decrease in hepatic CD56(+) T cells and V alpha 24(+) natural killer T cells in chronic hepatitis C viral infection. *J. Hepatol.* **37**:101–108.
13. Durante-Mangoni, E., et al. 2004. Hepatic CD1d expression in hepatitis C virus infection and recognition by resident proinflammatory CD1d-reactive T cells. *J. Immunol.* **173**:2159–2166.
14. Kawarabayashi, N., et al. 2000. Decrease of CD56(+)T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. *Hepatology.* **32**:962–969.
15. Rosen, H.R., et al. 2008. Pretransplantation CD56(+) innate lymphocyte populations associated with severity of hepatitis C virus recurrence. *Liver Transpl.* **14**:31–40.
16. Doherty, D.G., et al. 1999. The human liver contains multiple populations of NK cells, T cells, and CD3+CD56+ natural T cells with distinct cytotoxic activities and Th1, Th2, and Th0 cytokine secretion patterns. *J. Immunol.* **163**:2314–2321.
17. Mazzaferro, V., et al. 1996. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N. Engl. J. Med.* **334**:693–699.
18. Mercer, D.F., et al. 2001. Hepatitis C virus replication in mice with chimeric human livers. *Nat. Med.* **7**:927–933.
19. Kneteman, N.M., et al. 2006. Anti-HCV therapies in chimeric scid-Alb/uPA mice parallel outcomes in human clinical application. *Hepatology.* **43**:1346–1353.
20. Tateno, C., et al. 2004. Near completely humanized liver in mice shows human-type metabolic responses to drugs. *Am. J. Pathol.* **165**:901–912.
21. Tseng, C.T., and Klimpel, G.R. 2002. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. *J. Exp. Med.* **195**:43–49.
22. Crotta, S., et al. 2002. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. *J. Exp. Med.* **195**:35–41.
23. Wang, S.H., et al. 2008. Natural killer cells suppress full cycle HCV infection of human hepatocytes. *J. Viral Hepat.* **15**:855–864.
24. Kanto, T., and Hayashi, N. 2007. Innate immunity in hepatitis C virus infection: Interplay among dendritic cells, natural killer cells and natural killer T cells. *Hepatol. Res.* **37**(Suppl. 3):S319–S326.
25. Yamagiwa, S., et al. 2008. Sustained response to interferon-alpha plus ribavirin therapy for chronic hepatitis C is closely associated with increased dynamism of intrahepatic natural killer and natural killer T cells. *Hepatol. Res.* **38**:664–672.
26. Van Wauwe, J.P., De Mey, J.R., and Goossens, J.G. 1980. OKT3: a monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. *J. Immunol.* **124**:2708–2713.
27. Chang, T.W., Kung, P.C., Gingras, S.P., and Goldstein, G. 1981. Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells? *Proc. Natl. Acad. Sci. U. S. A.* **78**:1805–1808.
28. Chatenoud, L., et al. 1990. In vivo cell activation following OKT3 administration. Systemic cytokine release and modulation by corticosteroids. *Transplantation.* **49**:697–702.
29. Soza, A., et al. 2005. Pilot study of interferon gamma for chronic hepatitis C. *J. Hepatol.* **43**:67–71.
30. Jo, J., et al. 2009. Analysis of CD8+ T-cell-mediated inhibition of hepatitis C virus replication using a novel immunological model. *Gastroenterology.* **136**:1391–1401.
31. Penna, A., et al. 2007. Dysfunction and functional restoration of HCV-specific CD8 responses in chronic hepatitis C virus infection. *Hepatology.* **45**:588–601.
32. Ohdan, H., et al. 2003. Intraoperative near-infrared spectroscopy for evaluating hepatic venous outflow in living-donor right lobe liver. *Transplantation.* **76**:791–797.
33. Tanaka, Y., Ohdan, H., Onoe, T., and Asahara, T. 2004. Multiparameter flow cytometric approach for simultaneous evaluation of proliferation and cytokine-secreting activity in T cells responding to allo-stimulation. *Immunol. Invest.* **33**:309–324.
34. Guo, J.T., Bichko, V.V., and Seeger, C. 2001. Effect of alpha interferon on the hepatitis C virus replicon. *J. Virol.* **75**:8516–8523.
35. Tanabe, Y., et al. 2004. Synergistic inhibition of intracellular hepatitis C virus replication by combination of ribavirin and interferon- alpha. *J. Infect. Dis.* **189**:1129–1139.
36. Yokota, T., et al. 2003. Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. *EMBO Rep.* **4**:602–608.
37. Tsuge, M., et al. 2005. Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis B virus. *Hepatology.* **42**:1046–1054.
38. Hiraga, N., et al. 2007. Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis C virus and its susceptibility to interferon. *FEBS Lett.* **581**:1983–1987.
39. Kimura, T., et al. 2008. Establishment of an infectious genotype 1b hepatitis C virus clone in human hepatocyte chimeric mice. *J. Gen. Virol.* **89**:2108–2113.

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ORIGINAL ARTICLE

Successful hepatitis B vaccination in liver transplant recipients with donor-specific hyporesponsiveness

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Summary

Currently, patients are prescribed lifelong treatment with hepatitis B immunoglobulin (HBIg) after liver transplantation (LT) for hepatitis B virus (HBV)-related diseases in order to prevent reinfection with HBV. Active immunization with an HBV vaccine would be a preferable alternative; however, the immunosuppressive environment in LT recipients is believed to elicit a poor response to vaccination. Minimizing the exposure of the HBV-infected LT recipients to immunosuppressants would be beneficial in inducing adaptive immunity against HBV by vaccination. In this study, in addition to efforts to minimize immunosuppression, prophylaxis with HBV vaccination combined with continuous HBIg administration was performed in 17 LT recipients who had undergone transplantation attributable to HBV-related diseases. During the observation period, the overall response rate to HBV vaccination was 64.7%. The immune status of the recipients was evaluated by a mixed lymphocyte reaction assay in response to allostimulation. Patients showing a donor-specific hyporesponse with a well-maintained response to the third-party stimulus always achieved a sustained immune response to the vaccine, whereas patients showing a hyporesponse to both the donor and the third-party stimulus were unable to do so. Thus, inducing an anti-donor-specific immunosuppressive status by minimizing immunosuppression should enable post-transplant HBV vaccination to be a promising prophylactic strategy.

Introduction

Patients face a high risk of endogenous hepatitis B virus (HBV) reinfection in the absence of postoperative prophylaxis after liver transplantation (LT) caused by HBV-related disease. Combined treatment with either a nucleoside or nucleotide analog and hepatitis B immunoglobulins (HBIg) has been the gold standard for prophylaxis of HBV reinfection after LT [1–3]. According to current recommendations, HBIg should be administered indefinitely after LT [4–6]. However, indefinite prophylaxis with HBIg has substantial drawbacks, such as increasing costs [7] and the risk of emergence of HBV envelope protein mutations [8,9]. Therefore, induction of an active immune response against the hepatitis B surface antigen (HBsAg), leading to the continuous production of specific antibodies would be

an enormous advantage, and it would eliminate the need for lifelong replacement with HBIg [10,11].

Several groups have attempted vaccination of LT recipients against HBV [11–20]. In most of these studies, relatively low seroconversion rates as well as serum anti-HBs concentrations were observed among chronic HBV-infected LT recipients; only a minority of vaccinees developed stable antibody levels >100 IU/l, the maintenance of which is required for prevention of HBV reinfection [21]. The poor response to vaccination was probably because of the immunosuppressive environment in LT recipients. Minimizing the exposure of HBV-infected LT recipients to immunosuppressants appears to be beneficial in inducing adaptive immunity against HBV by vaccination; however, the relevance of the immune status of LT recipients to the outcome of HBV vaccination remains to be elucidated.

In this study, prophylactic HBV vaccination combined with continuous HBIg administration was performed in 17 LT recipients who had undergone transplantation because of an HBV-related disease and had not experienced signs of recurrence for at least 12 months after treatment with HBIg. The immune status of these patients was evaluated by a mixed lymphocyte reaction (MLR) assay in response to anti-donor and third-party allostimulation using an intracellular carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling technique.

Patients and methods

Patients

In this study, we included 17 living donor LT recipients at the Hiroshima University Hospital. All patients had normal liver function without any virologic and biochemical evidence of HBV recurrence. The following were the inclusion criteria: (i) at least 3 months of HBIg plus lamivudine (100 mg/day) with/without adefovir (10 mg/day) administration and (ii) no findings of recurrent infection and negativity for HBsAg and hepatitis B viral deoxyribonucleic acid (HBV DNA) (by PCR) at the time of vaccination. For prophylaxis against reinfection, all transplanted patients were on a stable schedule of 1000–2000 IU of intravenous HBIg every 4 weeks in order to maintain an anti-HBs titer of >100 IU/l. We attempted to minimize immunosuppression in all patients with good liver function by adopting the policy of tapering off the immunosuppressants. The study protocol was approved by the Ethics Committee of Hiroshima University, and all patients provided informed consent before entering into the trial. None of the vaccinees showed clinical evidence of recurrence of HBV graft infection and the episode of rejection throughout the follow-up period, and all of them were persistently negative for both HBsAg and HBV DNA, except for one vaccinee (Patient #3) who showed temporarily positive for HBV DNA.

Vaccination protocol

All participants received a yeast-derived recombinant, adsorbed HBV vaccine (Bimmugen®; Chemotherapy and Serotherapy Laboratories Inc., Kumamoto, Japan) subcutaneously every 4 weeks at a dose of 10–20 μg (0.5–1.0 ml) in combination with HBIg and lamivudine/adefovir. HBIg immunoprophylaxis was continued during primary immunization (dose, 1000–2000 IU every 4 weeks). The response to vaccination was defined as (i) a confirmed increase in the anti-HBs titer to >100 IU/l that could not be explained by HBIg administration and (ii) sustained anti-HBs titer to >100 IU/l after discontinuation of combined administration of the vaccine and HBIg. If the anti-HBs titer exceeded the responsive increasing level, HBIg substitution and vaccine administration were discontinued. Lamivudine/adefovir prophylaxis was additionally discontinued, if the anti-HBs titer was maintained effectively without HBIg administration. The vaccine was continuously and indefinitely administered till acquired immunity was elicited.

Serologic markers and virologic assays

Serum HBsAg, hepatitis Be antigen (HBeAg), hepatitis B core antibody (HBcAb), and anti-HBsAb were measured monthly using an enzyme-linked immunoassay (Abbott Diagnostics, Chicago, IL, USA). HBV DNA was detected by the Amplicor HBV monitor test (Roche Diagnostics, Tokyo, Japan). The measurement range of the assay is $10^{2.6}$–$10^{7.6}$ copies/ml (2.6–7.6 log copies/ml). These quantitative assays of HBV DNA were performed at the Special Reference Laboratory, Tokyo, Japan. Positive levels of HBV DNA were defined as levels >2.6 log copies/ml. HBV recurrence was diagnosed on the basis of appearance of HBsAg or HBV DNA.

Immune monitoring by *in vitro* CFSE-MLR assay

For patients who showed completely normal liver function, CFSE-MLR was performed to determine whether immunosuppression could be further minimized. In patients with hyporesponse of anti-donor T cells, immunosuppression was successfully reduced.

For CFSE-MLR, the peripheral blood mononuclear cells prepared from the blood of the LT recipients (autologous control), donors, and healthy volunteers with same blood type as the donors (third-party control) for use as the stimulator cells were irradiated with 30 Gy and those obtained from the recipients for use as the responder cells

were labeled with 5 μM CFSE (Molecular Probes Inc., Eugene, OR, USA), as described previously [22]. The stimulator and responder cells (2×10^6 each) were incubated in 24-well flat-bottomed plates (BD Labware, Franklin Lakes, NJ, USA) in a total volume of 2 ml of culture medium at 37 °C under 5% CO_2 for 5 days. After culture for MLR, the harvested cells were stained with either phycoerythrin (PE)-conjugated anti-human CD4 or PE-conjugated anti-human CD8 monoclonal antibodies (mAbs; BD Pharmingen, San Diego, CA, USA) and subjected to analysis by flow cytometry (FCM). All analyses were performed on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). Dead cells were excluded from the analysis by forward scatter or propidium iodide gating. T-cell proliferation was visualized by serial-halving of the fluorescence intensity of CFSE. $CD4^+$ and $CD8^+$ T-cell proliferation and stimulation index (SI) were quantified using a previously described method [23,24]. Briefly, the number of division precursors was extrapolated from the number of daughter cells of each division, and the number of mitotic events in each $CD4^+$ and $CD8^+$ T-cell subset was calculated. Using these values, the mitotic index was calculated by dividing the total number of mitotic events by the total number of precursors. The SIs of allogeneic combinations were calculated by dividing the mitotic index of a particular allogeneic combination by that of self-control.

Statistical analysis

The values are presented as the median and the range. The Mann–Whitney *U*-test was performed to analyze whether the age of the vaccinees at the time of vaccination, the time elapsed since LT, the anti-HBsAb titers at the start of the vaccination, the median tacrolimus trough levels, and the SI in anti-donor and anti-third-party MLR differed significantly between the good and poor responders and also between the moderate and poor responders. A Fisher's exact test was performed to determine whether there were differences between both the above groups with regard to gender, indication for LT, ratio of HBV DNA and HBeAg negative before LT, ratio of donor HBc and HBsAb positive before LT, and immunosuppressive monotherapy at the time of vaccine administration. *P*-values below 0.05 were considered statistically significant.

Results

Demographics

A total of 17 HBV vaccinees (four female- and 13 male subjects; age range, 20–65 years; median age, 49 years) participated in this study. The demographic and clinical data of the participants are shown in Table 1. Of them, 14 patients underwent LT for HBV-related cirrhosis and three underwent transplantation for HBV-related fulminant hepatic failure. Among the 17 vaccinees, five (29.4%) had been HBV DNA positive before LT with levels >2.6/ml, and five (29.4%) had been HBeAg positive before LT. Immunosuppressive treatment comprised either cyclosporine or tacrolimus monotherapy in 11 patients (64.7%) and additional steroid therapy (methylprednisolone, 2–4 mg/day) in six patients. Steroids were withdrawn at after a median duration of 13 months (range, 1–50 months) after LT. At the time of vaccination, a median duration of 21 months (range, 3–41 months) had elapsed since LT. The median follow-up time after commencement of vaccination was 26 months (range, 8–72 months). At the start of vaccination, a median anti-HBsAb titer was 161.4 (range, 37.7–328.4) IU/l.

Response to vaccination

During the observation period, 11 of the 17 HBV vaccinees (64.7%) achieved a sustained immune response to the HBV vaccine, which was defined as a confirmed increase in the anti-HBs titer to >100 IU/l that could not be explained by HBIg administration and no decrease in the anti-HBs titer to <100 IU/l even after discontinuation of combined administration of the vaccine and HBIg (Table 1). Within 1 year, 5/11 responders responded to the vaccine, and other six responded after 1 year from the commencement of vaccination (Fig. 1a and b). The other six HBV vaccinees did not respond to the vaccine during the study period (Fig. 1c). When the subjects were divided into three distinct groups, i.e., patients who responded to the vaccine within 1 year after commencement of vaccination (good responders), patients who responded to the vaccine after 1 year since commencement of vaccination (moderate responders), and patients who did not respond to the vaccine within 1 year and still remain receiving the vaccine (poor responders), the following factors did not exhibit statistically significant differences between the good and poor responders and also between the moderate and poor responders: age, gender, indication for LT, HBV viremia, donor HBcAb and HBsAb before LT, immunosuppressive regimen and tacrolimus trough levels and anti-HBsAb titers at the time of vaccination, duration between vaccination and transplantation and also duration between steroid withdrawal and transplantation. (Table 2) (Fig. 2).

Estimation of immunosuppressive status during vaccination by CFSE-MLR assay

Eleven patients (#1, 2, 4, 5, 7, 9, 11, 12, 13, 14 and 17) and their donors consented to be subjected to an

Table 1. The demographic and clinical characteristics of patients.

Patient	Age*	Gender	Underlying disease	HBV DNA before LT	Recipient HBeAg before LT	Donor HBcAb before LT	Donor HBsAb before LT	Time of vaccination†	Time of steroid withdrawal†	Duration of follow-up‡	Immuno-suppressive drugs*	Tac/CsA trough level (ng/ml)*	Anti-HBsAb titer (IU/l)*
Patients who responded to the vaccine within 1 year after commencement of vaccination (good responders)													
1	62	M	Cirrhosis/HCC	<2.6	Negative	NA	Negative	41	3	34	CsA 50 mg	39.3 (CsA)	152.6
2	54	M	Cirrhosis/HCC	<2.6	Negative	NA	NA	26	2	35	CsA 50 mg	15.0 (CsA)	189.1
3	58	M	Cirrhosis	6.4	Positive	Negative	Negative	9	2	43	Tac 2 mg	4.6	161.0
4	43	M	Cirrhosis/HCC	3.4	Negative	Negative	NA	35	45	20	Tac 3 mg + mPSL 2 mg	1.5	220.6
5	57	M	Fulminant	<2.6	Negative	Negative	Negative	9	1	15	Tac 2 mg	3.4	37.7
Patients who responded to the vaccine after 1 year since commencement of vaccination (moderate responders)													
6	34	M	Fulminant	<2.6	Negative	NA	NA	3	7	72	Tac 6 mg + mPSL 4 mg	4.2	152.1
7	38	M	Cirrhosis/HCC	<2.6	Negative	NA	NA	35	7	49	Tac 1 mg	4.4	146.6
8	57	M	Cirrhosis/HCC	<2.6	Negative	NA	Negative	40	50	31	Tac 2 mg + mPSL 4 mg	4.4	68.3
9	46	F	Cirrhosis/HCC	4.6	Positive	Negative	Positive	17	2	33	Tac 3 mg	4.7	93.4
10	46	F	Cirrhosis	<2.6	Negative	Positive	Positive	20	1	22	Tac 1 mg	4.2	214.9
11	53	M	Cirrhosis/HCC	<2.6	Negative	Positive	Positive	13	4	15	Tac 2 mg	4.2	160.5
Patients who did not respond to the vaccine during the study period (poor responders)													
12	20	M	Fulminant	>7.6	Positive	Negative	Positive	18	29	20	Tac 3 mg + mPSL 2 mg	5.0	222.7
13	46	M	Cirrhosis	<2.6	Negative	Negative	Negative	16	1	20	Tac 1 mg	6.6	188.9
14	58	F	Cirrhosis/HCC	<2.6	Negative	NA	NA	18	20	11	Tac 2 mg + mPSL 2 mg	1.5	92.3
15	65	M	Cirrhosis/HCC	4.5	Positive	NA	NA	12	21	13	Tac 4 mg + mPSL 4 mg	8.0	328.4
16	45	F	Cirrhosis/HCC	<2.6	Negative	NA	Negative	25	23	8	Tac 0.5 mg	3.7	193.3
17	54	M	Cirrhosis/HCC	<2.6	Positive	Positive	Positive	13	1	9	Tac 2 mg	2.9	122.2

LT, liver transplantation; Tac, tacrolimus; CsA, cyclosporine; mPSL, methylprednisolone; NA, not available.

*At the time of vaccination.

†Months after liver transplantation.

‡Months after commencement of vaccination.

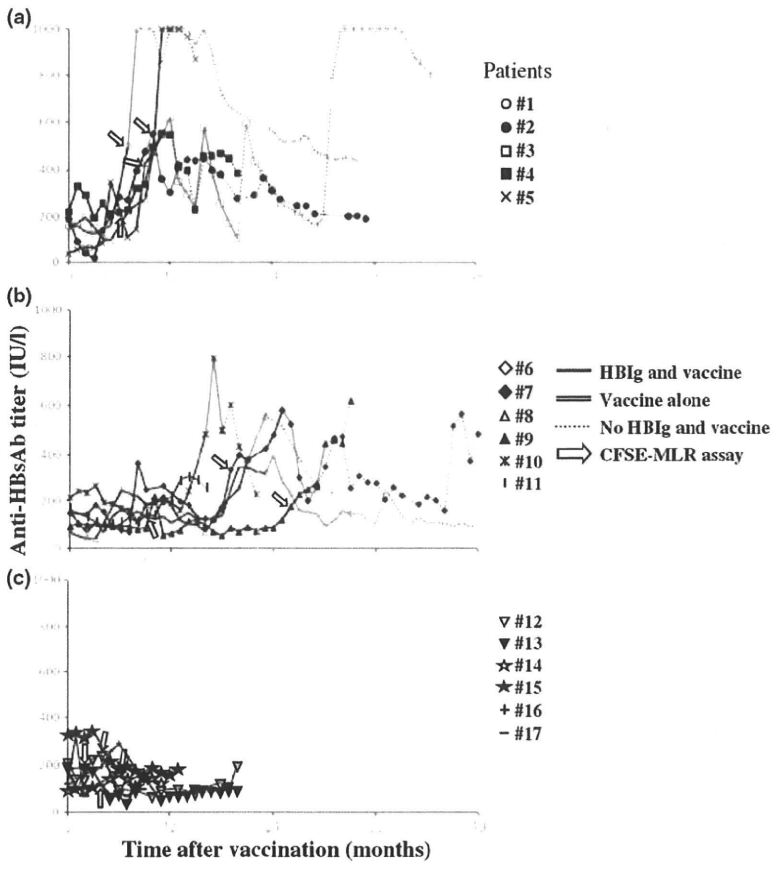

Figure 1 Anti-HBs titer kinetics in patients who responded to the vaccine within 1 year after commencement of vaccination (good responders) (a), in patients who responded to the vaccine after 1 year since the commencement of vaccination (moderate responders) (b), and in patients who did not respond to the vaccine (poor responders) (c).

Table 2. Age, gender, indication for LT, HBV viremia, immunosuppresive regimen, duration between vaccination and transplantation, and duration between steroid withdrawal and transplantation.

	Good responders (n = 5)	Moderate responders (n = 6)	Poor responders (n = 6)	P-value
Age at vaccination (years)*	55 (43–62)	46 (34–57)	48 (20–65)	NS
Gender (male/female)	5/0	4/2	4/2	NS
Indication for LT (fulminant hepatitis/cirrhosis)	1/4	1/5	1/5	NS
HBV DNA before LT (positive/negative)	2/3	2/4	2/4	NS
Recipient HBeAg before LT (positive/negative)	1/4	1/5	3/3	NS
Donor HBcAb before LT (positive/negative)	0/3	2/1	1/2	NS
Donor HBsAb before LT (positive/negative)	0/3	3/1	2/2	NS
CsA or Tac monotherapy/combination with steroid†	4/1	4/2	3/3	NS
Duration between vaccination and transplantation (months)*	24 (9–41)	21 (3–40)	17 (12–25)	NS
Duration between steroid withdrawal and transplantation (months)*	11 (1–45)	12 (1–50)	16 (1–29)	NS
Anti-HBsAb titer (IU/l)*†	152 (38–221)	139 (93–215)	191 (92–328)	NS

NS, not significant; LT, liver transplantation; CsA, cyclosporine A; Tac, tacrolimus.
*Median (range).
†At the time of vaccination.

MLR assay using a CFSE-labeling technique. In all the seven patients who responded to the HBV vaccine, limited $CD4^+$ T-cell proliferation was observed in the anti-donor MLR assay as compared with the anti-third-party MLR assay, i.e., a hyporesponse in the anti-donor MLR assay and a normal response in the anti-third-party MLR assay (Fig. 3). In these patients, the average of SIs for $CD4^+$ T cells in response to anti-third-party stimulation was >2 (average value in healthy volunteers without any immunosuppressive treatment). In contrast,

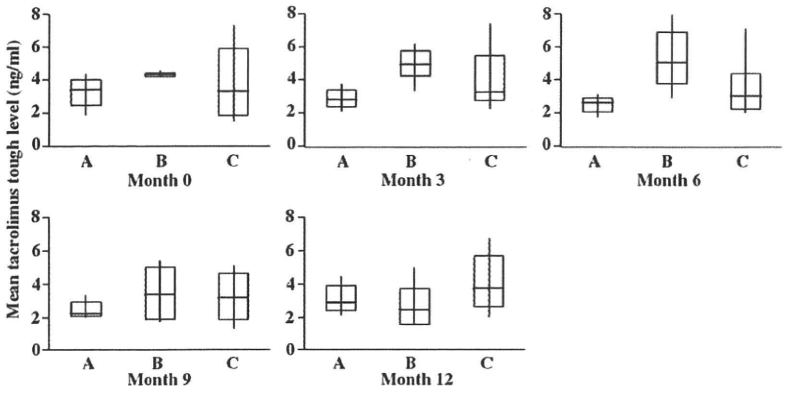

Figure 2 Tacrolimus trough levels in patients who responded to the vaccine within 1 year after commencement of vaccination (good responders) (A), in patients who responded to the vaccine after 1 year since the commencement of vaccination (moderate responders) (B), and in patients who did not respond to the vaccine (poor responders) (C). The Mann–Whitney *U*-test was used to compare the tacrolimus trough levels between the good and moderate responders with those of poor responders. The box plot represents the 25th to 75th percentile, the dark line is the median, and the extended bars represent the 10th to the 90th percentile. Statistical analyses at none of the time-points at 0, 3, 6, 9 and 12 months were significant.

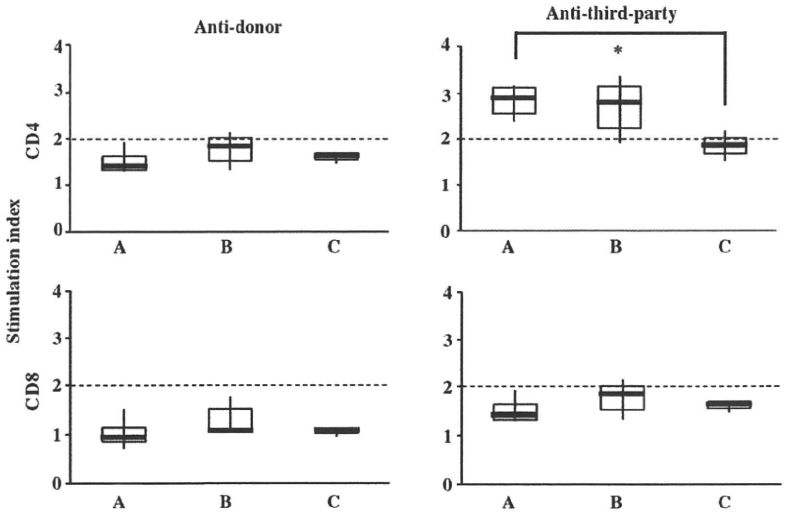

Figure 3 Stimulation indices (SIs) of each of the $CD4^+$ and $CD8^+$ T-cell subsets in the anti-donor and anti-third-party MLR in patients who responded to the vaccine within 1 year after commencement of vaccination (good responders) (A), in patients who responded to the vaccine after 1 year since the commencement of vaccination (moderate responders) (B), and in patients who did not respond to the vaccine (poor responders) (C). $CD4^+$ and $CD8^+$ T-cell proliferation and their SIs were quantified as follows. The number of division precursors was extrapolated from the number of daughter cells of each division, and the number of mitotic events in each of the $CD4^+$ and $CD8^+$ T-cell subsets was calculated. Using these values, the mitotic index was calculated by dividing the total number of mitotic events by the total number of precursors. The SIs of allogeneic combinations were calculated by dividing the mitotic index of a particular allogeneic combination by that of the self control. The Mann–Whitney *U*-test was used to compare the tacrolimus trough levels between the good and moderate responders with those of poor responders. The box plot represents the 25th to 75th percentile, the dark line is the median, and the extended bars represent the 10th to the 90th percentile. $*P = 0.04$.

in the four patients who did not respond to the HBV vaccine, limited $CD4^+$ and $CD8^+$ T-cell proliferation was observed in both the anti-donor and the anti-third-party MLR assay, i.e., a hyporesponse in both cases. In these patients, the average of SIs for $CD4^+$ T cells in response to both anti-donor and anti-third-party stimulation was <2. Thus, the SIs for $CD4^+$ T cells in response to anti-third-party stimulation in good responders was higher than that of poor responders ($P = 0.04$) (Fig. 3).

Discussion

The strategy of HB vaccination after LT to achieve protective immunity and to allow discontinuation of long-term HBIg administration has been investigated in a number of studies [7,11,12,15–20]. However, those attempts to immunize these patients with HB vaccine have been equivocal and generally less than successful. It is common practice to immunize these patients against hepatitis B; however, the response of LT recipients could be below adequate standard. Although the currently available HBV vaccines are extremely safe and have an efficacy of more than 90% in the general population, it has been reported that the response rate is slightly lower in obese individuals, smokers, and men and is significantly lower in patients with cirrhosis or chronic renal failure, patients undergoing long-term hemodialysis, organ transplant recipients, and immunocompromised patients [21]. In particular, because of the impairment in T-cell-dependent functions in cirrhotic patients, the results of vaccination in transplant candidates have been very disappointing [25–29]. Moreover, even in responder patients, immunosuppressive treatment frequently leads to a decrease in the serum antibody titers after transplantation [21]. Among the previous HBV vaccination trials in multiple institutions, most of the results did not show significant promise with regard to HBV vaccine response rates. Each vaccination protocol differed with respect to the dose of vaccine, the time of commencement and frequency of vaccination, the route of vaccination, combination with HBIg, and the immunosuppressive regimen at the time of vaccination. It has been reported that successful vaccination is attributed to the long time-interval that had elapsed after transplant, which allowed them to markedly reduce the immunosuppressive therapy [11]. It has also been proposed that the administration of the vaccine through the intradermal route in preference to the intramuscular route might prove to be more responsive to HB vaccination, because the epidermis is known to be rich with antigen-presenting cells, making it an appropriate target for vaccine delivery [18]. Based on these hypotheses in this study, vaccination through the intradermal route was administered to the LT recipients against HBV with an effort to minimize immunosuppression. In addition to the different vaccination protocols, the difference in the immune status of the subjects likely influences their HBV vaccine response.

In order to evaluate the immune status of the LT recipient vaccinees, we employed a MLR assay using a CFSE-labeling technique [22]. CFSE stably stains intracellular proteins without toxicity, and the fluorescence of each stained cell segregates equally to the daughter cells upon cell division, resulting in sequential halving of cellular fluorescence intensity with each successive generation [30]. When analyzed by FCM, this sequential halving of fluorescence is visualized as distinct peaks or populations of cells and can be used to track cell division in populations of proliferating cells. This, then, allows phenotypic analysis of the proliferating cells and determination of the number of cells produced in each generation by multicolor FCM analysis, i.e., the number of viable $CD4^+$ and $CD8^+$ responder T cells that proliferate in response to allostimulation can be quantified separately. The lack of proliferation of $CD4^+$ T cells in anti-donor MLR reflects the suppression of the anti-donor response [22]. In this study, all of the good responders showed a normal response of the anti-third-party $CD4^+$ T cells (Fig. 3). In contrast, the poor responders showed a hyporesponse of both anti-donor and anti-third-party $CD4^+$ T cells, suggesting an excessively immunosuppressive state. The development of an effective immune response to HB vaccination requires coordinated immune activity comprising the interaction of T cells, cytokines, antigen-presenting cells, and B cells [31]. It is important to note that these immunocompetent cells can be sufficiently activated to acquire immune activity at the time of vaccination even in a state of immunosuppression. T-cell interaction should lead to (i) activation of anti-HBsAg-specific T cells in order to achieve a successful response to vaccination and (ii) suppression of anti-donor-specific T cells to avoid transplant rejection. Patients showing a donor-specific hyporesponse with a well-maintained response to the third-party stimulus always achieved a sustained immune response to the vaccine in this study; based on this observation, we propose a concept that inducing anti-donor-specific immunosuppressive status by minimizing immunosuppression enables post-transplant HBV vaccination to become a promising prophylactic strategy, although further studies are needed to establish the optimal HBV vaccination protocol. A larger and prospective trial might be required to evaluate whether or not the MLR response can actually predict successful HBV vaccination. The higher rate of response to vaccination than that of this study has been shown in a previous report [17]. An adjuvant preparation of vaccine that used in the previous study is thought to attribute to the successful induction of a strong response. It remains to elucidate whether patients with hyporesponse to both anti-donor and anti-third-party $CD4^+$ T cells can respond to such an adjuvant preparation of vaccine.

Authorship

HT, KC, and HO: designed research. HT and YT: performed research. HT, KI, KI, MS, TI, YU, MO, MB,

HT, TI, and TA: collected data. HT, YT, and HO: analyzed data. HT and HO: wrote the paper.

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References

1. Shouval D, Samuel D. Hepatitis B immune globulin to prevent hepatitis B virus graft reinfection following liver transplantation: a concise review. *Hepatology* 2000; **32**: 1189.
2. Samuel D. Liver transplantation and hepatitis B virus infection: the situation seems to be under control, but the virus is still there. *J Hepatol* 2001; **34**: 943.
3. Markowitz JS, Martin P, Conrad AJ, *et al.* Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. *Hepatology* 1998; **28**: 585.
4. Samuel D, Muller R, Alexander G, *et al.* Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med* 1993; **329**: 1842.
5. Olivera-Martinez MA, Gallegos-Orozco JF. Recurrent viral liver disease (hepatitis B and C) after liver transplantation. *Arch Med Res* 2007; **38**: 691.
6. Coffin CS, Terrault NA. Management of hepatitis B in liver transplant recipients. *J Viral Hepat* 2007; **14**(Suppl. 1): 37.
7. Perrillo RP, Mason AL. Hepatitis B and liver transplantation. Problems and promises. *N Engl J Med* 1993; **329**: 1885.
8. Ghany MG, Ayola B, Villamil FG, *et al.* Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. *Hepatology* 1998; **27**: 213.
9. Hunt CM, McGill JM, Allen MI, Condreay LD. Clinical relevance of hepatitis B viral mutations. *Hepatology* 2000; **31**: 1037.
10. Gunther M, Neuhaus R, Bauer T, Jilg W, Holtz JA, Bienzle U. Immunization with an adjuvant hepatitis B vaccine in liver transplant recipients: antibody decline and booster vaccination with conventional vaccine. *Liver Transpl* 2006; **12**: 316.
11. Sanchez-Fueyo A, Rimola A, Grande L, *et al.* Hepatitis B immunoglobulin discontinuation followed by hepatitis B virus vaccination: a new strategy in the prophylaxis of hepatitis B virus recurrence after liver transplantation. *Hepatology* 2000; **31**: 496.
12. Starkel P, Stoffel M, Lerut J, Horsmans Y. Response to an experimental HBV vaccine permits withdrawal of HBIg prophylaxis in fulminant and selected chronic HBV-infected liver graft recipients. *Liver Transpl* 2005; **11**: 1228.
13. Sanchez-Fueyo A, Martinez-Bauer E, Rimola A. Hepatitis B vaccination after liver transplantation. *Hepatology* 2002; **36**: 257.
14. Rosenau J, Hooman N, Rifai K, *et al.* Hepatitis B virus immunization with an adjuvant containing vaccine after liver transplantation for hepatitis B-related disease: failure of humoral and cellular immune response. *Transpl Int* 2006; **19**: 828.
15. Rosenau J, Hooman N, Hadem J, *et al.* Failure of hepatitis B vaccination with conventional HBsAg vaccine in patients with continuous HBIG prophylaxis after liver transplantation. *Liver Transpl* 2007; **13**: 367.
16. Lo CM, Liu CL, Chan SC, Lau GK, Fan ST. Failure of hepatitis B vaccination in patients receiving lamivudine prophylaxis after liver transplantation for chronic hepatitis B. *J Hepatol* 2005; **43**: 283.
17. Bienzle U, Gunther M, Neuhaus R, *et al.* Immunization with an adjuvant hepatitis B vaccine after liver transplantation for hepatitis B-related disease. *Hepatology* 2003; **38**: 811.
18. Angelico M, Di Paolo D, Trinito MO, *et al.* Failure of a reinforced triple course of hepatitis B vaccination in patients transplanted for HBV-related cirrhosis. *Hepatology* 2002; **35**: 176.
19. Albeniz Arbizu E, Barcena Marugan R, Oton Nieto E, *et al.* Prophylaxis of recurrent hepatitis B virus by vaccination after liver transplant: preliminary results. *Transplant Proc* 2003; **35**: 1848.
20. Soejima Y, Ikegami T, Taketomi A, *et al.* Hepatitis B vaccination after living donor liver transplantation. *Liver Int* 2007; **27**: 977.
21. Castells L, Esteban R. Hepatitis B vaccination in liver transplant candidates. *Eur J Gastroenterol Hepatol* 2001; **13**: 359.
22. Tanaka Y, Ohdan H, Onoe T, *et al.* Low incidence of acute rejection after living-donor liver transplantation: immunologic analyses by mixed lymphocyte reaction using a carboxyfluorescein diacetate succinimidyl ester labeling technique. *Transplantation* 2005; **79**: 1262.
23. Tanaka Y, Ohdan H, Onoe T, Asahara T. Multiparameter flow cytometric approach for simultaneous evaluation of proliferation and cytokine-secreting activity in T cells responding to allo-stimulation. *Immunol Invest* 2004; **33**: 309.
24. Wells AD, Gudmundsdottir H, Turka LA. Following the fate of individual T cells throughout activation and clonal expansion. Signals from T cell receptor and CD28 differentially regulate the induction and duration of a proliferative response. *J Clin Invest* 1997; **100**: 3173.
25. Chalasani N, Smallwood G, Halcomb J, Fried MW, Boyer TD. Is vaccination against hepatitis B infection indicated in patients waiting for or after orthotopic liver transplantation? *Liver Transpl Surg* 1998; **4**: 128.

26. Horlander JC, Boyle N, Manam R, *et al.* Vaccination against hepatitis B in patients with chronic liver disease awaiting liver transplantation. *Am J Med Sci* 1999; **318**: 304.
27. Kallinowski B, Benz C, Buchholz L, Stremmel W. Accelerated schedule of hepatitis B vaccination in liver transplant candidates. *Transplant Proc* 1998; **30**: 797.
28. Van Thiel DH, el-Ashmawy L, Love K, Gavaler JS, Starzl TE. Response to hepatitis B vaccination by liver transplant candidates. *Dig Dis Sci* 1992; **37**: 1245.
29. Villeneuve E, Vincelette J, Villeneuve JP. Ineffectiveness of hepatitis B vaccination in cirrhotic patients waiting for liver transplantation. *Can J Gastroenterol* 2000; **14**(Suppl. B): 59.
30. Paramore CG, Turner DA, Madison RD. Fluorescent labeling of dissociated fetal cells for tissue culture. *J Neurosci Methods* 1992; **44**: 7.
31. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29.

LIVER TRANSPLANTATION 15:208-215, 2009

ORIGINAL ARTICLE

Significant Correlation Between Spleen Volume and Thrombocytopenia in Liver Transplant Patients: A Concept for Predicting Persistent Thrombocytopenia

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Interferon (IFN) therapy with or without ribavirin treatment is well established as a standard antiviral treatment for hepatitis C virus (HCV)–infected patients. However, susceptibility to thrombocytopenia is a major obstacle for initiating or continuing this therapy, particularly in liver transplant (LTx) recipients with HCV. Studies have reported that splenectomy performed concurrently with LTx is a feasible strategy for conditioning patients for anti-HCV IFN therapy. However, the relationship between the severity of splenomegaly and alterations in the blood cytopenia in LTx recipients remains to be clarified. Here, we analyzed the relationship between spleen volume (SV) and thrombocytopenia in 45 patients who underwent LTx at Hiroshima University Hospital. The extent of pre-LTx splenomegaly [the SV to body surface area (BSA) ratio in an individual] was inversely correlated with both the post-LTx white blood cell count and platelet (PLT) count ($P < 0.001$). Furthermore, the PLT count of patients with thrombocytopenia (PLT count $\leq 5 \times 10^4/mm^3$) increased significantly in the group without splenomegaly (SV/BSA value < 400) versus that in the group with splenomegaly ($P = 0.005$). Thus, if both splenomegaly and thrombocytopenia coexist (PLT count $\leq 5 \times 10^4/mm^3$ and SV/BSA value ≥ 400), persistent thrombocytopenia is predictable after LTx. ***Liver Transpl 15:208-215, 2009.*** © **2009 AASLD.**

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Postoperative thrombocytopenia is a common feature in liver transplant (LTx) patients.[1,2] The mechanism underlying this thrombocytopenia is considered to involve the peripheral destruction and/or consumption of platelets (PLTs)[2,3] because megakaryotic hyperplasia has been observed in the bone marrow aspirates of LTx recipients.[2] Severe thrombocytopenia resulting from bleeding complications during the postoperative period may lead to increased morbidity and mortality.[4,5] Furthermore, the PLT count is one of the crucial determinants for the discontinuation of interferon (IFN) administration, which is used as a preemptive measure or as a treatment strategy for recurrent hepatitis C virus (HCV) infections.[6] Thrombocytopenia in patients with cirrhosis has been reported to be caused by an increased PLT pool in the enlarged spleen.[7-9] Splenectomy may alleviate the postoperative thrombocytopenia in LTx patients; however, the septic complications following this procedure have generally been reported to have an adverse effect on LTx outcome.[10-13] Therefore,

Abbreviations: ALT, alanine aminotransferase; BSA, body surface area; HCV, hepatitis virus C; Hgb, hemoglobin; IFN, interferon; LTx, liver transplant; MELD, Model for End-Stage Liver Disease; PLT, platelet; PSE, partial splenic embolization; SD, standard deviation; SV, spleen volume; T-Bil, total bilirubin; WBC, white blood cell.

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the conditions under which splenectomy can be performed to prevent the development of thrombocytopenia following LTx should be carefully defined. Analyzing the association between the extent of splenomegaly and thrombocytopenia in LTx recipients would provide important information in this respect.

In this study, we report an analysis of the relationship between preoperative spleen volume (SV) and blood cytopenia in 45 patients who underwent LTx at the Hiroshima University Hospital.

PATIENTS AND METHODS

Patients

Between January 2002 and May 2007, 83 LTx on 81 patients were underwent at the University of Hiroshima. Of these, 36 patients were excluded from the study because of death within 1 year (n = 13), fulminant hepatitis as the primary disease (n = 7), retransplantation (n = 2), splenectomy that had already been performed at LTx (n = 2), or insufficient clinical examinations (n = 12). The remaining 45 patients who had undergone LTx because of liver cirrhosis were analyzed. The profiles of these patients are shown in Table 1. Computed tomography was performed preoperatively and at 1 and 6 months after LTx. The hemoglobin (Hgb) levels and the serial white blood cell and PLT counts were obtained from the medical charts of the LTx recipients. The SV was measured from computed tomography images obtained with a workstation (Virtual Place Advance 300, AZE, Ltd.). The body surface area (BSA) was calculated as follows with the equation of Whitington et al.[14]:

$$BSA\ (m^2) = Body\ weight\ (kg)^{0.425} \times Body\ height\ (cm)^{0.725} \times 0.007184$$

In this study, the SV/BSA value was used as a parameter for assessing splenomegaly.

Statistical Analysis

The postoperative data were compared with an unpaired Student t test. The correlations between variables were assessed with the Spearman rank order correlation coefficient, and a P value < 0.05 was considered statistically significant. The data are expressed as mean ± standard deviation.

RESULTS

The extent of thrombocytopenia and splenomegaly prior to LTx varied in the 45 patients. This might reflect various degrees of liver cirrhosis. The PLT count ranged from $2.6 \times 10^4/mm^3$ to $18.0 \times 10^4/mm^3$, and the SV ranged from 98 to 1299 mL. The average PLT count and SV of the 45 patients before and after LTx are shown in Table 2. The PLT count was observed to increase significantly 1 month after LTx. However, no further increase was observed thereafter. In contrast, the SV values demonstrated a downward trend until 1 month after LTx and plateaued thereafter.

Because both the PLT count and SV stabilized at 1 month after LTx, we investigated the correlation be-

TABLE 1. Perioperative Clinical Characteristics of Liver Transplant Recipients

Number of patients	45
Gender (male/female)	26/19
Recipient age (years, mean ± SD)	54.5 ± 6.3
Donor age (years, mean ± SD)	34.4 ± 12.9
MELD score (mean ± SD)	13.8 ± 7.5
Blood loss (mL, mean ± SD)	4245 ± 3806
Graft weight/standard liver volume (%, mean ± SD)	51.0 ± 10.9
Spleen volume (cm^3, mean ± SD)	516 ± 304
SV/BSA (mean ± SD)	306.5 ± 177.6
Portal venous pressure (mm Hg, mean ± SD)	
Initial	22.9 ± 6.3
Closure	17.1 ± 6.3
WBC count (mean ± SD)	3901 ± 2097
Hgb (mean ± SD)	10.0 ± 1.4
T-Bil (mg/dL, mean ± SD)	6.0 ± 9.4
ALT (IU/L, mean ± SD)	40.8 ± 28.2
Platelet count ($\times 10^4/mm^3$, mean ± SD)	6.6 ± 3.0
Etiology of liver disease	
Alcoholic	4
Primary biliary cirrhosis	1
Autoimmune liver disease	3
Chronic hepatitis B	15
Chronic hepatitis C	18
Hepatocellular carcinoma	27
Other	3

Abbreviations: ALT, alanine aminotransferase; Hgb, hemoglobin; MELD, Model for End-Stage Liver Disease; SD, standard deviation; SV/BSA, spleen volume to body surface area ratio; T-Bil, total bilirubin; WBC, white blood cell.

TABLE 2. Changes in the Spleen Volume and Platelet Count After Liver Transplantation

Spleen volume (cm^3, mean ± SD)	
Before LTx	516 ± 304
1 month after LTx	421 ± 220
6 months after LTx	417 ± 212
Platelet count ($\times 10^4/mm^3$, mean ± SD)	
Before LTx	6.6 ± 3.0
1 month after LTx	12.4 ± 6.0
6 months after LTx	12.3 ± 5.7

Abbreviations: LTx, liver transplant; SD, standard deviation.

TABLE 3. Correlation Between Postoperative Thrombocytopenia and Clinical Variables

	Correlation Coefficient	*P* Value
Recipient age	0.20	0.180
Donor age	−0.09	0.574
MELD score	−0.05	0.745
Blood loss	−0.15	0.333
Graft weight/standard liver volume	0.38	0.010
Portal venous pressure		
Initial	−0.18	0.260
Closure	−0.26	0.101
Pre-LTx WBC count	0.37	0.012
Pre-LTx hemoglobin	−0.10	0.500
Pre-LTx PLT count	0.61	0.00001
Pre-LTx T-Bil	−0.24	0.111
Pre-LTx ALT	−0.13	0.400
SV/BSA	−0.67	0.000006

Abbreviations: ALT, alanine aminotransferase; LTx, liver transplant; MELD, Model for End-Stage Liver Disease; PLT, platelet; SV/BSA, spleen volume to body surface area ratio; T-Bil, total bilirubin; WBC, white blood cell.

tween the thrombocytopenia at 1 month after LTx and the perioperative clinical variables by a simple linear regression analysis. The PLT count at 1 month after LTx was clearly inversely related to the pre-LTx SV/BSA value and positively related to the PLT count prior to LTx (Table 3).

The relationship between the pre-LTx SV/BSA value and the PLT count at 1 month after LTx is shown in Fig. 1A. On the basis of the regression line, thrombocytopenia of less than 10×10^4 PLTs/mm^3 at 1 month after LTx could be expected in patients who demonstrated pre-LTx SV/BSA levels of >400. The patients were divided into 2 groups: those with a pre-LTx SV/BSA value < 400 (SV < 400 group; n = 33) and those with a pre-LTx SV/BSA value ≥ 400 (SV ≥ 400 group; n = 12). No significant differences were observed in the Hgb concentrations between the groups. The PLT count in the SV < 400 group significantly increased immediately after LTx and was maintained until 6 months. In contrast, during the observation period, the PLT count was maintained at a lower level and the SV was maintained at a high level in the SV ≥ 400 group ($P < 0.01$; Fig. 1). Thus, preoperative splenomegaly may influence the SV and PLT count at 1 and 6 months after LTx.

A plot of the PLT count before LTx versus the PLT count 1 month after LTx is shown in Fig. 2A. As indicated by the regression line, thrombocytopenia of $<10 \times 10^4$ PLTs/mm^3 at 1 month after LTx could be expected in patients who demonstrated pre-LTx PLT counts of less than 5×10^4/mm^3. The patients were divided into 2 groups: those in whom the PLT count prior to LTx was greater than 5×10^4/mm^3 (PLT > 50K group; n = 28) and those in whom the PLT count prior to LTx was less than or equal to 5×10^4/mm^3 (PLT ≤ 50K group; n = 17). During the observation period, the white blood cell and PLT counts in the PLT > 50K group were significantly higher than those in the PLT ≤ 50K group ($P < 0.05$ and $P < 0.01$, respectively). Furthermore, the SV in the PLT > 50K group was lower than that in the PLT ≤ 50K group ($P < 0.05$). Among the various immunosuppressants, inhibitors of nucleic acid synthesis such as mycophenolate mofetil and azathioprine possibly worsen thrombocytopenia.[15] In this study, 23, 7, 22, and 8 patients were orally administered mycophenolate mofetil within 6 months after LTx in the SV < 400 group, SV ≥ 400 group, PLT > 50K group, and PLT ≤ 50K group, respectively. The dosage of this immunosuppressant was not significantly different among the groups.

Thus, the pre-LTx values of both the SV/BSA level and the PLT count had a significant impact on the PLT count at 1 month after LTx. We further examined whether these factors mutually influence the PLT count at 1 month after LTx. The patients were categorized as follows: the PLT > 50K, SV < 400 group, which consisted of 26 patients without severe thrombocytopenia (pre-LTx PLT count > 5×10^4/mm^3) and with an SV/BSA value < 400; the PLT > 50K, SV ≥ 400 group, which consisted of 2 patients without severe thrombocytopenia and with an SV/BSA value ≥ 400; the PLT ≤ 50K, SV < 400 group, which consisted of 7 patients with severe thrombocytopenia (pre-LTx PLT count ≤ 5×10^4/mm^3) and an SV/BSA value < 400; and the PLT ≤ 50K, SV ≥ 400 group, which consisted of 10 patients with severe thrombocytopenia and an SV/BSA value ≥ 400 (Fig. 3A). The PLT > 50K, SV < 400 group did not suffer from severe splenomegaly, and their PLT count was 15.2 ± 6.2/mm^3 at 1 month after LTx (data not shown). The number of patients in the PLT > 50K, SV ≥ 400 group was too small for a meaningful analysis. The PLT ≤ 50K, SV ≥ 400 group suffered from splenomegaly, and their PLT count at 1 month after LTx was only 7.0 ± 2.1/mm^3. The PLT ≤ 50K, SV < 400 group did not suffer from splenomegaly, and their PLT count increased to 11.3 ± 3.0/mm^3 at 1 month after LTx. The PLT count in the PLT ≤ 50K, SV < 400 group was observed to be significantly elevated versus the PLT ≤ 50K, SV ≥ 400 group ($P = 0.005$; Fig. 3B). Thus, in LTx recipients without splenomegaly, the PLT count can be expected to increase shortly after the operation.

At our institute, preemptive IFN therapy for HCV-infected recipients has been practiced since 2005. We decided to administer preemptive IFN therapy to 9 HCV-infected recipients within 6 months after LTx. In 8 of the 9 HCV patients, neither pre-LTx splenomegaly (SV/BSA value ≥ 400) nor thrombocytopenia (PLT count ≤ 5×10^4/mm^3) existed. They were able to continuously receive IFN therapy without severe thrombocytopenia. In the remaining HCV patient, pre-LTx splenomegaly and thrombocytopenia coexisted. This particular patient suffered from persistent thrombocy-

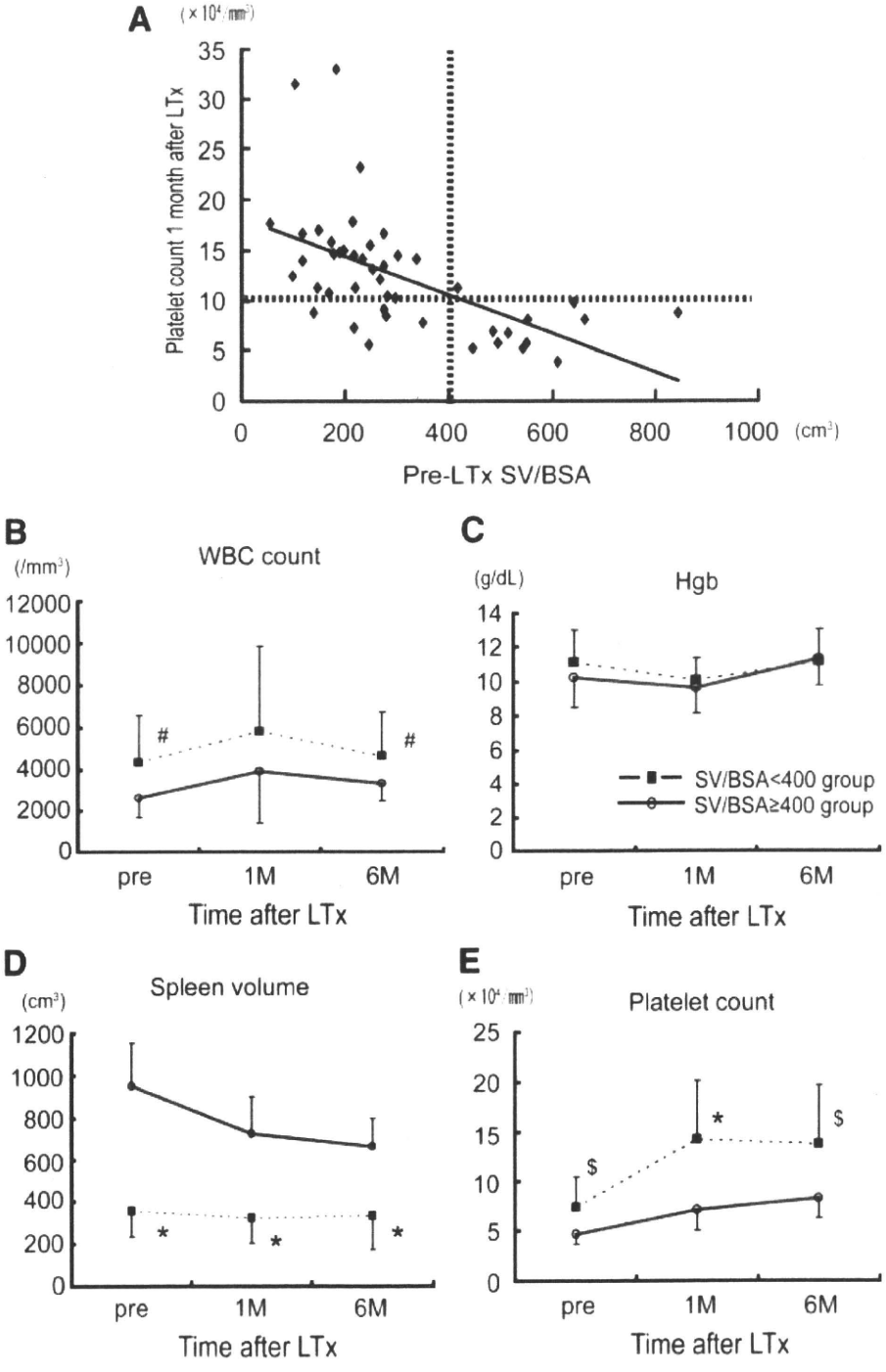

Figure 1. (A) Correlation between the pre-LTx SV/BSA value and PLT count at 1 month after LTx ($r = 0.67$, $P < 0.0001$). A regression line is superimposed on the plot: $y = -0.02x + 18.23$ (x axis: SV/BSA value; y axis: post-LTx PLT count at 1 month). Changes in (B) the WBC count, (C) hemoglobin concentration, (D) spleen volume, and (E) PLT count. The post-LTx values of these variables in the SV < 400 group (broken line with closed squares) and SV ≥ 400 group (thick line with open circles) are shown. There was a significant difference between the groups with respect to the WBC count, PLT count, and spleen volume ($^{\#}P < 0.05$, $^{\$}P < 0.01$, and $^{*}P < 0.001$ for the SV < 400 group versus the SV ≥ 400 group). Abbreviations: BSA, body surface area; Hgb, hemoglobin; LTx, liver transplant; PLT, platelet; SV, spleen volume; WBC, white blood cell.

topenia and eventually underwent splenectomy so that IFN therapy could be commenced only 9 months after LTx.

DISCUSSION

Thrombocytopenia is an extremely common complication in LTx patients. Several causes have been postulated for this reduced concentration of PLTs, including hypersplenism,[16,17] decreased thrombopoietin levels,[18,19] and destruction by anti-PLT antibodies.[20,21] It has also been reported that serum thrombopoietin levels or anti-PLT antibodies levels correlate with the spleen size.[22-24] This fact is consistent with the finding that the spleen size correlates with portal hypertension and the PLT count in patients with cirrhosis.[16] Our data also demonstrate that pre-LTx splenomegaly is associated with the pre-LTx PLT count. Uneventful LTx is expected to improve splenomegaly.[25,26] However, our data show that splenomegaly remained unchanged in

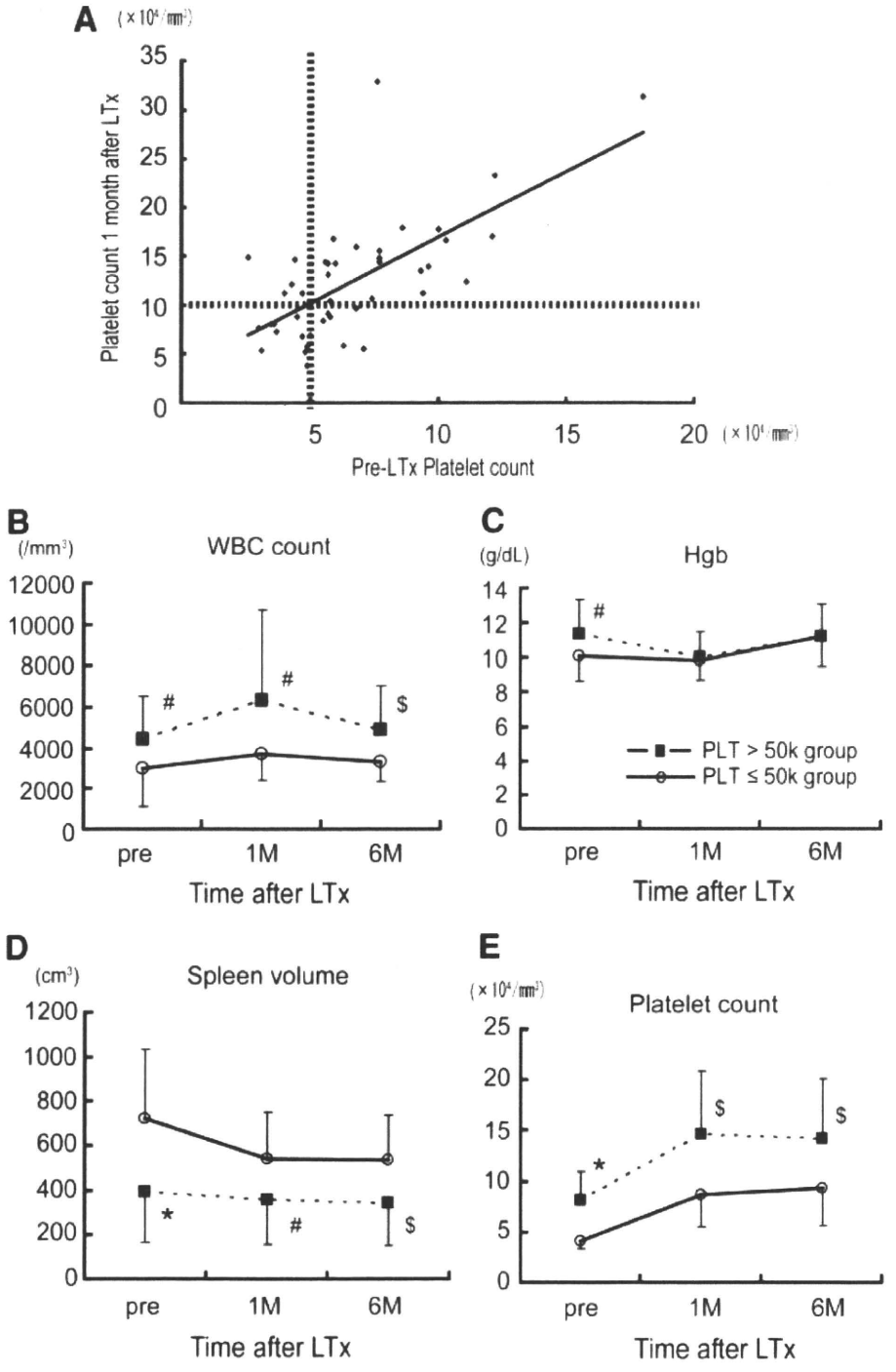

Figure 2. (A) Correlation between the pre-LTx PLT count and PLT count at 1 month after LTx ($r = 0.61$, $P < 0.0001$). A regression line is superimposed on the plot: $y = 1.35x + 3.48$ (x axis: pre-LTx PLT count; y axis: post-LTx PLT count at 1 month). Changes in (B) the WBC count, (C) hemoglobin concentrations, (D) spleen volume, and (E) PLT count. The values of these variables after LTx in the PLT > 50K group (broken line with closed squares) and PLT ≤ 50K group (thick line with open circles) are shown. There was a significant difference between the groups with respect to the WBC count, hemoglobin concentration, PLT count, and spleen volume ($^{\#}P < 0.05$, $^{\$}P < 0.01$, and $^{*}P < 0.001$ for the PLT > 50K group versus the PLT ≤ 50K group). Abbreviations: Hgb, hemoglobin; LTx, liver transplant; PLT, platelet; WBC, white blood cell.

LTx recipients whose pre-LTx SV/BSA level exceeded 400. Among the various perioperative clinical factors, the SV/BSA level was the most significant determinant of the PLT count after LTx. In the present analysis, the PLT count of patients with pre-LTx thrombocytopenia (PLT count $\leq 5 \times 10^4/mm^3$) increased significantly after LTx in the group with no pre-LTx splenomegaly (SV/BSA value < 400) versus the group with pre-LTx splenomegaly ($P < 0.01$).

We recently reported that splenectomy should be performed simultaneously with LTx in HCV patients with a PLT count $< 6 \times 10^4/mm^3$ in order to complete preemptive IFN therapy at an earlier time point in the postoperative period.[27] Several authors have reported that the only indication for simultaneous splenectomy in LTx is the preoperative PLT count[12,28,29] because thrombocytopenia in the immediate posttransplant period is correlated with a low preoperative PLT count.[30]

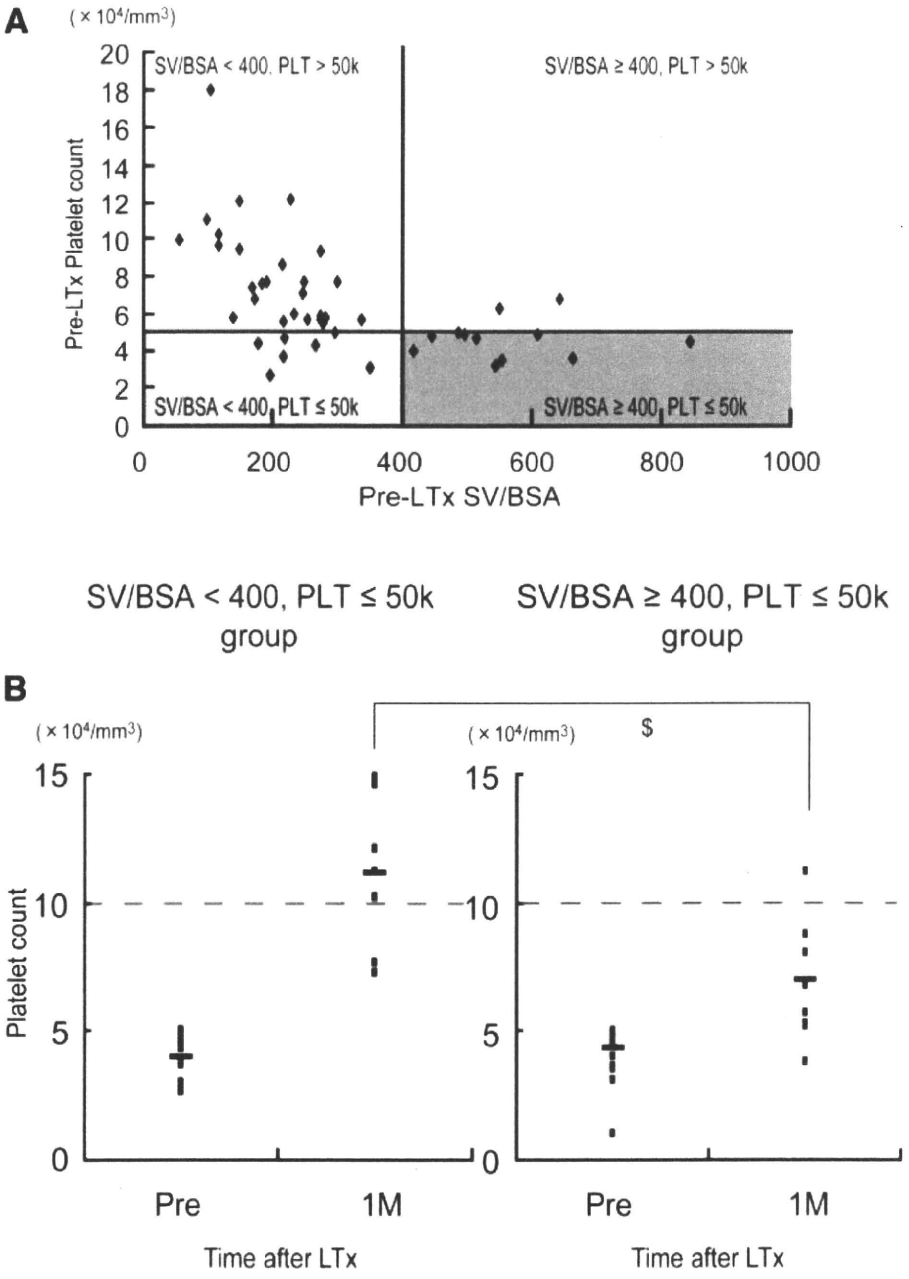

Figure 3. (A) Correlation between the pre-LTx SV/BSA value and pre-LTx PLT count. The patients were categorized as follows: the PLT > 50K, SV < 400 group, which consisted of patients without severe thrombocytopenia (pre-LTx PLT count > 5 × 10^4/mm^3) and without severe splenomegaly (pre-LTx SV/BSA level < 400); the PLT > 50K, SV ≥ 400 group, which consisted of patients without severe thrombocytopenia and with severe splenomegaly (pre-LTx SV/BSA value ≥ 400); the PLT ≤ 50K, SV < 400 group, which consisted of patients with severe thrombocytopenia (pre-LTx PLT count ≤ 5 × 10^4/mm^3) and without severe splenomegaly; and the PLT ≤ 50K, SV ≥ 400 group, which consisted of patients with severe thrombocytopenia and with severe splenomegaly. (B) Changes in the PLT count in the PLT ≤ 50K, SV < 400 group and PLT ≤ 50K, SV ≥ 400 group. The PLT count in the PLT ≤ 50K, SV < 400 group was significantly elevated versus that in the PLT ≤ 50K, SV ≥ 400 group ($^{\$}P$ < 0.01). Abbreviations: BSA, body surface area; LTx, liver transplant; PLT, platelet; SV, spleen volume.

Studies have also reported that the routine administration of simultaneous splenectomy and LTx in all HCV patients conditions them for anti-HCV IFN therapy.[31] Although splenectomy strongly affects thrombocytopenia, it might predispose patients to develop portal vein thrombosis or increase the risk of sepsis, which is particularly lethal for immunosuppressed subjects.[32] Thus, caution is advised when recommending splenectomy for patients undergoing LTx. Compared with splenectomy, splenic artery ligation is a technically simpler procedure that can easily be included in a complicated transplant operation.[33] However, the benefit of splenic artery ligation in reducing posttransplant thrombocytopenia is controversial.[34,35] Recently, partial splenic embolization (PSE) has been described as a useful procedure for severe post-LTx thrombocytopenia,[36,37] and PSE could also be an option for pre-LTx.[38] However, several groups have reported complications generally observed after PSE, including splenic infarction, abscess formation, reduced immunity-related septic complications, and portal thrombosis.[39,40] Thus, the most appropriate methods among the strategies or alternative methods for avoiding persistent thrombocytopenia remain to be elucidated.

In conclusion, the pre-LTx SV/BSA value and PLT count have been correlated with post-LTx thrombocytopenia. If both splenomegaly and thrombocytopenia coexist (PLT count ≤ 5 × 10^4/mm^3 and SV/BSA

value ≥ 400), persistent thrombocytopenia is predictable after LTx.

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REFERENCES

1. Plevak DJ, Halma GA, Forstrom LA, Dewanjee MK, O'Connor MK, Moore SB, et al. Thrombocytopenia after liver transplantation. Transplant Proc 1988;20(suppl 1):630-633.
2. McCaughan GW, Herkes R, Powers B, Rickard K, Gallagher ND, Thompson JF, et al. Thrombocytopenia post liver transplantation. Correlations with pre-operative platelet count, blood transfusion requirements, allograft function and outcome. J Hepatol 1992;16:16-22.
3. Richards EM, Alexander GJ, Calne RY, Baglin TP. Thrombocytopenia following liver transplantation is associated with platelet consumption and thrombin generation. Br J Haematol 1997;98:315-321.
4. Mor E, Jennings L, Gonwa TA, Holman MJ, Gibbs J, Solomon H, et al. The impact of operative bleeding on outcome in transplantation of the liver. Surg Gynecol Obstet 1993;176:219-227.
5. Tabasco-Minguillan J, Jain A, Naik M, Weber KM, Irish W, Fung JJ, et al. Gastrointestinal bleeding after liver transplantation. Transplantation 1997;63:60-67.
6. Shergill AK, Khalili M, Straley S, Bollinger K, Roberts JP, Ascher NA, et al. Applicability, tolerability and efficacy of preemptive antiviral therapy in hepatitis C-infected patients undergoing liver transplantation. Am J Transplant 2005;5:118-124.
7. Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. J Clin Invest 1966;45:645-657.
8. Kutti J, Weinfeld A, Westin J. The relationship between splenic platelet pool and spleen size. Scand J Haematol 1972;9:351-354.
9. Wadenvik H, Denfors I, Kutti J. Splenic blood flow and intrasplenic platelet kinetics in relation to spleen volume. Br J Haematol 1987;67:181-185.
10. Neumann UP, Langrehr JM, Kaisers U, Lang M, Schmitz V, Neuhaus P. Simultaneous splenectomy increases risk for opportunistic pneumonia in patients after liver transplantation. Transpl Int 2002;15:226-232.
11. Lusebrink R, Blumhardt G, Lohmann R, Bachmann S, Knoop M, Lemmens HP, et al. Does concomitant splenectomy raise the mortality of liver transplant recipients? Transpl Int 1994;7(suppl 1):S634-S636.
12. Troisi R, Colle I, Van Vlierberghe H, Hesse UJ, Cuomo O, de Hemptinne B. Splenectomy and liver transplantation. Transplant Proc 2001;33:1500-1501.
13. Samimi F, Irish WD, Eghtesad B, Demetris AJ, Starzl TE, Fung JJ. Role of splenectomy in human liver transplantation under modern-day immunosuppression. Dig Dis Sci 1998;43:1931-1937.
14. Whitington PF, Emond JC, Whitington SH, Broelsch CE, Baker AL. Small-bowel length and the dose of cyclosporine in children after liver transplantation. N Engl J Med 1990; 322:733-738.
15. Danesi R, Del Tacca M. Hematologic toxicity of immunosuppressive treatment. Transplant Proc 2004;36:703-704.
16. Adinolfi LE, Giordano MG, Andreana A, Tripodi MF, Utili R, Cesaro G, et al. Hepatic fibrosis plays a central role in the pathogenesis of thrombocytopenia in patients with chronic viral hepatitis. Br J Haematol 2001;113:590-595.
17. Bashour FN, Teran JC, Mullen KD. Prevalence of peripheral blood cytopenias (hypersplenism) in patients with nonalcoholic chronic liver disease. Am J Gastroenterol 2000;95:2936-2939.
18. Martin TG III, Somberg KA, Meng YG, Cohen RL, Heid CA, de Sauvage FJ, et al. Thrombopoietin levels in patients with cirrhosis before and after orthotopic liver transplantation. Ann Intern Med 1997;127:285-288.
19. Tsukahara A, Sato Y, Yamamoto S, Suzuki S, Nakatsuka H, Watanabe T, et al. Thrombopoietin levels and peripheral platelet counts following living related donor liver transplantation. Hepatogastroenterology 2003;50:227-230.
20. Pockros PJ, Duchini A, McMillan R, Nyberg LM, McHutchison J, Viernes E. Immune thrombocytopenic purpura in patients with chronic hepatitis C virus infection. Am J Gastroenterol 2002;97:2040-2045.
21. Nagamine T, Ohtuka T, Takehara K, Arai T, Takagi H, Mori M. Thrombocytopenia associated with hepatitis C viral infection. J Hepatol 1996;24:135-140.
22. Giannini E, Borro P, Botta F, Fumagalli A, Malfatti F, Podesta E, et al. Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. J Hepatol 2002;37:572-577.
23. Kuwana M, Okazaki Y, Kaburaki J, Kawakami Y, Ikeda Y. Spleen is a primary site for activation of platelet-reactive T and B cells in patients with immune thrombocytopenic purpura. J Immunol 2002;168:3675-3682.
24. Sanjo A, Satoi J, Ohnishi A, Maruno J, Fukata M, Suzuki N. Role of elevated platelet-associated immunoglobulin G and hypersplenism in thrombocytopenia of chronic liver diseases. J Gastroenterol Hepatol 2003;18:638-644.
25. Egami S, Sugawara Y, Mizuta K, Kaneko J, Kawarasaki H, Makuuchi M. Effect of pediatric living-donor liver transplantation on splenomegaly. Transplantation 2002;74: 1639-1642.
26. Kaneko J, Sugawara Y, Akamatsu N, Kokudo N, Makuuchi M. Spleen volume and platelet number changes after living donor liver transplantation in adults. Hepatogastroenterology 2004;51:262-263.
27. Tashiro H, Itamoto T, Ohdan H, Fudaba Y, Kohashi T, Amano H, et al. Should splenectomy be performed for hepatitis C patients undergoing living-donor liver transplantation? J Gastroenterol Hepatol 2007;22:959-960.
28. Cescon M, Sugawara Y, Takayama T, Seyama Y, Sano K, Imamura H, et al. Role of splenectomy in living-donor liver transplantation for adults. Hepatogastroenterology 2002; 49:721-723.
29. Hashikura Y, Kawasaki S, Okumura N, Ishikawa S, Matsunami H, Ikegami T, et al. Prevention of hepatic artery thrombosis in pediatric liver transplantation. Transplantation 1995;60:1109-1112.
30. Chatzipetrou MA, Tsaroucha AK, Weppler D, Pappas PA, Kenyon NS, Nery JR, et al. Thrombocytopenia after liver transplantation. Transplantation 1999;67:702-706.
31. Kishi Y, Sugawara Y, Akamatsu N, Kaneko J, Tamura S, Kokudo N, et al. Splenectomy and preemptive interferon therapy for hepatitis C patients after living-donor liver transplantation. Clin Transplant 2005;19:769-772.
32. Settmacher U, Nussler NC, Glanemann M, Haase R, Heise M, Bechstein WO, et al. Venous complications after orthotopic liver transplantation. Clin Transplant 2000;14:235-241.
33. Lo CM, Liu CL, Fan ST. Portal hyperperfusion injury as the cause of primary nonfunction in a small-for-size liver graft—successful treatment with splenic artery ligation. Liver Transpl 2003;9:626-628.
34. Matsukura A, Kita Y, Harihara Y, Kubota K, Takayama T, Kawarasaki H, et al. Is splenic artery ligation effective for thrombocytopenia early after liver transplantation? Transplant Proc 1999;31:2906-2907.

35. Troisi R, Cammu G, Militerno G, De Baerdemaeker L, Decruyenaere J, Hoste E, et al. Modulation of portal graft inflow: a necessity in adult living-donor liver transplantation? Ann Surg 2003;237:429-436.
36. Herrero JI, Sangro B, Quiroga J, Bilbao JI, Yuste JR, Longo J, et al. Partial splenic embolization in the treatment of thrombocytopenia after liver transplantation. Transplantation 1997;63:482-484.
37. Sangro B, Bilbao I, Herrero I, Corella C, Longo J, Beloqui O, et al. Partial splenic embolization for the treatment of hypersplenism in cirrhosis. Hepatology 1993;18:309-314.
38. Umeda Y, Yagi T, Sadamori H, Matsukawa H, Matsuda H, Shinoura S, et al. Preoperative proximal splenic artery embolization: a safe and efficacious portal decompression technique that improves the outcome of live donor liver transplantation. Transpl Int 2007;20:947-955.
39. Sekikawa T, Shatney CH. Septic sequelae after splenectomy for trauma in adults. Am J Surg 1983;145:667-673.
40. Bader-Meunier B, Gauthier F, Archambaud F, Cynober T, Mielot F, Dommergues JP, et al. Long-term evaluation of the beneficial effect of subtotal splenectomy for management of hereditary spherocytosis. Blood 2001;97:399-403.

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ORIGINAL ARTICLE

Prolongation of interferon therapy for recurrent hepatitis C after living donor liver transplantation: Analysis of predictive factors of sustained virological response, including amino acid sequence of the core and NS5A regions of hepatitis C virus

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Abstract

Objective. The aim of the present retrospective study was to evaluate the therapeutic efficacy and predictive factors of prolongation of treatment with peginterferon (PEGIFN) combined with ribavirin (RBV) for recurrent hepatitis C after living donor liver transplantation (LDLT). ***Methods.*** Fifty-three patients underwent LDLT due to HCV-related end-stage liver disease. Sixteen patients were removed from the study as a result of early death ($n = 14$), no recurrence of HCV ($n = 1$) and refusal of antiviral therapy ($n = 1$). Therapy is ongoing in another 10 patients. The remaining 27 patients were available to establish the efficacy of IFN therapy. HCV genotype was 1b in 24 patients. All patients with genotype 1b were treated with IFN therapy for at least 48 weeks after HCV RNA levels had become undetectable. Amino acid substitutions in the HCV core region and NS5A region were analyzed by direct sequencing before LDLT. ***Results.*** The rate of sustained virological response (SVR) was 37.0% (10/27). SVR rate in patients with genotype 1 was 29.2% (7/24) and 100% (3/3) in patients with genotype 2. Most patients with genotype 1b whose HCV RNA reached undetectable levels achieved SVR (87.5%; 7/8). However, mutation of the HCV core region and number of ISDR mutations were not associated with SVR rate in LDLT in our study. ***Conclusions.*** Prolonged IFN therapy for more than 48 weeks after HCV RNA reached undetectable levels might prevent virological relapse of HCV.

Key Words: *Core and NS5A regions, HCV, IFN, LDLT*

Introduction

Hepatitis C virus (HCV)-related end-stage liver disease is currently the leading indication for liver transplantation (LT). Unfortunately, prevention of HCV infection after transplantation is difficult and, unlike the situation with the prevention of hepatitis B virus after transplantation [1], HCV re-infection after LT is almost universal, with histological evidence of chronic hepatitis in approximately 50% of patients within 1 year and cirrhosis in about 30% after 5 years. This in turn yields an excess risk of death or

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