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Impact of Donor and Recipient Single Nucleotide Polymorphisms in Living Liver Donor Transplantation for Hepatitis C

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ABSTRACT

Introduction. Recently, several studies have shown that specific single nucleotide polymorphisms (SNPs) affect liver fibrosis progression in patients with hepatitis C virus (HCV) infection. In this study, we examined the impact of donor and recipient SNPs on the progression of fibrosis after liver transplantation for HCV infection.

Methods. This cohort study enrolled 43 patients with HCV infection who underwent liver transplantation at our hospital. We evaluated 5 genotypes (rs4374383, rs2629751, rs9380516, rs8099917, and rs738409) that have been reported to be significant predictors of fibrosis in HCV infection using a Taqman assay.

Results. Liver fibrosis (stage $\geq$ F1, New Inuyama classification) was detected at 1 year after liver transplantation in 30 cases (70%). The rs2629751 non–AA-genotype was found to be significantly associated with fibrosis progression at 1 year after liver transplantation (AA:GG or GA = 46%:88%, $P < .05$). The primary outcome was stage $\geq$F2 (portoportal septa) or liver-related mortality in 22 patients. The time to stage $\geq$F2 fibrosis or liver-related mortality was significantly different only in terms of the donor rs2629751 genotype (AA:GG or GA = 5.5 $\pm$ 0.6 years:3.6 $\pm$ 0.7 years, $P = .025$).

Conclusions. The rs2629751 genotype may be an important predictor of posttransplant outcome in HCV-infected patients. This result might be useful in donor selection for liver transplantation in HCV-infected patients and may guide therapeutic decisions regarding early antiviral treatment.

HEPATITIS C VIRUS (HCV) infects 170 million people worldwide, and is the most indication for liver transplantation [1,2]. Moreover, HCV reinfection occurs in almost all liver transplantation recipients and is associated with a poor survival outcome [3,4]. Although different host and viral factors have been associated with poor prognosis [5], predictive factors for fibrosis progression after liver transplantation for HCV infection have not been fully elucidated.

Several single-nucleotide polymorphisms (SNPs) have been reported to be potential predictors of severe fibrosis in patients with HCV infection through a genome-wide association study (GWAS). SNPs located near the *IL28* gene have been strongly associated with efficacy of interferon (IFN) therapy [6]. Recently, these SNPs have also been associated with progression to cirrhosis [7].

The SNPs in the patatin-like phospholipase 3 gene (*PNPLA3*) have been identified as predictors of severity of non-alcoholic fatty liver disease in a GWAS [8,9]. Recent studies have also reported an association of this SNP with fibrosis, hepatocellular carcinoma, and response to antiviral treatment in patients with HCV infection [10,11]. Moreover, the recent GWAS in Europe and United States identified several SNPs that were associated with steatosis and fibrosis progression in patients with HCV [12]. However, the role of these SNPs in recurrent HCV after living liver donor transplantation has not been sufficiently

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Table 1. Baseline Characteristics

Variable	Mean ± SD or Ratio
Age (y)	57.83 ± 6.3
Sex male:female	24:19
HCV-RNA (log IU/mL)	5.9 ± 1.4
HCV serotype (1:2)	40:3
AST (IU/L)	69.02 ± 39.8
ALT (IU/L)	59.14 ± 44.6
γ-GTP (IU/L)	146.73 ± 354.39
MELD score	15.61 ± 6.87
Child-Pugh score	9.4 ± 6.7

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltransferase; MELD, Model for End-stage Liver Disease.

elucidated. Therefore, in the present study, we examined the presence of these SNPs in both donors and in recipients who developed recurrent HCV after living donor liver transplantation.

PATIENTS AND METHODS

Between January 1, 2005, and September 30, 2012, a total of 43 patients with HCV infection underwent living donor liver transplantation. Eight of 43 patients were given IFN therapy before liver transplantation, but none of them showed a clearance of HCV RNA before liver transplantation. Table 1 shows patients characteristics. The follow-up period ended in October 1, 2014. Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

SNP Genotyping

Genomic DNA from recipients and donors were extracted from peripheral blood mononuclear cells. Real-time polymerase chain reaction was performed using a Thermal Cycler and a Taqman probe (Life Technologies Japan, Tokyo, Japan) according to the manufacturer's protocol.

Histopathologic Examination of the Liver

Patients underwent liver biopsies 1 year after liver transplantation for HCV infection and yearly thereafter, or when abnormal liver enzyme levels were detected. Ultrasound-guided liver biopsy specimens were fixed in 10% formalin, embedded in paraffin, cut to a thickness of 4 μm, and stained with hematoxylin and eosin and Azan stain. All liver tissue specimens were evaluated by a single pathologist, who was blinded to the clinical condition of the patient. Fibrosis was graded and staged according to the New Inuyama classification system [13] as follows: F1 (periportal expansion), F2 (portoportal septa), F3 (portocentral linkage or bridging fibrosis), and F4 (cirrhosis).

Table 2. Genotyping and Progression of Fibrosis (Stage ≥ F1) on Liver Biopsies 1 Year After Liver Transplantation

Genotype	Stage ≥ F1 Cases/All (%)	*P*
rs8099917 recipients TT:TG+GG	25/33 (75):5/10 (50)	NS
Donors TT:TG+GG	21/31 (67):10/12 (83)	NS
rs738409 recipients CC:CG+GG	6/8 (75):24/35 (68)	NS
Donors CC:CG+GG	7/13 (53):23/30 (76)	NS
rs4374383 recipients AA:AG+GG	13/22 (59):17/21 (80)	NS
Donors AA:AG+GG	13/20 (65):17/23 (73)	NS
rs9380516 recipients TT:CT+CC	0/0 (0):30/43 (69)	NS
Donors TT:CT+CC	1/2 (50):29/41 (70)	NS
rs2629751 recipients AA:AG+GG	9/15 (60):21/28 (75)	NS
Donors AA:AG+GG	15/26 (57):15/17 (87)	.044

Statistical Analysis

Progression of the liver fibrosis was defined as fibrosis (≥F1) on liver biopsies 1 year after liver transplantation. The time to stage ≥ F2 (portoportal septa) or all-cause mortality, and the time to stage ≥ F2 or liver-related mortality were evaluated. The data were analyzed using the chi-square test. Kaplan–Meier survival curves were generated and compared using log-rank test. All analyses were performed using IBM SPSS statistics software, version 20.0 (SPSS Inc., Chicago, Ill).

RESULTS

The rs8099917 TT variant was present in 72% of donors and 76% of recipients. The rs738409 CC variant was detected in 27% of donors and 17% of recipients. The rs4374383 AA variant was detected in 46% of donors and 51% of recipients. The rs9380516 TT variant was detected in 4% of donors and none of the recipients. The rs2629751 AA variant was detected in 60% of donors and 34% of recipients.

Genotyping and Progression of Fibrosis (Stage ≥ F1) on Liver Biopsies at 1 Year After Liver Transplantation

A total of 30 patients had fibrosis (≥F1) on liver biopsies 1 year after liver transplantation (Table 2). The SNPs rs8099917, rs738409, rs4374383, and rs9380516 in donors and recipients showed no association with the progression of liver fibrosis on liver biopsies at 1 year after liver transplantation.

In contrast, progression of liver fibrosis on liver biopsies at 1 year after liver transplantation was seen in a

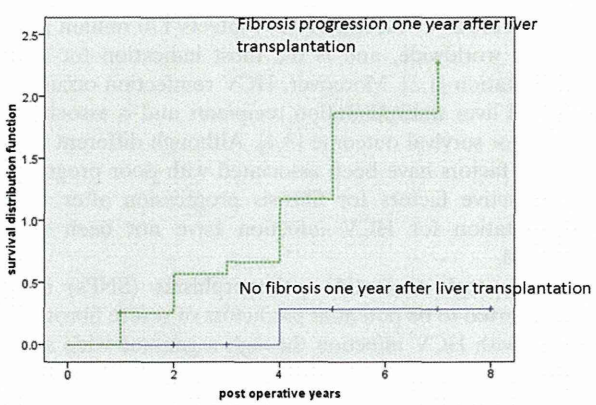

Fig 1. Time to stage 2 fibrosis or all-cause mortality. The time to stage ≥ F2 (portoportal septa) or all-cause mortality differed significantly between patients with and without liver fibrosis progression 1 year after liver transplantation (fibrosis progression:no fibrosis = 3.0 ± 0.4 years:6.6 ± 0.6 years; $P = .025$).

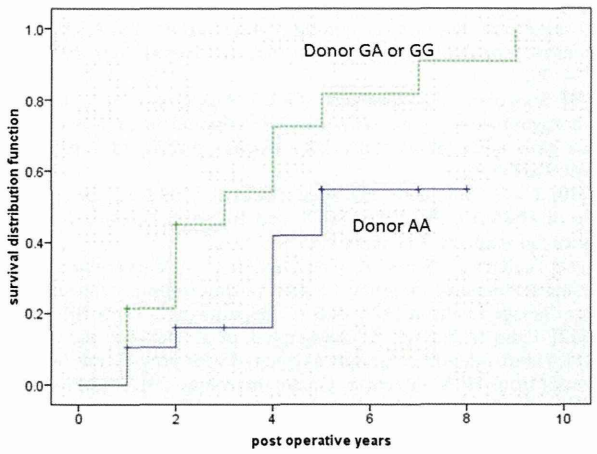

Fig 2. Time to stage 2 fibrosis or liver-related mortality. Patients with time to stage ≥ F2 or liver-related mortality fibrosis had significant differences only with respect to the donor's rs2629751 genotype (AA:GG or GA = 5.5 ± 0.6 years:3.6 ± 0.7 years; $P = .025$).

significantly greater number of donors with rs2629751 AG or GG alleles than in those with AA alleles (AA:GG ratio or GA = 57%:87.5%; $P < .05$). There was no difference in recipients with the rs2629751 genotype.

Time to Stage ≥ F2 (Portoportal Septa) or All-cause Mortality and F2 (Portoportal Septa) or Liver-related Mortality

The time to stage ≥ F2 (portoportal septa) or all-cause mortality differed significantly between patients with and without liver fibrosis progression at 1 year after liver transplantation (fibrosis progression:no fibrosis = 3.0 ± 0.4 years:6.6 ± 0.6 years; $P = .025$; Fig 1). Fibrosis progression (≥F2) or all-cause mortality was seen in 30 cases (70%). This endpoint occurred in 3 of 12 patients (25%) without fibrosis and in 27 of 31 patients (87%) with fibrosis progression. None of the SNPs showed an association with the time to stage ≥ F2 (portoportal septa) or all-cause mortality.

Fibrosis progression (≥F2) or liver-related mortality was found in 22 cases (66%). The time to stage ≥ F2 or liver-related mortality fibrosis was significantly different only in donors with the rs2629751 genotype (AA:GG ratio or GA = 5.5 ± 0.6 years:3.6 ± 0.7 years; $P = .025$; Fig 2).

Donor Factors and the rs2629751 Genotypes

Donor age was not different in donors with the rs2629751 SNPs (AA 40.8 ± 13.9: 36.4 ± 11.1). No other donor factors (gender, graft type) were significantly different.

Antiviral Therapy and the rs2629751 Genotype

Twenty-one patients received antiviral therapy and 6 (28%) achieved a sustained viral response. Sustained viral response rates were not different in recipients with the rs2629751 SNPs (AA:GG or GA = 28%:28%; $P = 1.00$).

DISCUSSION

HCV infection is the most common indication for liver transplantation in Japan. Fibrosis deposition after liver transplantation is accelerated, with 30% of patients developing graft cirrhosis at 5 years after transplantation [3]. Therefore, it is important to identify predictive factors for fibrosis progression in patients after liver transplantation for HCV infection.

Blasco et al. [14] showed that the presence of portal hypertension or significant fibrosis at 1 year after transplantation were accurate indicators of risk for clinical decompensation and graft loss owing to recurrent hepatitis C (rapid fibrosis), whereas patients with normal portal pressure and/or mild fibrosis had excellent outcomes [14]. Indeed, in our study progression of fibrosis detected on liver biopsies 1 year after liver transplantation was a significant predictive factor for fibrosis progression (stage ≥ F2) or all-cause mortality. Therefore, we believe that the identification of predictive factors for fibrosis progression 1 year after liver transplantation is of essential prognostic value.

In the present study, we examined 5 SNPs in recipients and donors that were associated with fibrosis development in patients with HCV infection. We identified the rs2629751 donor genotype to be associated significantly with fibrosis progression at 1 year after liver transplantation. Moreover, the rs2629751 donor genotype was also associated with fibrosis progression (stage ≥ F2) or HCV infection-related mortality after liver transplantation for HCV infection. The rs2629751 SNP was identified as a risk factor for fibrosis in patients with chronic hepatitis C in 2012 by a GWAS [12]. This SNP is located within the glycosyltransferase 8 domain containing 2 (*GLT8D2*) gene. The GLT8D2 protein was found to localize in the endoplasmic reticulum, interact with ApoB100, and positively regulate the levels of ApoB100 protein in vitro [15]. Huang et al. [16] showed that HCV secretion is dependent on both *ApoB* expression and very low-density lipoprotein cholesterol assembly in a chromosomally integrated complementary DNA model of HCV secretion. Taken together, our results suggest that *ApoB* expression might be associated with HCV replication and HCV-induced fibrosis after liver transplantation.

HCV clearance after liver transplantation can decrease the risk of subsequent HCV-related complications such as progression to cirrhosis and graft loss [17–19]. To date, the standard of care in the treatment of HCV recurrence after liver transplantation is represented by IFN-containing regimens; however, the response rate of 13%–42% is not high [19–22]. In the future, IFN-free antiviral therapy will be expected to result in a high sustained viral response rate [23,24]. Therefore, it is important for clinicians to decide when to begin antiviral treatment.

In our study, about 90% of patients with the rs2629751 AG or GG genotype had fibrosis progression at 1 year after

liver transplantation. If the donor had the rs2629751 AG or GG genotype, the possibility of fibrosis progression was also high. In our study, 40% of donors had the rs2629751 AG or GG genotype. These results suggest that, based on the donor's rs2629751 genotype, recipients with rs2629751 genotype AG or GG would require antiviral therapy.

In posttransplant patients, HCV-related events are diverse (eg, rejection, HCV recurrence, opportunistic infection). In this study, with the endpoint of stage ≥ F2 (portoportal septa) or all-cause mortality, the effects associated with genotype disappeared. In addition, our results suggested that the cause of death could be unrelated to HCV. Indeed, in this study, most of the causes of death were owing to infection and graft failure owing to small transplant size. Limiting this analysis was the small sample size. Further studies with larger sample sizes will be needed to validate these markers.

In conclusion, the donor rs2629751 genotype is an important predictor of posttransplant outcome in patients with HCV infection. This result might guide clinical decisions regarding antiviral treatment in HCV-infected patients.

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REFERENCES

[1] Brown RS. Hepatitis C and liver transplantation. Nature 2005;436:973–8.

[2] Marubashi S, Dono K, Miyamoto A, et al. Liver transplantation for hepatitis C. J Hepatobiliary Pancreat Surg 2006;13:382–92.

[3] Prieto M, Berenguer M, Rayon JM, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. Hepatology 1999;29:250–6.

[4] Moonka DK, Kim D, Kapke A, et al. The influence of induction therapy on graft and patient survival in patients with and without hepatitis C after liver transplantation. Am J Transplant 2010;10:590–601.

[5] Russo MW, Galanko J, Beavers K, et al. Patient and graft survival in hepatitis C recipients after adult living donor liver transplantation in the United States. Liver Transpl 2004;10:340–6.

[6] Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–9.

[7] Noureddin M, Wright EC, Alter HJ, et al. Association of IL28B genotype with fibrosis progression and clinical outcomes in patients with chronic hepatitis C: a longitudinal analysis. Hepatology 2013;58:1548–57.

[8] Valenti L, Alisi A, Galmozzi E, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology 2010;52:1274–80.

[9] Sookoian S, Castano GO, Burgueno AL, et al. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. J Lipid Res 2009;50:2111–6.

[10] Cai T, Dufour JF, Muellhaupt B, et al. Viral genotype-specific role of PNPLA3, PPARG, MTTP, and IL28B in hepatitis C virus-associated steatosis. J Hepatol 2011;55:529–35.

[11] Valenti L, Rumi M, Galmozzi E, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology 2011;53:791–9.

[12] Patin E, Kutalik Z, Guergnon J, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. Gastroenterology 2012;143:1244–52. e1241–e1212.

[13] Ichida F, Tsuji T, Omata M, et al. New Inuyama classification: new criteria for histological assessment of chronic hepatitis. Int Hepatol Commun 1996;6:112–9.

[14] Blasco A, Forns X, Carrion JA, et al. Hepatic venous pressure gradient identifies patients at risk of severe hepatitis C recurrence after liver transplantation. Hepatology 2006;43:492–9.

[15] Wei HS, Wei HL, Zhao F, et al. Glycosyltransferase GLT8D2 positively regulates ApoB100 protein expression in hepatocytes. Int J Mol Sci 2013;14:21435–46.

[16] Huang H, Sun F, Owen DM, et al. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. Proc Natl Acad Sci U S A 2007;104:5848–53.

[17] Crespo G, Marino Z, Navasa M, et al. Viral hepatitis in liver transplantation. Gastroenterology 2012;142:1373–1383.e1371.

[18] Carrion JA, Navasa M, Garcia-Retortillo M, et al. Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. Gastroenterology 2007;132:1746–56.

[19] Berenguer M, Palau A, Aguilera V, et al. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. Am J Transplant 2008;8:679–87.

[20] Gordon FD, Kwo P, Ghalib R, et al. Peginterferon-alpha-2b and ribavirin for hepatitis C recurrence postorthotopic liver transplantation. J Clin Gastroenterol 2012;46:700–8.

[21] Xiao N, Shi S, Zhuang H. A meta-analysis that compares the use of either peginterferon-alpha2a or peginterferon-alpha2b plus ribavirin for HCV infection. Hepat Med 2010;2:99–109.

[22] Ferenci P, Formann E, Laferl H, et al. Randomized, double-blind, placebo-controlled study of peginterferon alfa-2a (40KD) plus ribavirin with or without amantadine in treatment-naive patients with chronic hepatitis C genotype 1 infection. J Hepatol 2006;44:275–82.

[23] Zeuzem S, Jacobson IM, Baykal T, et al. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med 2014;370:1604–14.

[24] Kumada H, Suzuki Y, Ikeda K, et al. Daclatasvir plus asunaprevir for chronic HCV genotype 1b infection. Hepatology 2014;59:2083–91.

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Original Article

Relationship between immune function recovery and infectious complications in patients following living donor liver transplantation

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Aim: The ImmuKnow (IK) assay enables the evaluation of peripheral blood $CD4^+$ adenosine triphosphate activity to facilitate an objective assessment of the cellular immune function in immunosuppressed patients. However, it is unclear whether the IK assay is utilized during the acute postoperative periods following living donor liver transplantation (LDLT).

Methods: The IK values of 43 LDLT recipients were measured during the month following LDLT to evaluate the relationship between the measured IK values and infectious events.

Results: The IK values after LDLT were significantly increased compared with the IK values before LDLT ($P < 0.01$). During the month following transplantation, the rate of bacterial infection in the recipients with IK values of more than 225 ng/mL was significantly lower than that in the recipients with IK values of 225 ng/mL or less (42.1% vs 91.7%, respectively; $P < 0.01$). The rate of severe infections among the recipients who maintained IK values of more than 150 ng/mL was significantly lower than that among the recipients with IK values of 150 ng/mL or less during the month following transplantation (3.7% vs 56.3%, respectively; $P < 0.01$).

Conclusion: The immune system of LDLT recipients dramatically improved following transplantation. The IK values of LDLT recipients were associated with the incidence of infectious events during the perioperative period after LDLT. Monitoring IK values was useful during both the acute and long-term postoperative periods.

Key words: bacterial and fungal infection, immunosuppression, ImmuKnow, living donor liver transplantation

INTRODUCTION

IN PATIENTS WITH end-stage liver disease that is considered to be an indication for living donor liver transplantation (LDLT), immune dysfunction may be present due to long-term malnutrition or pancytopenia due to hypersplenism.[1] LDLT recipients must also take immunosuppressants to prevent acute rejection even though infection is a major cause of death during the acute postoperative period.[2,3] In most institutions, the immunosuppressant dose is determined based on therapeutic drug monitoring (TDM). However, TDM does not always reflect the effects of immunosuppression due to the lack of an objective immune function evaluation. To improve the management of LDLT recipients, a better parameter is required.

Kowalski *et al.* reported the utility of the ImmuKnow (IK) assay (Cylex Inc., Columbia, MD, USA) for postoperative management following organ transplantation.[4] The IK assay uses phytohemagglutinin (PHA) to stimulate lymphocyte activation. Most immune cell functions depend on a cellular energy supply; thus, the assay is designed to measure the increase in intracellular adenosine triphosphate following mitogen activation or antigenic or allogenic stimulation. The IK values were not related to the TDM of calcineurin-inhibitor drug.[5–7] The efficacy of IK values has been reported for the late phase following liver transplantation.[7–10] However, it still remains unclear how IK values change after LDLT. Furthermore, the efficacy of the IK assay during perioperative periods for LDLT recipients remains insufficient. In this study, we examined perioperative IK values in LDLT recipients and evaluated the relationship between IK values and clinical outcomes,

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which were primarily the occurrence of bacterial and fungal infections.

METHODS

Study design

BETWEEN MAY 2010 and May 2013, 65 adult patients underwent LDLT at Nagasaki University Hospital. Blood samples for the IK assay were obtained from 43 of these patients prior to LDLT. The IK assay was also conducted in 21 healthy volunteers, who served as the control group (Fig. 1). The IK values of the LDLT recipients were chronologically measured at postoperative weeks 1, 2, 3 and 4. The IK values were also evaluated during infectious events.

This study protocol was approved by the institutional review board of Nagasaki University Hospital, and informed consent was obtained from each recipient and healthy volunteer.

Demographics of the LDLT recipients

The 43 LDLT recipients consisted of 25 men and 18 women with a mean age of 57.6 ± 7.6 years. The indications for LDLT were as follows: type C cirrhosis in 26 patients; type B cirrhosis in five patients; non-B, non-C cirrhosis in four patients; primary biliary cirrhosis in four patients; Wilson's disease in one patient; alcoholic cirrhosis in one patient; acute liver failure in one patient; and multiple liver metastases of a rectal carcinoid in one patient. The patient demographics are summarized in Table 1. Splenectomy was performed in 33 patients with severe thrombocytopenia (platelet counts <50 000/μL) or type C liver cirrhosis prior to postoperative interferon therapy. Splenectomy had been performed in three patients prior to LDLT.

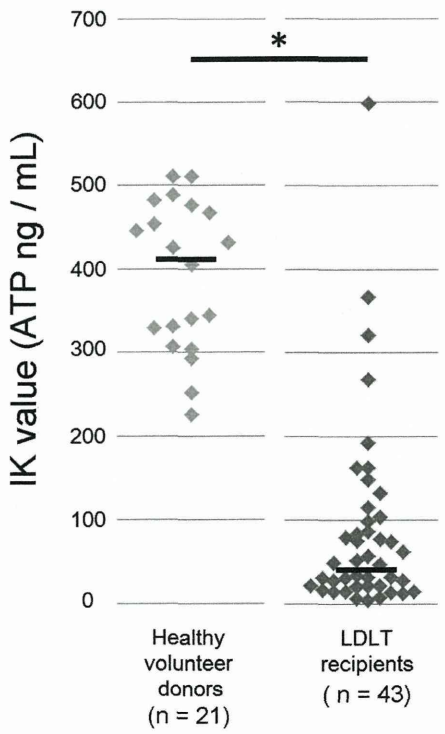

Figure 1 Comparison of the IK values between healthy volunteer donors and LDLT recipients. Small bars indicate the median IK values. IK, ImmuKnow; LDLT, living donor liver transplantation. *$P = 0.01$.

Immunosuppressive protocols

As a basic perioperative immunosuppressive protocol, bitherapy with tacrolimus and steroids was administrated to most patients. A steroid bolus was administrated intraoperatively. Tacrolimus was initiated on postoperative day 1 while tapering the steroid dose. The target trough level of the p.o. administrated tacrolimus was between 10 and 15 ng/mL within the first month following LDLT and 5–10 ng/mL thereafter. The corticosteroid was gradually decreased within the first 3–4 months. The immunosuppressant doses were adjusted after considering the clinical course and blood tacrolimus trough levels, regardless of the IK values. For patients with severe renal dysfunction, an interleukin-2 receptor inhibitor (basiliximab) was also administrated on days 1 and 4 without a calcineurin inhibitor. Adjunctive mycophenolate mofetil (MMF) was also administrated. When triple therapy consisting of

Table 1 Pretransplant characteristics of the 43 LDLT recipients

Age (years)	57.6 ± 7.6
Sex (male : female)	25:18
Primary disease	
Hepatitis B virus-associated liver cirrhosis	5
Hepatitis C virus-associated liver cirrhosis	26
Acute liver failure	1
Others	11
Child–Pugh score	10.0 ± 2.3
MELD score	17.6 ± 8.8
Incompatible blood type	13
Other viral carrier	
HIV infection	0
HTLV-1 infection	3
Splenectomy	36
ImmuKnow (ATP ng/mL)	88.6 ± 114.7

Splenectomy had been performed in three patients prior to LDLT. ATP, adenosine triphosphate; HTLV-1, human T-cell leukemia virus type 1; LDLT, living donor liver transplant; MELD, Model for End-Stage Liver Disease.

tacrolimus, MMF and steroids was administrated, the target trough level of tacrolimus was adjusted to 5–10 ng/mL.

Postoperative bacterial and fungal infections

Post-transplant bacterial and fungal infections were defined as cases that required an additional antibacterial or antifungal agent due to a positive culture after the discontinuation of antibacterial prophylaxis. Severe infections were defined as cases requiring mechanical ventilation or surgical drainage with septic shock.

IK measurement

The perioperative immune status was evaluated using the IK assay. The ImmuKnow assay protocol has been described in detail elsewhere.[2,4] Briefly, 250 μL of whole blood with 750 μL of sample diluent was dispensed in 100-μL aliquots to four wells of a 96-well microtiter plate and was incubated for 15–18 h with PHA in a 37°C, 5% CO_2 incubator. The $CD4^+$ T cells were positively selected within the microwells using magnetic particles coated with antihuman CD4 monoclonal antibodies (Dynabeads; Dynal, Oslo, Norway) and a strong magnet (Cylex Magnet Tray 1050; Cylex Inc., Columbia, MD, USA). The selected $CD4^+$ T cells were lysed to release intracellular adenosine triphosphate (ATP). Released ATP was measured using luciferin/luciferase and a luminometer (Berthold [Knoxville, TN, USA] or Turner Biosystems [Sunnyvale, CA, USA]). The concentration of ATP was determined from the relative light units of an ATP calibration curve according to the IK date analysis calculator. The concentrations were averaged after outliers were excluded.

Evaluating the recovery of post-transplant IK values related to infections

We evaluated the relationship between the chronological changes in post-transplant IK values and infections during the month following LDLT. According to a previous report by Kowalski *et al.*, the immune cell response can be stratified as strong (≥525 ng/mL), moderate (226–524 ng/mL) or low (≤ 225 ng/mL).[4] Consequently, the 225 ng/mL IK value was defined as the cut-off value in the immunosuppressive group. The patients were divided into two groups according to the IK value at 2–4 weeks after LDLT to evaluate the recovery of IK values following post-LDLT bacterial and fungal infections. The low IK group demonstrated low IK values (≤225 ng/mL) at least once within the 2–4 weeks after LDLT, and the remaining group underwent an evaluation to determine the sensitivity of the IK values to the bacterial and fungal infections.

Evaluating the recovery of post-transplant IK values relative to severe infections

We evaluated the relationship between chronological changes in the post-transplant IK values and severe infections during the month following LDLT.

The 150 ng/mL IK value was defined as the cut-off value based on receiver–operator curves. The patients were divided into two groups. The first group contained patients with an average IK value of 150 ng/mL in the 2–4 weeks following LDLT. This group was used to evaluate the recovery of IK values following LDLT. The second group contained patients who experienced severe post-transplant infections. Moreover, the patients were also divided into a very low IK group, who demonstrated IK values of less than 150 ng/mL at least once within the 2–4 weeks following LDLT, and the remaining group, who were evaluated to determine the sensitivity of the IK values to severe infections.

Statistical analyses

The ages, Child–Pugh scores, the Model for End-Stage Liver Disease (MELD) scores, operation time, intraoperative bleeding and IK values of the patients are reported as means and standard deviations. The Wilcoxon rank sum test or Student's *t*-test were used for non-categorical variables. Spearman's rank correlation coefficient was used to analyze ordinal qualitative variable. For the categorical analyses, Fisher's exact test or χ^2-test were used. One-way ANOVA with post-hoc Tukey honestly significant difference test was used for comparing multiple groups. $P < 0.05$ was considered to be significant. The SAS-JMP software program (Cary, North Carolina, USA) was used to perform all statistical analyses.

RESULTS

Comparison of the preoperative IK values

THE MEDIAN PREOPERATIVE IK value of the 43 LDLT recipients was 48 ng/mL (range, 4–598), which was significantly lower than that of the 21 healthy volunteers (415 ng/mL; range, 311–438) ($P = 0.01$) (Fig. 1). The IK values of the male LDLT recipients prior to LDLT (79 ng/mL; range, 27–115) were significantly higher than those of the females (31.5 ng/mL; range, 15–54.5) ($P = 0.01$). There were no significant relationships between IK values and age, DM, Child–Pugh score or MELD score.

Post-transplant trends in the IK values

The IK value was 86.9 ± 113.9 ng/mL before LT and 204.7 ± 169.1 ng/mL at 1 week, 303.9.7 ± 192.2 ng/mL at 2 weeks, 302.8 ± 180.5 ng/mL at 3 weeks and 309.8

± 217.6 ng/mL at 4 weeks following LDLT. The IK values following LDLT significantly increased with time (Fig. 2a). At 4 weeks following LDLT, the IK values of the patients who had undergone splenectomy were significantly higher than those of the patients who did not undergo splenectomy ($P < 0.01$) (Fig. 2b). There were no significant relationships between IK values after LDLT and other factors such as age, sex, operation time, blood loss, DM, Child-Pugh score, MELD score or pre-IK values.

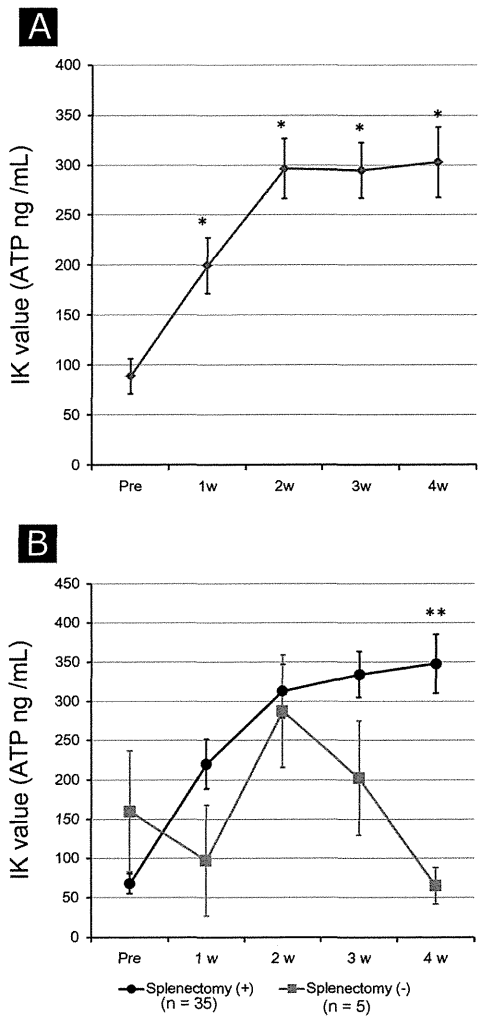

Figure 2 Chronological trends in the IK values during the perioperative period in LDLT recipients. (a) Recovery of IK values following LDLT. (b) Longitudinal changes in IK values in patients who did or did not undergo splenectomy. IK values are mean ± standard error. IK, ImmuKnow; LDLT, living donor liver transplantation; Ope, operation; Pre, preoperative state; w, week. *$P < 0.01$ vs Pre, **$P < 0.01$ vs splenectomy (-).

IK values and postoperative bacterial and fungal infections

Bacterial and fungal infections within 1 month following LDLT were observed at a high rate (42 episodes in 30 patients, 69.8%). Positive bacterial cultures were detected in samples from blood ($n = 18$), sputum ($n = 12$) and the abdominal cavity ($n = 12$). Severe infections within 1 month following LDLT were observed in 10 patients (23.3%). There were no significant relationships between IK values after LDLT and other factors such as age, sex, operation time, blood loss, DM, Child-Pugh score, MELD score or pre-IK values.

The recovery of postoperative IK values in the patients who developed bacterial and fungal infections was significantly slower than that in the remaining patients at 2 and 3 weeks ($P = 0.007$, $P = 0.014$) (Fig. 3a). Based on the average postoperative IK values, the occurrence of bacterial and fungal infections significantly increased the IK values in patients with an IK value less than 225 ng/mL ($P = 0.001$) (Fig. 3b). The rate of bacterial and fungal infections in the low IK group (91.67%) was significantly higher than that in the remaining group (45%) ($P < 0.001$) (Fig. 3c).

The postoperative IK values in patients who developed severe infections were significantly lower than those in the remaining patients at 1–4 weeks ($P = 0.006$, $P = 0.001$, $P = 0.003$, $P = 0.001$) (Fig. 4a). Based on the average postoperative IK values, the occurrence of severe infections was significantly increased in the group of patients with IK values less than 150 ng/mL ($P < 0.001$) (Fig. 4b). The rate of bacterial and fungal infections in the very low IK group (56.3%) was significantly higher than that in the remaining group (3.6%) ($P < 0.001$) (Fig. 4c). Representative cases from the very low IK group and the remaining group are presented in Figure 5.

The IK values significantly differed between the three groups (patients without infections [$n = 13$], patients with infections [$n = 20$] and patients with severe infections [$n = 10$] at 2–4 weeks after LDLT) (Fig. 6).

Other than bacterial infections, cytomegalovirus infection (CMV) was recognized in nine patients. Furthermore, biopsy-proven acute cellular rejection (ACR) was detected in three patients. However, the IK values were not significantly different between patients with CMV infections or ACR and patients without these events.

DISCUSSION

THIS STUDY DEMONSTRATED that the IK values dramatically improved in almost all LDLT recipients, and the improvements in the IK values differed due to bacterial and fungal infections during the acute perioperative

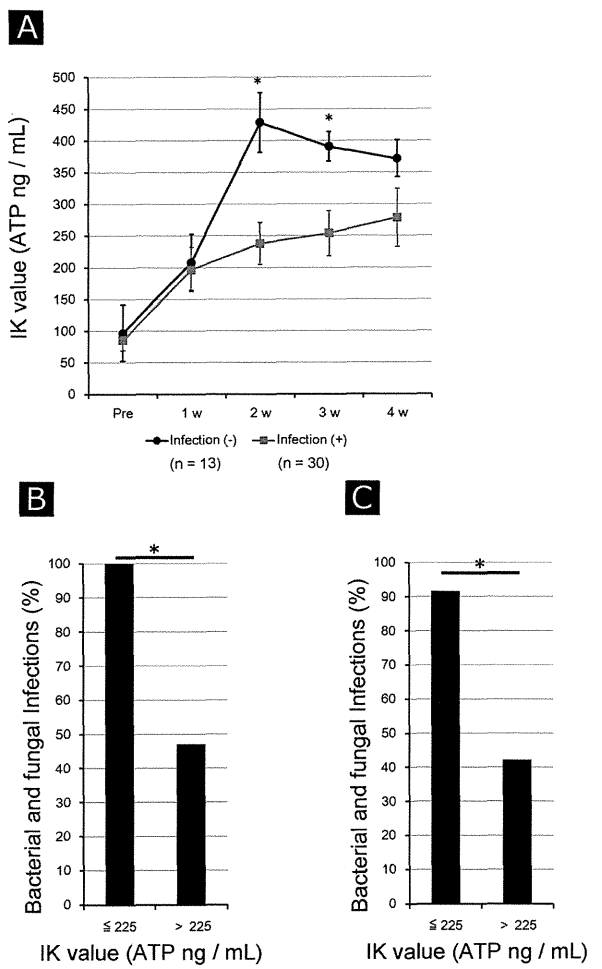

Figure 3 Development of post-transplant bacterial and fungal infections according to IK values following LDLT. (a) The IK values following LDLT. IK value recovery was delayed in patients with bacterial and fungal infections. Bars indicate the standard error. (b) The rate of bacterial and fungal infections among the patients with average IK values less than 225 ng/mL or not. (c) The rate of bacterial and fungal infections in patients with low IK values (≤225 ng/mL). IK values are mean ± standard error. IK, ImmuKnow; LDLT, living donor liver transplantation; Pre, preoperative state; w, week. $^{*}P < 0.01$.

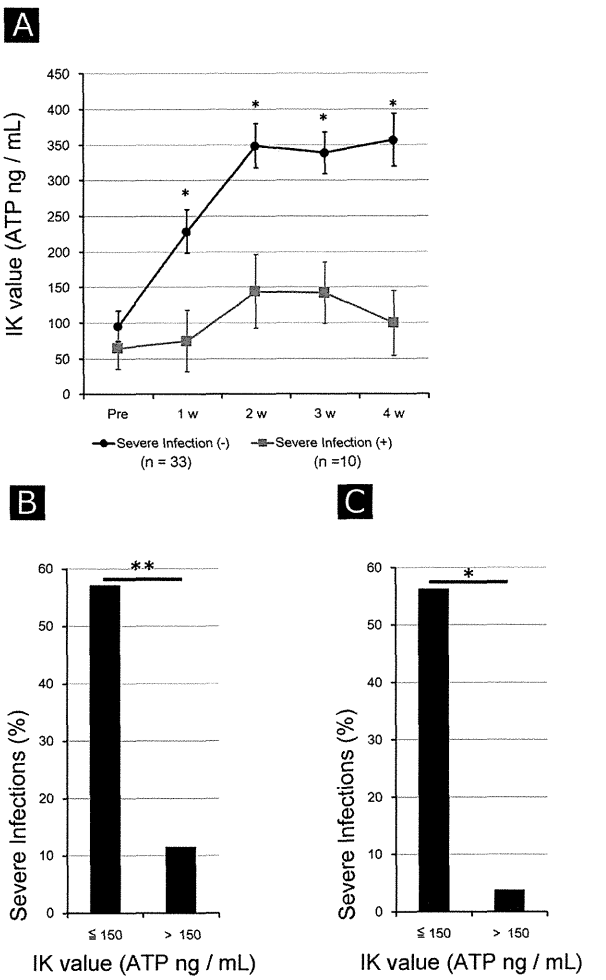

Figure 4 Development of severe infections according to the IK values after LDLT. (a) IK values following LDLT. Recovery of IK values was markedly delayed in patients with severe infections. Bars indicate the standard error.(b) The rate of severe infections among patients with average IK values less than 150 ng/mL or not. (c) The rate of severe infections among patients with and without low IK values (≤150 ng/mL), IK values are mean ± standard error. IK, ImmuKnow; LDLT, living donor liver transplantation; Pre, preoperative state; w, week. $^{**}P < 0.05$, $^{*}P < 0.01$.

periods. Specifically, the patients who developed severe infections demonstrated almost no improvement in their IK values. These results suggest that the IK assay could be useful during not only the postoperative period but also during the acute perioperative periods.

Because patients with liver cirrhosis are often immunocompromised, various types of bacterial infections, such as spontaneous bacterial peritonitis, urinary infection and pneumonia, can occur. Severe infectious diseases cause the high death rate during end-stage liver disease.[1] This study used the IK assay to objectively demonstrate the preoperative immunocompromised status in LDLT recipients with end-stage liver disease. Although the preoperative IK values in LDLT recipients were significantly lower than those in healthy volunteers, most recipients demonstrated recovery within a month after transplantation. Measuring the concentration of ATP in activated T cells is clinically useful for evaluating T-cell function.[11,12] Therefore, patients with end-stage liver disease who demonstrate

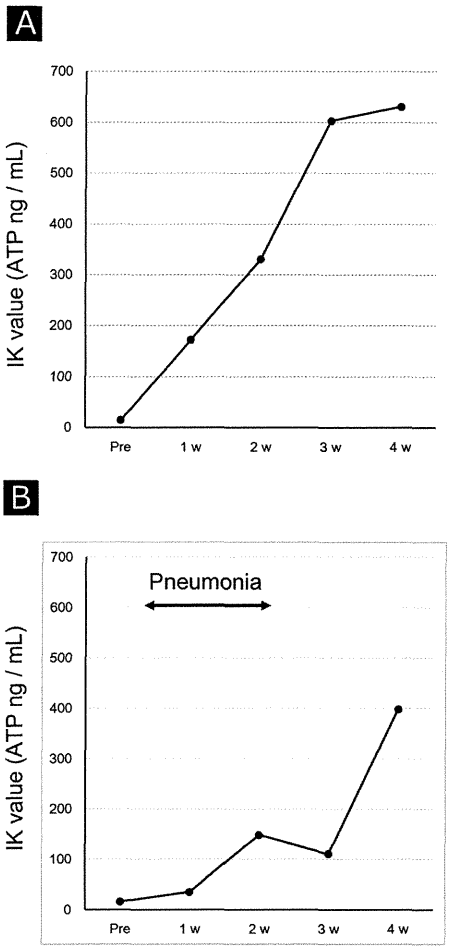

Figure 5 IK value changes following LDLT among the patients who did and did not develop severe infections. (a) A 59-year-old man underwent LDLT for liver cirrhosis. In this patient, no infectious events occurred during the first month following LDLT. (b) A 62-year-old woman underwent LDLT for liver cirrhosis. In this patient, postoperative pneumonia occurred during the 2 weeks following LDLT. IK values quickly increased after the pneumonia was cured. IK, ImmuKnow; LDLT, living donor liver transplantation; Pre, preoperative state; w, week.

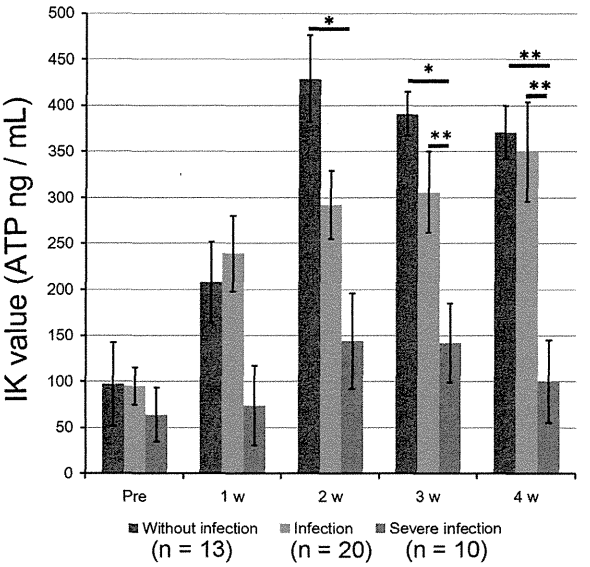

Figure 6 IK value changes of post-transplant infections with or without severe infections according to IK values following LDLT. IK values are the means ± standard error. IK, ImmuKnow; LDLT, living donor liver transplantation; Pre, preoperative state; w, week. $^{**}P < 0.05$, $^{*}P < 0.01$.

significantly low ATP levels are in a state of $CD4^+$ T-cell dysfunction. Although patients with end-stage liver disease often experience leukocytopenia, there was no relationship between the IK values and lymphocyte counts (data not shown). In patients with liver cirrhosis accompanied by hypersplenism, the balance of immune function often changes; the absolute number of T cells and the CD4/CD8 ratio decrease.[13,14] As helper T cells, $CD4^+$ T cells adjust the immune response. The risk of sepsis increases following abdominal surgery in HIV patients with a low number of $CD4^+$ T cells.[14,15] In this study, the IK values of the patients who developed severe infections were extremely low, even though the IK values of the other patients improved to the same level as that in healthy volunteers. At 4 weeks post-LDLT, the IK values in the patients who underwent splenectomy were significantly higher than those in the patients who did not undergo splenectomy, although LDLT without splenectomy was performed in only five patients. Splenectomy may improve portal vein hemodynamics and the function of $CD4^+$ T cells.[16,17]

In this study, only one patient experienced a postoperative decrease in IK values. This patient had multiple metachronous metastases from a rectal neuroendocrine tumor and had normal liver function. All of the other patients with primary liver disease demonstrated a post-transplant increase in the IK values. This result demonstrated the efficacy of liver transplantation in liver transplant recipients with end-stage liver disease with respect to the recovery of their immune function. Goralzyk *et al.* also reported patients who had quickly recovered from preoperative pneumonia following liver transplantation.[18]

The postoperative increase in IK values in the present study explains the above-mentioned findings. Li *et al.* also reported an immunofunctional assessment based on IK values and $CD4^+$ cell counts, and the $CD4^+/CD8^+$ ratio