Table 1: Continued

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association without unknown)	Odds ratio	95% CI	p-Value	p-Value (global association without unknown)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Splenectomy	No	135	1.000	–	–	–	1.000	–	–	–
	Yes	241	0.841	0.599–1.181	0.317		1.094	0.564–2.122	0.0790	
	Unknown	5	0.874	0.213–3.587	0.852		0.000	N/A	N/A	
Rituximab prophylaxis	No	119	1.000	–	–	–	1.000	–	–	–
	Yes	259	0.501	0.358–0.702	<0.001*		0.214	0.111–0.414	<0.001*	
	Unknown	3	1.554	0.380–6.358	0.540		0.000	N/A	N/A	
Prophylactic IVIG after transplantation	No	325	1.000	–	–	–	1.000	–	–	–
	Yes	56	0.859	0.523–1.409	0.547		0.392	0.117–1.313	0.129	
Anti-lymphocyte antibodies	No	345	1.000	–	–	–	1.000	–	–	–
	Yes	36	1.232	0.732–2.073	0.432		0.953	0.320–2.836	0.931	
Plasmapheresis	No	47	1.000	–	–	–	1.000	–	–	–
	Yes	320	0.723	0.454–1.152	0.172		1.132	0.422–3.038	0.806	
	Unknown	14	0.913	0.368–2.263	0.844		0.646	0.069–6.041	0.702	
Plasmapheresis (times)	0	47	1.000	–	–	0.240	1.000	–	–	0.247
	1	68	0.639	0.353–1.155	0.138		0.813	0.233–2.837	0.745	
	2	89	0.865	0.505–1.483	0.277		1.185	0.386–3.637	0.767	
	3	93	0.622	0.355–1.091	0.098		0.684	0.205–2.283	0.537	
	4	28	1.159	0.597–2.249	0.664		2.801	0.793–9.888	0.110	
	≥5	28	0.659	0.302–1.439	0.295		1.008	0.222–4.584	0.992	
	Unknown	28	0.616	0.282–1.346	0.224		1.826	0.478–6.973	0.378	
Short-term outcomes										
IgM (peak posttransplantation)	Low (<64)	251	1.000	–	–	–	1.000	–	–	–
	High (≥64)	94	1.689	1.180–2.418	0.004*		7.935	3.973–15.85	<0.001*	
	Unknown	36	1.046	0.571–1.916	0.884		0.000	N/A	N/A	
IgG (peak posttransplantation)	Low (<64)	205	1.000	–	–	–	1.000	–	–	–
	High (≥64)	126	1.484	1.043–2.110	0.028*		10.453	4.467–24.46	<0.001*	
	Unknown	50	1.142	0.671–1.945	0.624		1.805	0.450–7.244	0.405	
Acute rejection	No	296	1.000	–	–	–	1.000	–	–	–
	Yes	78	0.964	0.640–1.453	0.862		1.133	0.533–2.408	0.745	
	Unknown	7	2.023	0.746–5.487	0.166		0.000	N/A	N/A	
Chronic rejection	No	349	1.000	–	–	–	1.000	–	–	–
	Yes	5	1.905	0.703–5.158	0.205		1.827	0.199–16.74	0.594	
	Unknown	27	1.750	1.006–3.044	0.048		0.281	0.037–2.126	0.219	
Bacterial infection	No	254	1.000	–	–	–	1.000	–	–	–
	Yes	124	4.160	2.965–5.835	<0.001*		1.843	0.975–3.485	0.060	
	Unknown	3	3.650	0.890–14.97	0.072		0.000	N/A	N/A	
Fungal infection	No	342	1.000	–	–	–	1.000	–	–	–
	Yes	34	5.718	3.772–8.667	<0.001*		3.776	1.666–8.558	0.002*	
	Unknown	5	1.394	0.344–5.648	0.641		0.000	N/A	N/A	
CMV disease	No	199	1.000	–	–	–	1.000	–	–	–
	Yes	180	0.784	0.562–1.095	0.153		0.911	0.485–1.713	0.773	
	Unknown	2	1.233	0.171–8.870	0.835		0.000	N/A	N/A	
Antibody-mediated rejection	No	337	1.000	–	–	–	–	–	–	–
	Yes	44	2.493	1.654–3.759	<0.001*		–	–	–	

CMV, cytomegalovirus; IVIG, intravenous immunoglobulin; MELD, Model for End-Stage Liver Disease.
*$p < 0.05$.

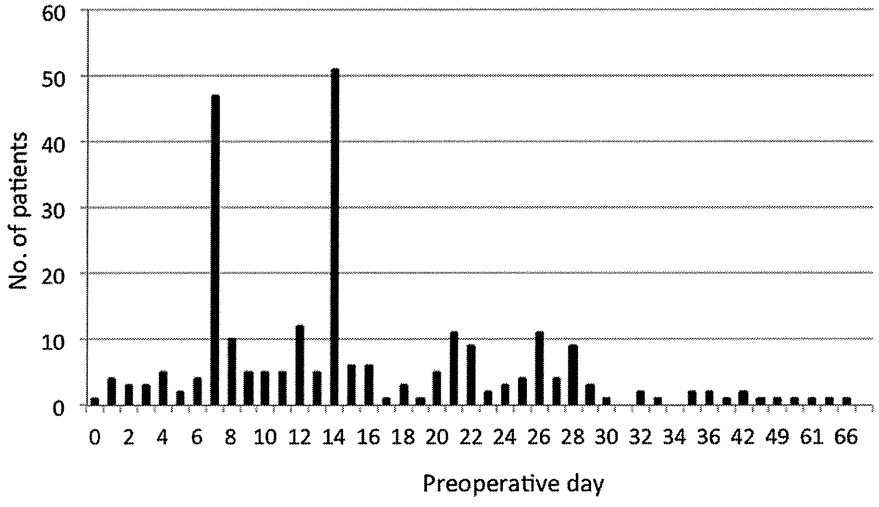

Figure 2: The timing of initial administration of rituximab ranged from preoperative days 0 to 66 and was within 6 days before transplantation in 22 cases.

Impact of rituximab on clinical outcomes

The AMR incidence was significantly lower in the rituximab group (6%) than in the nonrituximab group (23%) ($p < 0.001$; Figure 4, top); a significant difference was also observed for the subset of patients with hepatic necrosis-type AMR ($p < 0.001$; Figure 4, top). There were no significant differences between the incidences of ACR (Figure 4, top), bacterial infection or CMV disease (Figure 4, bottom) between the rituximab and nonrituximab groups. The rate of fungal infection was significantly lower in the rituximab group (4%) than in the nonrituximab group (19%) ($p < 0.001$; Figure 4, bottom).

Adverse effects of rituximab (kidney dysfunction, sepsis, neutropenia or lung edema) were observed in four patients, whose ages ranged from 56 to 62 years. Neutropenia occurred after a single dose of 300 mg/body, and the other complications manifested after the second or third dose of 500 mg/body. The patient with renal dysfunction died from a massive thrombus of the superior mesenteric artery on postoperative day 63, and the patient with sepsis died on postoperative day 202 from sepsis with an unknown focus. The other two patients are doing well.

Subgroup analysis of rituximab group

Because most ABO-I LDLT patients are currently administered rituximab, we analyzed the effects of additional desensitization therapies and the manner of rituximab administration to elucidate a better regimen. In a subgroup analysis of the rituximab group, local infusion, splenectomy, anti-lymphocyte antibodies and IVIG had no significant impact on overall survival or AMR incidence (Table 4).

Patients who were administered multiple doses of rituximab, or a regular dose of 500 mg/body or 375 mg/m^2, tended toward a lower incidence of AMR, but this was not

Table 2: Prognostic factors for overall survival: multivariate analysis (n = 381)

Characteristics	Category	N	5-Year survival (%)	Hazard ratio	95% CI	p-Value
Era	Up to 2000	20	40.0	1.000	–	–
	2001–2004	79	50.6	0.766	0.378–1.551	0.459
	2005 onwards	282	67.5	0.742	0.346–1.591	0.443
Preoperative status	At home	143	65.8	1.000	–	–
	In-hospital	178	63.6	1.087	0.735–1.606	0.676
	In-ICU	40	44.3	1.355	0.765–2.398	0.297
	Unknown	20	60.0	0.883	0.395–1.974	0.762
MELD	Low (<23)	240	66.9	1.000	–	–
	High (≥23)	88	57.2	1.364	0.894–2.080	0.149
	Unknown	53	48.8	1.420	0.827–2.437	0.203
Rituximab prophylaxis	No	119	48.4	1.000	–	–
	Yes	259	69.6	0.629	0.377–1.051	0.077
	Unknown	3	33.3	1.875	0.445–7.900	0.391

MELD, Model for End-Stage Liver Disease.

Table 3: Prognostic factors for antibody-mediated rejection: multivariate analysis (n = 381)

Characteristics	Category	N	AMR (%)	Odds ratio	95% CI	p-Value
Era	Up to 2000	20	30.0	1.000	–	–
	2001–2004	79	21.5	0.656	0.170–2.534	0.541
	2005 onwards	282	7.5	0.625	0.143–2.742	0.534
Autoimmune disease	No	304	9.5	1.000	–	–
	Yes	74	20.3	2.023	0.940–4.356	0.072
	Unknown	3	0.0	0.000	N/A	N/A
Preoperative status	At home	143	8.4	1.000	–	–
	In-hospital	178	11.8	0.929	0.404–2.134	0.862
	In-ICU	40	25.0	1.430	0.473–4.320	0.526
	Unknown	20	5.0	0.322	0.030–3.443	0.349
IgG (preoperative)	Low (<64)	155	7.7	1.000	–	–
	High (≥64)	182	16.5	1.805	0.724–4.505	0.205
	Unknown	44	4.6	0.744	0.100–5.555	0.773
IgG (at operation)	Low (<16)	191	7.9	1.000	–	–
	High (≥16)	124	18.6	1.933	0.790–4.731	0.149
	Unknown	66	9.1	1.066	0.269–4.234	0.927
MELD	Low (<23)	240	7.5	1.000	–	–
	High (≥23)	88	20.5	2.026	0.878–4.675	0.098
	Unknown	53	15.1	0.936	0.278–3.154	0.915
Rituximab prophylaxis	No	119	23.5	1.000	–	–
	Yes	259	6.2	0.248	0.089–0.690	0.008*
	Unknown	3	0.0	0.000	N/A	N/A

AMR, antibody-mediated rejection; MELD, Model for End-Stage Liver Disease.
*$p < 0.05$.

statistically significant (Table 4). In contrast, patients given multiple doses had significantly greater incidences of fungal infection and CMV disease than those given a single dose, and patients given the regular dose had a greater incidence of CMV disease than those given a small dose of 300 mg/body or less (Table 5). Patients subjected to local infusion together with rituximab prophylaxis (RI and RIS) had greater incidences of CMV disease than patients without local infusion or splenectomy (R) (Table 5). Finally, there were no significant differences among rituximab regimens in terms of AMR incidence or patient survival (Table 4; Figure 5).

Early administration of rituximab had no significant impact on AMR incidence or patient survival (Table 4). Twenty-two FHF patients underwent LDLT, and six of them were given

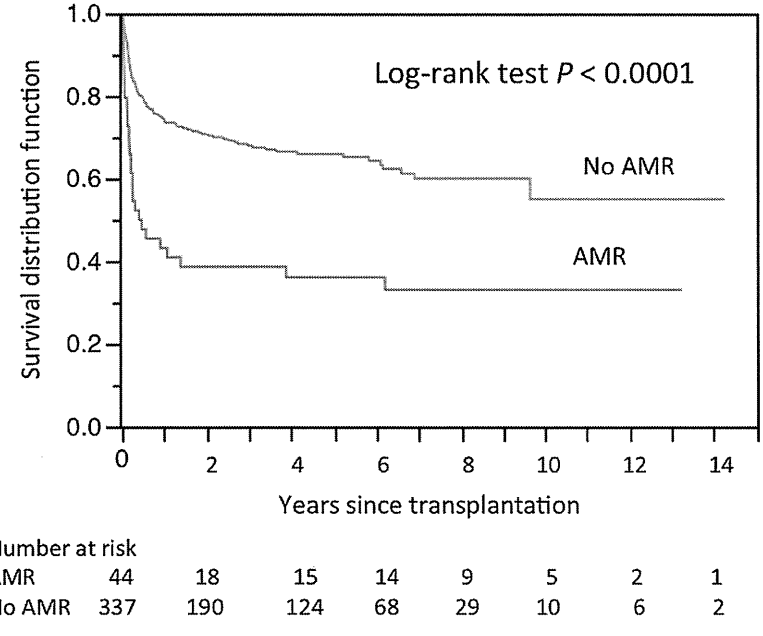

Figure 3: Comparison of overall survival between patients with and without antibody-mediated rejection. Patients with antibody-mediated rejection (AMR) had a significantly higher overall survival risk than those without AMR, $p < 0.001$.

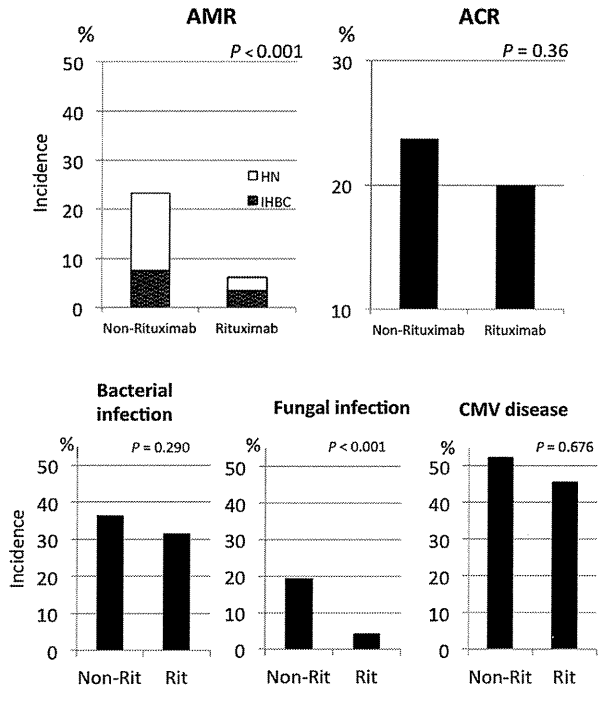

Figure 4: Comparison of incidences of complications between rituximab and nonrituximab groups. The incidences of antibody-mediated rejection (AMR) and acute cellular rejection (ACR) are shown (top); rates of intrahepatic biliary complication (IHBC) and hepatic necrosis (HN) type AMR were lower in the rituximab group than in the nonrituximab group (chi-squared test, $p < 0.0001$). The incidences of bacterial infection, fungal infection and cytomegalovirus (CMV) disease are shown (bottom); rates of bacterial infection and CMV disease were similar between the two groups (chi-squared test, $p = 0.36$), but the rate of fungal infection was significantly lower in the rituximab group (chi-squared test, $p < 0.0001$).

rituximab immediately before or during transplantation (three treated with RIS, two with RI and one with RS). All 6 patients survived transplantation without AMR, whereas AMR occurred in 7 patients and 1-year survival was 44% in the other 16 patients who were not given rituximab.

Peak IgG DSA titer before transplantation, IgG DSA titer at transplantation and peak IgG and IgM DSA titers post-transplantation showed a significant positive association with AMR incidence in the total cohort of adult ABO-I LDLT patients in the univariate analysis (Table 1). In the rituximab group, peak IgG and IgM DSA titers posttransplantation were significantly greater in patients with AMR than in those without AMR (Table 6). When the AMR incidence in the rituximab group was compared between high and low titers according to optimum cut-off values calculated from ROC curves, there were significant differences in peak IgG titers before transplantation (10% [10/104] vs. 3% [4/125] titer $\geq$128 vs. <128, $p = 0.042$), peak IgM titers post-transplantation (22% [10/45] vs. 3% [6/194], titer $\geq$64 vs. <64, $p < 0.001$) and peak IgG titers posttransplantation (19% [10/54] vs. 2% [3/171], titer $\geq$128 vs. <128, $p < 0.001$).

Discussion

Worldwide, the first case report of rituximab prophylaxis in kidney transplantation was published in Japan in 2002 (9); many rituximab protocols for kidney transplantation have been reported since. Monteiro et al (10) reported the first case of ABO-I liver transplantation using rituximab in 2003, and Usuda et al (3) reported the first case of rituximab prophylaxis in ABO-I LDLT in 2005. In the Japanese registry, the first adult case of rituximab prophylaxis was reported in November 2003. In our previous multicenter study (1) of 291 patients who underwent ABO-I LDLT up to and including March 2006, 44 adult patients were administered rituximab. The current study includes 259 adult patients who underwent rituximab prophylaxis up to and including December 2011.

After 2000, the evolution of innovation in the treatment of small-for-size syndrome in adult LDLT and desensitization for DSA was achieved (11–13). The era effect on overall survival is significant. In the total cohort of 381 adult patients, after adjustment for era effects in the multivariate analysis, only rituximab prophylaxis was a significant prognostic factor for AMR, but it was not a prognostic factor for overall survival. A prospective study is required to elucidate the effect of rituximab on patient survival; however, it would be difficult to remove rituximab prophylaxis when the current results are so much improved in the most recent era and when this may be attributable to rituximab.

To find the best regimen for rituximab, the impact of additional desensitization therapies and times and doses of rituximab were addressed. Splenectomy used to be considered an essential component of a successful ABO-I desensitization regimen for renal transplantation (14); however, it has been reported that rituximab can be used in place of splenectomy with similar outcomes (15,16). The Kyoto group suggested that splenectomy should be avoided in 2007 (2,17). In LDLT, however, splenectomy is performed not only for desensitization but also for portal flow adjustment in patients with small-for-size syndrome and for future anti-viral treatment using interferon in hepatitis C patients. An assessment of the effects of preserving the spleen is required in patients without small-for-size syndrome or hepatitis C infection in future.

Plasma exchange is a standard procedure to reduce DSA titers, but the titer required to prevent AMR is not defined. If titers increase again after plasmapheresis, another plasmapheresis is often performed. When peak titer before transplantation is very low, plasmapheresis is not performed. In other words, the more times the plasmapheresis

Table 4: Prognostic factors for antibody-mediated rejection and overall postsurgical survival: univariate analysis of 259 patients given rituximab prophylaxis

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Local infusion	No	40	1.000	–	–	–	1.000	–	–	–
	Yes	218	1.329	0.635–2.779	0.451	–	2.882	0.370–22.450	0.312	–
	Unknown	1	–	–	–	–	–	–	–	–
Splenectomy	No	90	1.000	–	–	–	1.000	–	–	–
	Yes	169	0.985	0.614–1.579	0.948	–	0.881	0.309–2.506	0.812	–
Anti-lymphocyte antibodies	No	244	1.000	–	–	–	1.000	–	–	–
	Yes	15	0.838	0.306–2.298	0.731	–	0.447	0.023–8.547	0.593	–
Prophylactic IVIG after transplantation	No	214	1.000	–	–	–	1.000	–	–	–
	Yes	45	0.984	0.529–1.830	0.960	–	0.664	0.146–3.031	0.598	–
Timing of rituximab administration before transplantation	≤6 days	22	1.000	–	–	–	1.000	–	–	–
	>7 days	236	1.241	0.535–2.883	0.615	–	1.425	0.179–11.330	0.738	–
	Unknown	1	–	–	–	–	–	–	–	–
Number of doses of rituximab	1	225	1.000	–	–	0.443	1.000	–	–	0.922
	2	22	1.504	0.747–3.031	0.253	–	0.947	0.161–5.560	0.730	–
	3	12	1.377	0.550–3.448	0.494	–	0.543	0.027–10.77	0.689	–
Dose of rituximab	Regular	162	1.000	–	–	–	1.000	–	–	–
	Small	66	1.282	0.745–2.207	0.370	–	2.655	0.952–7.404	0.062	–
	Unknown	31	–	–	–	–	–	–	–	–
Dose and number of doses of rituximab	Regular × 1	134	1.000	–	–	0.461	1.000	–	–	0.409
	Regular × 2	16	1.408	0.589–3.366	0.442	–	0.451	0.023–8.902	0.601	–
	Regular × 3	12	1.506	0.580–3.910	0.400	–	0.595	0.029–12.240	0.737	–
	Small × 1	60	1.264	0.694–2.310	0.444	–	2.086	0.738–5.897	0.165	–
	Small × 2	6	2.755	0.844–8.993	0.093	–	4.058	0.512–32.19	0.185	–
	Unknown	31	–	–	–	–	–	–	–	–
Regimen	RS	30	1.000	–	–	0.700	1.000	–	–	0.938
	R	10	2.053	0.490–8.597	0.325	–	0.937	0.031–28.37	0.970	–
	RI	81	1.568	0.596–4.128	0.362	–	1.693	0.266–10.790	0.577	–
	RIS	137	1.691	0.667–4.285	0.268	–	1.454	0.242–8.743	0.683	–
	Unknown	1	–	–	–	–	–	–	–	–

IVIG, intravenous immunoglobulin; R, only rituximab; regular dose, 500 mg/body or 375 mg/m^2; RI, rituximab and infusion; RIS, rituximab and infusion and splenectomy; RS, rituximab and splenectomy; small dose, 300 mg/body or less.

Table 5: Prognostic factors for infectious complications: univariate analysis of 259 patients given rituximab prophylaxis

			Bacterial infection				Fungal infection				CMV disease			
Characteristics	Category	N	Odds ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)
			Logistic regression analysis				Logistic regression analysis				Logistic regression analysis			
Local infusion	No	40	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	218	1.449	0.671–3.128	0.345		0.830	0.173–3.993	0.816		2.945	1.373–6.319	0.006*	
	Unknown	1	–	–	–		–	–	–		–	–	–	
Splenectomy	No	90	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	169	0.588	0.342–1.011	0.055		0.913	0.260–3.208	0.887		1.071	0.641–1.791	0.793	
Anti-lymphocyte antibodies	No	244	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	15	2.010	0.703–5.747	0.193		1.650	0.197–13.82	0.644		1.049	0.369–2.982	0.929	
Prophylactic IVIG after transplantation	No	214	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	45	1.792	0.925–3.471	0.084		1.922	0.489–7.559	0.350		1.626	0.851–3.106	0.141	
Timing of rituximab administration before transplantation	≤ 6 days	22	1.000	0.383–2.501	0.964	–	1.000	–	–	–	1.000	–	–	–
	>7 days	236	0.979	–	–		0.402	0.081–1.988	0.264		1.012	0.421–2.435	0.978	
	Unknown	1	–	–	–		–	–	–		–	–	–	
Number of doses of rituximab	1	225	1.000	–	–	0.513	1.000	–	–	0.010*	1.000	–	–	0.004*
	2	22	0.638	0.227–1.798	0.396		1.543	0.181–13.17	0.692		3.038	1.256–7.980	0.019*	
	3	12	1.549	0.475–5.050	0.468		10.288	2.278–46.47	0.002*		36.742	4.737–999.9	0.017*	
Dose of rituximab	Regular	162	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Small	66	1.742	0.948–3.203	0.074		0.122	0.000–0.984	0.152		0.455	0.249–0.832	0.011*	
	Unknown	31	–	–	–		–	–	–		–	–	–	
Dose and number of doses of rituximab	Regular × 1	134	1.000	–	–	0.283	1.000	–	–	0.040*	1.000	–	–	0.001*
	Regular × 2	16	0.679	0.182–2.526	0.563		2.243	0.220–12.32	0.412		14.802	3.517–137.3	0.003*	
	Regular × 3	12	2.101	0.625–7.058	0.230		8.542	1.756–37.86	0.006*		35.805	4.548–999.9	0.018*	
	Small × 1	60	1.828	0.955–3.501	0.069		0.192	0.001–1.734	0.270		0.780	0.412–1.451	0.440	
	Small × 2	6	1.471	0.258–8.390	0.664		2.108	0.015–23.08	0.657		0.110	0.000–0.964	0.167	
	Unknown	31	–	–	–		–	–	–		–	–	–	
Regimen	RS	30	1.000	–	–	0.266	1.000	–	–	0.685	1.000	–	–	0.034*
	R	10	2.611	0.574–11.71	0.221		3.105	0.232–41.87	0.366		2.609	0.574–11.71	0.221	
	RI	81	2.351	0.929–6.670	0.089		0.900	0.141–9.567	0.917		3.176	1.264–8.982	0.021*	
	RIS	137	1.566	0.642–4.318	0.357		0.980	0.195–9.654	0.983		4.053	1.688–11.07	0.004*	
	Unknown	1	–	–	–		–	–	–		–	–	–	

IVIG, intravenous immunoglobulin; R, only rituximab; regular dose, 500 mg/body or 375 mg/m^2; RI, rituximab and infusion; RIS, rituximab and infusion and splenectomy; RS, rituximab and splenectomy; small dose, 300 mg/body or less.
*$p < 0.05$.

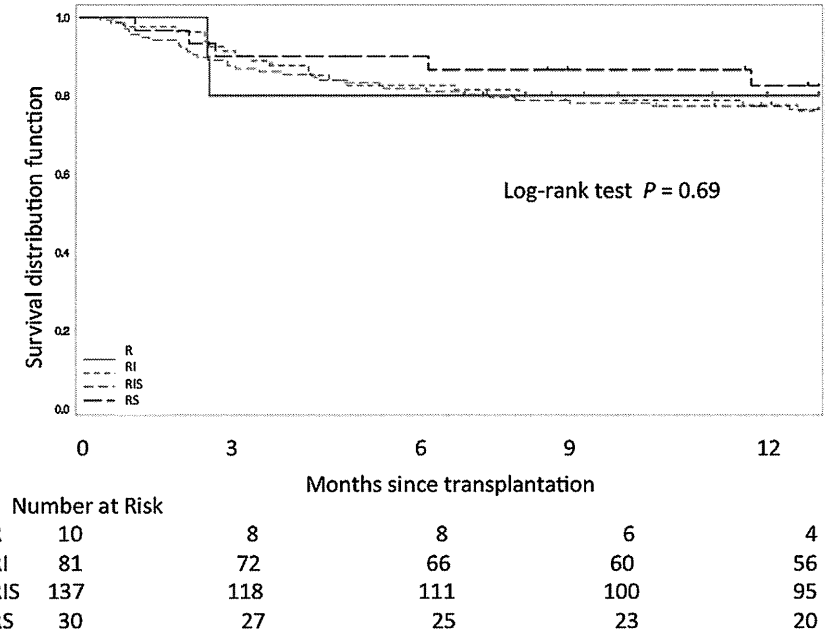

Figure 5: One-year survival of patients in the rituximab group. R, rituximab without splenectomy or local infusion (n = 10); RI, rituximab with infusion but without splenectomy (n = 81); RIS, rituximab with both infusion and splenectomy (n = 137); RS, rituximab with splenectomy but without infusion (n = 30). There were no significant differences among regimens with additional desensitization in patients with rituximab prophylaxis.

is performed, the greater the potential for an increase in DSA titer. However, we observed no significant relationship between the number of plasmapheresis procedures and clinical outcomes (Table 1).

IVIG is also a standard procedure, especially for human leukocyte antigen-related DSA in kidney transplantation, and the IVIG dose often ranged from 0.1 to 2 g/kg (18,19). In liver transplantation, Ikegami et al (4) reported a small series with desensitization by rituximab and IVIG (0.8 g/kg), and their cases were included here. We found no significant effect of IVIG on overall survival or AMR in the entire adult cohort (Table 1) and no additional effects in the rituximab group (Table 5). We analyzed the AMR incidence in each regimen with IVIG versus without IVIG (Figure 6). The AMR incidence was reduced from 26% to 9% in the local infusion and splenectomy (IS; no rituximab) regimen when IVIG was added, but this difference was not significant (p = 0.19). Among regimens with rituximab (R, RI, RIS and RS), the incidences were similar between with IVIG and without IVIG. IVIG is not approved in Japan and is not covered by insurance. IVIG costs 1.5–2.0 million yen per injection, whereas 500 mg of rituximab costs 0.3 million yen. A prospective study is required to elucidate the effects of IVIG in patients after rituximab prophylaxis.

The incidence of adverse effects of rituximab was 1.6% (4/258), and all patients recovered and underwent LDLT. Rituximab prophylaxis could be tolerated by patients with end-stage liver diseases. The incidences of bacterial

Table 6: Comparison of antibody titers between patients with and without AMR under rituximab prophylaxis

		AMR+			AMR−			
		N	Median	Mean ± SD	N	Median	Mean ± SD	p-Value
IgM	Peak before transplantation	15	64	158 ± 255	211	64	147 ± 199	0.881
	At transplantation	16	4	7 ± 8	213	4	16 ± 48	0.700
	Peak posttransplantation	16	64	593 ± 1091	223	8	49 ± 181	<0.001*
IgG	Peak before transplantation	14	128	408 ± 584	215	64	319 ± 771	0.221
	At transplantation	13	16	27 ± 35	210	8	34 ± 96	0.265
	Peak posttransplantation	13	256	1002 ± 2196	212	16	68 ± 187	<0.001*

AMR, antibody-mediated rejection. p-values are derived from Wilcoxon sum-rank test.
*$p < 0.05$ for AMR+ versus AMR−.

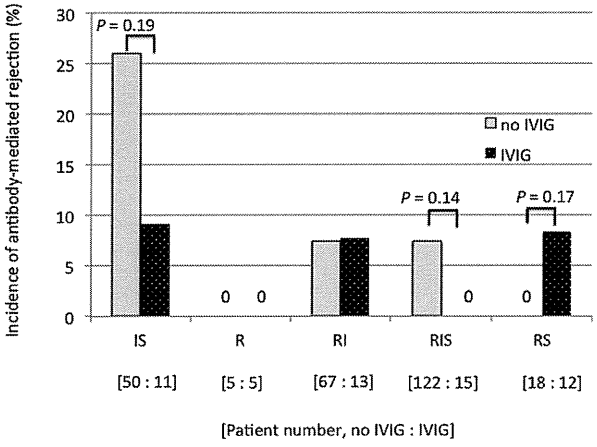

Figure 6: Comparison of the incidences of antibody-mediated rejection (AMR) with and without intravenous immunoglobulin (IVIG) in each regimen. IS, local infusion with splenectomy without rituximab; R, rituximab without splenectomy or local infusion; RI, rituximab with infusion but without splenectomy; RIS, rituximab with both infusion and splenectomy; RS, rituximab with splenectomy but without infusion. There were no significant differences in the incidence of AMR.

infections and CMV disease after transplantation were similar between the nonrituximab and rituximab groups, but the incidence of fungal infection was significantly lower in the rituximab group. Although data for the amount of steroid and trough levels of calcineurine inhibitors were not collected here, the total amount of conventional immunosuppressant might be reduced in light of the expected beneficial effects of rituximab. Lower amounts of conventional immunosuppressants might be a reason for the lower fungal infections.

In this study, half the patients were given 500 mg/body, a quarter were given 300 mg/body and a quarter were given 375 mg/m^2 (corresponding to 430–762 mg/body; median, 600 mg/body). One reason for dose reduction could be concern about potential adverse effects in patients with end-stage liver diseases. In kidney transplantation, Shirakawa et al (20) reported a successful trial to reduce rituximab from 500 to 200 mg/body. Here, there was a tendency toward a higher incidence of AMR in patients treated with $\leq$300 mg/body compared with 500 mg/body or 375 mg/m^2; however, three patients treated with 130 mg/body or 200 mg/body belonged to the same center, and one of them died from severe AMR. More evidence is needed before we can recommend reducing the rituximab dose below 300 mg/body in liver transplantation.

Multiple administrations of rituximab are standard in the treatment of B cell lymphoma. However, because the amount of targeted B cells is expected to be much smaller in transplant patients, a single dose is usually applied. A single dose is standard in kidney transplantation. Here, there were patients with two administrations in six centers and with three administrations in three centers, but the majority of these patients underwent transplantations in 2010 or earlier. All three centers changed their policy to one dose in 2012 on the basis of our data. The current study clearly demonstrates that multiple doses provide no significant benefit in terms of AMR incidence or survival, whereas they increase the incidences of fungal and CMV infections.

The Kyoto group recommended early administration of rituximab to deplete B cells, although the incidence of clinical AMR did not increase significantly in patients with late administration (2). Here, the timing of rituximab administration had no significant effect on AMR incidence on patient survival. Furthermore, 6 of 22 patients with FHF were given rituximab within 6 days before transplantation and survived without AMR. Hence, administration of rituximab immediately before transplantation is a promising therapeutic strategy.

The titers decrease after desensitization before transplantation and increase or do not change immediately after transplantation, and they usually decrease thereafter when patients survive (1). Hence, the optimum cut-off values vary among time points, between IgM and IgG. In rituximab-treated patients, peak IgG and IgM DSA titers posttransplantation were significantly greater in those with AMR, and the AMR incidence was significantly higher in patients with peak titers posttransplantation above optimum cut-off values calculated from ROC curves (i.e. IgM, $\geq$64; IgG, $\geq$128). Theoretically, it is an option to treat patients preemptively by using other desensitization methods such as IVIG and plasmapheresis when antibody titers are above the cut-off values; however, the decision is still difficult.

This study had limitations. It was an uncontrolled retrospective observational study with many confounders, some of which may have been nonrandom and unaccounted for, and thus despite the use of appropriate multivariate statistics unknown bias was possible. Because of the extent of co-linearity between rituximab and era, estimates of regression coefficients still might be unstable, although we tried to adjust era effects as much as possible. Prospective studies are required to examine the causality of the relationships found.

In conclusion, outcomes in adult ABO-I LDLT have significantly improved in the latest era coincident with the introduction of rituximab.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Case Report

Two patients treated with pegylated interferon/ribavirin/ telaprevir triple therapy for recurrent hepatitis C after living donor liver transplantation

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It is difficult to use protease inhibitors in patients with recurrent hepatitis C virus (HCV) infection after liver transplantation (LT) due to interaction with immunosuppressive drugs. We report our experience with two patients treated with telaprevir (TVR) combined with pegylated interferon/ribavirin (PEG IFN/RBV) for recurrent HCV genotype 1 infection after LT. The first was a 63-year-old man with HCV-related liver cirrhosis, who failed to respond to IFN-β plus RBV after LT. Treatment was switched to PEG IFN-α-2b plus RBV and TVR was started. The donor had TT genotype of interleukin (IL)-28 single nucleotide polymorphisms (SNP) (rs8099917). The recipient had TT genotype of IL-28 SNP (rs8099917). Completion of 12-week triple therapy was followed by PEG IFN-α-2b plus RBV for 36 weeks. Finally, he had sustained viral response. The second was a 70-year-old woman with HCV-related liver cirrhosis and hepatocellular carcinoma. She failed to respond to PEG IFN-α-2b plus RBV after LT, and was subsequently switched to PEG IFN-α-2b/RBV/TVR. Genotype analysis showed TG genotype of IL-28 SNP for the donor, and TT genotype of IL-28 SNP for the recipient. Serum HCV RNA titer decreased below the detection limit at 5 weeks. However, triple therapy was withdrawn at 11 weeks due to general fatigue, which resulted in HCV RNA rebound 4 weeks later. Both patients were treated with cyclosporin, starting with a small dose to avoid interactions with TVR. TVR is a potentially suitable agent for LT recipients who do not respond to PEG IFN-α-2b plus RBV after LT.

Key words: hepatitis C virus, liver transplantation, telaprevir

INTRODUCTION

THE HEPATITIS C virus (HCV) has infected 170 million people worldwide, which progresses in some patients to liver cirrhosis and/or hepatocellular carcinoma (HCC).[1] The current treatment for patients infected with HCV genotype 1 is the combination of pegylated interferon-α and ribavirin (PEG IFN/RBV) for 48 weeks.[2] However, this treatment produces sustained viral response (SVR) in only approximately 50% of patients with genotype 1 HCV infection. In 2011, the first direct-acting antiviral agent (DAA) for the treatment of HCV genotype 1, telaprevir (TVR), was approved and treatment with this agent improved SVR to approximately 70–80% of patients with genotype 1 HCV infection.[3,4]

Recurrence of HCV infection after liver transplantation (LT) is one of the major causes of morbidity and allograft loss after LT.[5,6] Because the outcome of post-LT therapy with the classic antiviral agents PEG IFN/RBV are at most moderate with respect to SVR, LT patients constitute one of the classic difficult-to-treat groups.[7–9] The newly introduced triple therapy of protease inhibitors (PEG IFN/RBV/TVR) offers promising perspectives

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for the management of LT patients, although TVR is not yet approved for use in LT patients.

Although there is urgent need for effective treatment of HCV recurrence after LT, significant concern has been expressed about the safety and efficacy of HCV protease inhibitors in this setting because of the side-effect profile and the potential for drug–drug interactions with immunosuppressive agents.[10] Both cyclosporin and tacrolimus are substrates of cytochrome P450 3A and P-glycoprotein. Thus, co-administration of TVR, a potent cytochrome P450 3A4 substrate and inhibitor with the potential to saturate or inhibit intestinal P-glycoprotein, substantially increases the blood levels of cyclosporin and tacrolimus.[11] Consequently, the blood concentration of tacrolimus increased 78-fold, and that of cyclosporin increased fourfold by interaction with TVR.[11] In their recent pilot study, Werner *et al.*[10] described the response to 12-week treatment with TVR plus tacrolimus, cyclosporin or sirolimus in nine patients. Pungpapong *et al.*[12] also reported the preliminary data of 35 patients treated with TVR plus cyclosporin and those of another group of 25 patients treated with boceprevir. Here, we report our preliminary data on protease inhibitors used in combination with PEG IFN/RBV for the treatment of recurrent HCV genotype 1 infection after LT.

CASE REPORT

Case 1

THIS PATIENT WAS a 63-year-old man with HCV-related liver cirrhosis. Living donor LT (LDLT) was performed after obtaining informed consent at May 2009. In August 2009, the patient was started on IFN-β (600 μg) plus RBV (200 mg) due to depression. Because serum HCV RNA titer never fell below the detection limit (1.2 log IU/mL) over the 48-month treatment period, tacrolimus was switched to cyclosporin. In April 2012, treatment was changed to PEG IFN-α-2b (100 μg) plus RBV (200 mg, due to anemia) and TVR (1500 mg) because of depression. At the start of triple therapy, the platelet count was $24.6 \times 10^4/\mu L$, alanine aminotransferase (ALT) was 45 IU/L, genotype was 1b and HCV RNA was 6.8 log IU/mL. Further analysis showed six amino acid (a.a.) substitutions in interferon sensitivity-determining region (ISDR), and mutant- and wild-type amino acids at a.a.70 and a.a.91 in the core region, respectively. The donor had TT genotype of IL-28 single nucleotide polymorphisms (SNP) (rs8099917) and TT/TT genotype of λ4 (ss469415590). The recipient had TT genotype of interleukin (IL)-28 SNP (rs8099917) and TT/TT genotype of λ4 (ss469415590) (Table 1, Fig. 1). Cyclosporin was started at 10 mg/day after triple

Table 1 Laboratory data of patient 1 at start of triple therapy after LT

CBC		LDH	219 IU/L	Tumor marker	
WBC	4630/μL	ALP	357 IU/L	AFP	4.8 ng/mL
RBC	$4.01 \times 10^6/\mu L$	γ-GT	20 IU/L		
Hb	12.4 g/dL	TP	7.3 g/dL	HCV virus markers	
Ht	37.8%	Alb	4.0 g/dL	HCV RNA	6.8 KIU/mL
Plt	$24.6 \times 10^4/\mu L$	TC	164 mg/dL	Genotype	1b
		TTT	12 U		
Blood coagulation test		ZTT	15 U		
PT	120%	BUN	24.6 mg/dl	a.a. substitution in ISDR	6
		Cr	1.07 mg/dl	a.a.70 in the core region	Mutant
Blood chemistry		CRP	0.10 mg/dl	a.a.91 in the core region	Wild
T-Bil	0.5 mg/dL	NH_3	32 μg/mL	*IL-28B* donor	TT genotype
AST	30 IU/L			*IL-28B* recipient	TT genotype
ALT	45 IU/L			ss469415590 donor	TT/TT genotype
FBS	98 mg/dL			ss469415590 recipient	TT/TT genotype
HbA1c	5.5%			AUC of telaprevir	103 μgh/mL

γ-GT, γ-glutamyltransferase; a.a. substitution in ISDR, amino acid substitutions in the interferon sensitivity-determining region; AFP, α-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under curve; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; CRP, C-reactive protein; FBS, fasting blood sugar; Hb, hemoglobin; HbA1c, hemoglobin A1c; Ht, hematocrit; LDH, lactate dehydrogenase; LT, liver transplantation; RBC, red blood cells; Plt, platelets; PT, prothrombin time; T-Bil, total bilirubin; TC, total cholesterol; TP, total protein; TTT, thymol turbidity test; WBC, white blood cells; ZTT, zinc sulfate turbidity test.

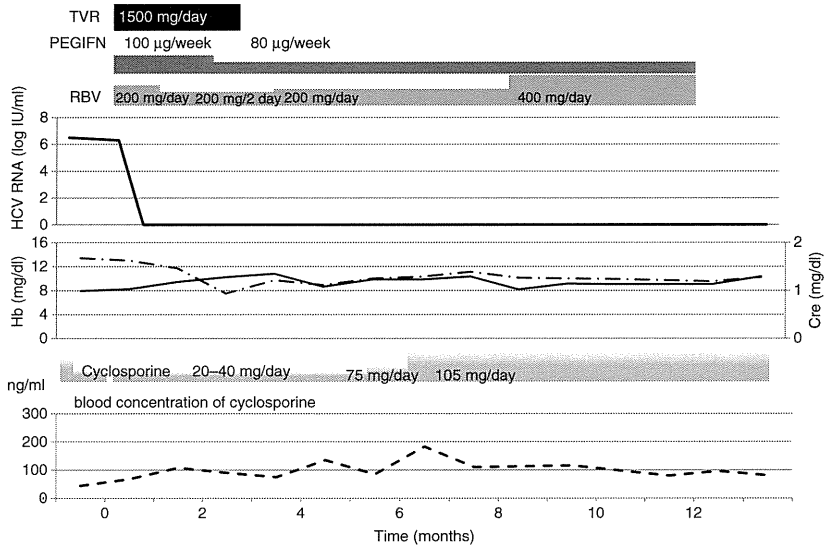

Figure 1 Clinical course of patient 1. Cre, creatinine; Hb, hemoglobin; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir. ——, Cre; — ·, Hb.

therapy, but subsequently increased (based on measurement of its level in the peripheral blood during follow up) to 105 mg/day. The area under the curve (AUC) of TVR was 103 μgh/mL. Serum HCV RNA titer fell below the detection limit (1.2 log IU/mL) at 2 weeks after triple therapy. After 12-week triple therapy, PEG IFN-α-2b and RBV were continued for 36 weeks until April 2013. Finally, he achieved SVR.

Case 2

The patient was a 70-year-old woman with HCV-related liver cirrhosis and HCC. LDLT was performed in May 2006 after obtaining informed consent. Postoperatively, the patient was treated with PEG IFN-α-2b (80 μg) plus RBV (200 mg, due to anemia), which commenced in August 2006. Because serum HCV RNA titer never decreased below the detection limit (1.2 log IU/mL) in the subsequent 48 months, tacrolimus was changed to cyclosporin, and PEG IFN-α-2b plus RBV was changed to the combination of PEG IFN-α-2b (100 μg), RBV (200 mg, due to anemia) and TVR (1500 mg). At the start of triple therapy, platelet count was $19.8 \times 10^4/\mu L$, ALT was 15 IU/L, genotype was 1b, and HCV RNA was 6.2 log IU/mL. Further analysis showed no a.a. substitutions in the ISDR, but mutant- and wild-type a.a. at a.a.70 and a.a.91 in the core region, respectively were detected. The donor had TG genotype of IL-28 SNP (rs8099917) and TT/ΔG genotype of λ4 (ss469415590), while the recipient had TT genotype of IL-28 SNP (rs8099917) and TT/TT genotype of λ4 (ss469415590) (Table 2, Fig. 2). Cyclosporin was started at 10 mg/day, and based on measurement of its concentration in peripheral blood, the dose was increased gradually to 40 mg/day. Subsequent analysis showed a rise in serum creatinine and uric acid, but parameters improved following transfusion. Skin rashes of grade 2 appeared during the triple therapy, which was successfully treated with steroid cream. On the other hand, serum HCV RNA titer decreased below the detection limit (1.2 log IU/mL) at 5 weeks. However, triple therapy was stopped at 11 weeks due to general fatigue. HCV RNA rebounded 4 weeks later.

DISCUSSION

THE SVR RATE has improved since the introduction of PEG IFN/RBV for patients who undergo LT for HCV-related end-stage liver disease. The current estimated SVR rate for LT patients with history of HCV genotype 1 infection is 30–50%.[13–18] These results are much better than those reported in the 1990s and early 2000s, however, more than half of recipients still suffer from recurrent chronic hepatitis C.

It is often difficult to use protease inhibitors for HCV recipients after LT due to potential interaction with immunosuppressive drugs. We reported here our experience with two patients treated with protease inhibitors combined with PEG IFN/RBV for the treatment of recurrent post-LT hepatitis caused by genotype 1 HCV.

A recent study that examined the effect of TVR on the pharmacokinetics of cyclosporin and tacrolimus

Table 2 Laboratory data of Patient 2 at start of triple therapy after LT

CBC		LDH	241 IU/L	Tumor marker	
WBC	7530/μL	ALP	294 IU/L	AFP	5.6 ng/mL
RBC	4.23×10^6/μL	γ-GT	17 IU/L		
Hb	13.3 g/dL	TP	6.4 g/dL	HCV virus markers	
Ht	39.7%	Alb	3.5 g/dL	HCV RNA	6.2 log IU/mL
Plt	17.8×10^4/μL	TC	219 mg/dL	genotype	1b
		TTT	7 U		
Blood coagulation test		ZTT	12 U		
PT	121%	BUN	12.6 mg/dL	a.a. substitution in ISDR	0
		Cr	0.50 mg/dL	a.a.70 in the core region	Mutant
Blood chemistry		CRP	0.11 mg/dL	a.a.91 in the core region	Wild
T-Bil	0.7 mg/dL	FBS	106 mg/dL	*IL-28B* donor	TG genotype
AST	20 IU/L	HbA1c	6.9%	*IL-28B* recipient	TT genotype
ALT	15 IU/L	NH_3	57 μg/mL	ss469415590 door	TT/⊿G genotype
				ss469415590 recipient	TT/TT genotype

γ-GT, γ-glutamyltransferase; a.a. substitution in ISDR, amino acid substitutions in the interferon sensitivity-determining region; AFP, α-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under curve; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; CRP, C-reactive protein; FBS, fasting blood sugar; Hb, hemoglobin; HbA1c, hemoglobin A1c; Ht, hematocrit; LDH, lactate dehydrogenase; LT, liver transplantation; RBC, red blood cells; Plt, platelets; PT, prothrombin time; T-Bil, total bilirubin; TC, total cholesterol; TP, total protein; TTT, thymol turbidity test; WBC, white blood cells; ZTT, zinc sulfate turbidity test.

reported a 78-fold increase in tacrolimus blood concentration and fourfold rise in cyclosporin blood concentration through interaction with TVR.[11] For this reason, we changed tacrolimus to cyclosporin before triple therapy. We also started cyclosporin using a small dose and checked the blood concentration of cyclosporin on a daily basis. Based on these measures, cyclosporin blood concentration remained at approximately 100 ng/mL. Considered collectively, it is important to change the dose of immunosuppressive drugs and frequently monitor cyclosporin blood concentrations.

It is noteworthy that the blood concentration of TVR also increased by interaction with cyclosporin. The AUC of TVR in patient 1 was 103 μgh/mL, while the AUC of TVR of 10 chronic hepatitis C patients treated with PEG IFN/RBV was 52 μgh/mL in our hospital (data not shown). These findings highlight the need for awareness of the potential side-effects of TVR. In fact, various side-

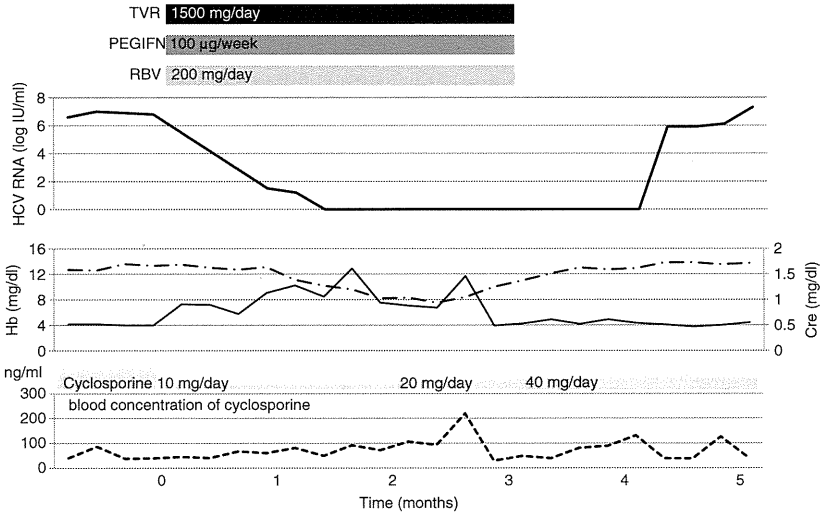

Figure 2 Clinical course of patient 2. Cre, creatinine; Hb, hemoglobin; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir. ——, Cre; — ·, Hb.

effects were reported by patient 2, including anemia, renal dysfunction and skin rashes. Consequently, the triple therapy was discontinued at 11 weeks in this patient.

What are the indications for triple therapy? While there are no standardized rules for the initiation of this mode of treatment, we believe that triply therapy should be used under the following conditions: (i) laboratory tests should show normal hemoglobin and serum creatinine levels to avoid potential side-effects of TVR; and (ii) recipients who develop HCV RNA relapse while receiving PEG IFN/RBV dual therapy after LT. In naïve cases, we recommend PEG IFN/RBV therapy. There are some reports of triple therapy for recipients after LT.[19–21] However, there is no evidence in safety of triple therapy for recipients. Furthermore, Coilly *et al.* recommends PEG IFN/RBV dual therapy for naïve cases in review.[22]

Third, both the donor and recipient must have good SNP (IL28B or λ4). On the other hand, we recommend withholding triple therapy for patients who fail to respond to PEG IFN/RBV and those who have minor SNP (IL28B or λ4) of donor and recipient. In this regard, several groups have reported that *IL28B* of both recipients and donors influenced the SVR to PEG IFN/RBV in patients with recurrent hepatitis C after LT.[23–26]T.[19–22]

Another important question regarding treatment of recurrent post-LT HCV infection is the duration of IFN therapy. The answer to this question is difficult and currently there are no data on the ideal duration of triple therapy. However, we recommend long-term PEG IFN/RBV therapy following triple therapy from 12 to 36 weeks, with a total duration of treatment of 48 weeks. This is based on our previous finding that the majority of patients with genotype 1b in whom HCV RNA reached undetectable levels were able to achieve SVR (87.5%; 7/8).[23] Eradication of HCV by triple therapy should increase the SVR rate. In fact, Pungpapong *et al.* used 12-week triple therapy followed by 36-week PEG IFN/RBV therapy and reported an SVR rate associated with this regimen of 100% (7/7) for genotype 1b recipients.[12]

On the other hand, for such hard-to-treat patients after LT, DAA will become a standard therapy in the future. Because SVR rate and safety of DAA therapy is more higher than triple therapy.[27–29] However, there is a problem of mutation of HCV against DAA therapy.[30,31] In these instances, it may be necessary to recommence triple therapy. The experience of the present study provides a good reference for such an occurrence (e.g. dose of TVR and dose of immunosuppressive agents).

In conclusion, we reported our experience with two patients who developed recurrent HCV genotype 1 infection after LT and were treated with protease inhibitors combined with PEG IFN/RBV. The results point to possible achievement of SVR by triple therapy; however, more studies are needed to evaluate the clinical benefits and side-effects of triple therapy for recurrent post-LT HCV infection.

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Attenuation of Portal Hypertension by Continuous Portal Infusion of PGE1 and Immunologic Impact in Adult-to-Adult Living-Donor Liver Transplantation

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Background. Small-for-size syndrome remains the greatest limiting factor of expanding segmental liver transplantation from living donors. Portal hyperperfusion is considered to substantially contribute to small-for-size syndrome. We investigated the impact of continuous portal infusion of prostaglandin E1 (PGE1) on small-for-size grafts (SFSGs) in adult-to-adult living-donor liver transplantation (LDLT).

Methods. From July 2003 to December 2009, LDLT was performed in 122 patients. We introduced continuous portal infusion of PGE1 to five SFSG patients (PG group) from November 2007 to December 2009 and retrospectively compared them with a historical control group of eight relevant SFSG patients without PGE1 infusion (non-PG group) from July 2003 to October 2007 to determine the safety and efficacy of continuous PGE1 portal infusion for SFSGs. Splenectomy cases were excluded from analysis.

Results. The PG group demonstrated significantly lower postoperative portal pressure than the non-PG group. Moreover, the PG group demonstrated significantly improved liver function in the early posttransplantation period and significantly better recovery from hyperammonemia at 1 week after transplantation and from hyperbilirubinemia in the late posttransplantation period. Overall survival was significantly better in the PG group than in the non-PG group. Three patients in the non-PG group died of rejection-related reasons. Interestingly, immunomonitoring assay revealed that antidonor immune responses were significantly accelerated in the non-PG group compared with the PG group after LDLT. In contrast, the PG group showed well-suppressed antidonor immune responses.

Conclusion. Continuous portal infusion of PGE1 for SFSG attenuated portal hypertension, improved graft function, and suppressed antidonor immune responses, resulting in better survival.

Keywords: Living-donor liver transplantation, Small-for-size graft, Portal hypertension, Alloimmune response, Prostaglandin E1.

(*Transplantation* 2013;95: 1521–1527)

Segmental liver transplantation based on cadaveric splitting or living-donor liver transplantation (LDLT) has been developed for treating patients with end-stage liver disease. It is also a means of overcoming organ shortage and wait-list mortality. However, small-for-size syndrome (SFSS) remains the greatest limiting factor for the expansion of segmental liver transplantation from either cadaveric or living donors (*1, 2*). If the volume of the engrafted liver is considerably less than the standard liver weight in patients with end-stage liver disease who are undergoing partial liver transplantation, excessive portal venous inflow might cause early portal hypertension (*3, 4*) and increased morbidity and mortality due to SFSS (*5*). Previous data have suggested that, in recipients of adult-to-adult LDLT, one of the most challenging tasks is to match a good size graft. Emphasis has more recently been placed not only on the evaluation of the ratio between donor and recipient liver volume but also on the degree of portal hypertension and the stage of liver disease in the recipient, consistent with the result in a pig model (*6–8*). Therefore, the importance of portal pressure during LDLT is now recognized.

We have demonstrated that continuous portal infusion of prostaglandin E1 (PGE1) considerably improved the congestion

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of the residual liver after extended hepatectomy in a rat model (*9*). Based on this result, we applied a continuous portal infusion of PGE1 for small-for-size grafts (SFSGs) in LDLT in the clinical setting.

We here investigated the clinical significance of controlling portal pressure by continuous portal infusion of PGE1 after surgery in LDLT with SFSGs, focusing on portal decompression, postoperative liver function, survival, and the antidonor immune status of the recipient retrospectively.

RESULTS

Patients' Demographic and Clinical Characteristics

Thirteen patients receiving SFSGs were retrospectively analyzed in this study. The patients' demographic and clinical characteristics are shown in Table 1. Of these patients, five received a continuous portal injection of PGE1 after transplantation (PG group) from November 2007 to December 2009 (era 2), whereas eight were historical controls from July 2003 to October 2007 (era 1) without PGE1 infusion (non-PG group). There was no significant difference in age or underlying disease between the two groups. Preoperative examination of the hepatic reserve showed similar Child-Pugh scores (PG group, 10.0±0.71; non-PG group, 9.00±0.83). Patients' model for end-stage liver disease scores, which were used as recipient severity indices, was similar between groups (mean [range], 16.8 [8–30] and 15.1 [9–28], respectively). Portal vein pressure (PVP) at laparotomy was also similar between the two groups (25.2 [17–34] and 20.3 [17–24] mm Hg, respectively). Concerning the graft, one patient in each group showed minimal fatty metamorphosis (<0.1%) on histology and there was no significant difference in graft-to-recipient body weight ratio (GRWR) between the two groups (0.680 [0.63–0.71] and 0.655 [0.51–0.72], respectively).

Furthermore, factors related to surgical invasiveness in those two groups, such as hemorrhage level, operation time, and graft ischemia duration, were similar. No donor had donor-specific antigens, and there was no difference in the number of human leukocyte antigen (HLA) mismatch (Table 1). Three donor candidates in each group underwent liver biopsy. Among them, one in each group showed minimal fatty metamorphosis (<0.1%) on histology. Of note, three of five patients in the PGE1 group and three of eight patients in the non-PGE1 group received right-lobe grafts. All patients receiving right lobes in both groups had grafts with middle hepatic vein (MHV) tributaries more than 5 mm in diameter, and all draining tributaries were reconstructed with the

TABLE 1. Patients' demographic and clinical characteristics

Variables	PG group (n=5)	Non-PG group (n=8)	*P*
Recipient factors			
Age, years	56.4±3.4	57.9±4.4	0.510[a]
Gender, male/female	5/0	3/5	0.075[b]
Child-Pugh score	10.0±1.6	9.0±1.9	0.325[a]
MELD score	16.8±8.2	15.1±5.8	0.702[a]
PVP, mm Hg, at laparotomy	25.2±6.1	20.9±3.0	0.199[a]
Disease background			
Viral hepatitis (B/C)	1/2	1/5	>0.999[b]
Alcoholic	1	1	>0.999[b]
Acute hepatic failure	1	0	0.385[b]
Cholestatic disease	0	1	>0.999[b]
Donor factors			
Age, years	26.2±3.3	33.3±10.5	0.113[a]
Gender, male/female	0/5	5/3	0.075[b]
Graft factors			
Graft type, right/left	3/2	3/5	0.592[b]
GRWR, %	0.68±0.03	0.66±0.09	0.510[a]
Reconstruction of hepatic vein	3	3	0.592[b]
HLA class I mismatch	1.20±0.49	1.63±0.23	0.453[a]
HLA class II mismatch	0.60±0.24	1.00±0.00	—
DSA	0	0	—
Surgical factors			
Operation time, min	781.0±153.6	755.9±106.0	0.758[a]
Bleeding, mL	5322.0±2295.3	5751.4±6371.2	0.866[a]
Total ischemia time, min	117.0±35.5	118.9±31.4	0.925[a]

[a] Unpaired *t* test with Welch's correction.
[b] Fisher's exact test.
DSA, donor-specific antibody; HLA, human leukocyte antigen; GRWR, graft-to-recipient body weight ratio; MELD, model for end-stage liver disease; PVP, portal vein pressure.

recipients' native MHV trunk as reported previously (*10*). There was no thrombosis in those reconstructed tributaries after surgery. One patient of each group had grafts with inferior right hepatic vein, which were reconstructed using direct anastomosis to inferior vena cava in each case.

Continuous PGE1 Infusion Attenuated Portal Hypertension After Reperfusion in SFSGs

After laparotomy, we inserted a catheter from the mesenteric vein to the distal side of the portal vein and measured the PVP during the operation. All patients exhibited portal hypertension during laparotomy. In the PG group, after reflow of the portal and hepatic veins was confirmed, we started PGE1 infusion into the portal vein through a catheter. Continuous infusion of PGE1 resulted in a significant reduction of PVP at the time of abdominal closure in the PG group compared with the non-PG group ($P<0.005$; Fig. 1A). The mean PVP at the time of abdominal closure was 15.4±1.17 mm Hg in the PG group and 20.5±1.47 mm Hg in the non-PG group (Fig. 1A). Furthermore, the PVP ratio at the end of the operation, compared with that at laparotomy, showed effective portal decompression in the PG group and non-PG group, respectively (0.62±0.04 vs. 0.99±0.06; $P<0.001$; Fig. 1B). Importantly, none of the patients in the PG group developed hypoperfusion after PGE1 portal infusion.

Clinical Course of Graft Liver Function

Graft liver function markers, including serum transaminases, arterial ketone body ratio (AKBR), ammonia, and total bilirubin, after surgery were compared between the PG group and the non-PG group.

Elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were significantly attenuated in the PG group compared with the non-PG group on days 1 and 2 (Fig. 2). Similarly, the AKBR, which reflects the hepatic mitochondrial redox state and is considered an accurate index of the functional reserve of the graft liver after transplantation, was significantly higher in the PG group. However, these values became comparable between the two groups after day 3. Strikingly, significantly better recovery from hyperammonemia was seen in the PG group for 1 week after surgery. The serum total bilirubin level was comparable between the two groups by day 28 after LDLT. Nonetheless, hyperbilirubinemia was significantly improved in the PG group after day 28 but remained prolonged in the non-PG group. These results indicate that continuous infusion of PGE1 significantly improved the liver function after LDLT with SFSGs.

Complications and Prognosis

In the PG group, no complications associated with the portal vein catheter were observed after surgery (e.g., post-removal bleeding, catheter infection, or portal thrombosis). One patient in the non-PG group and none in the PG group developed SFSS. Postoperative death occurred in 5 patients of the non-PG group and in none in the PG group. In the non-PG group, the 1- and 2-year survival rates were 62.5% and 37.5%, respectively. In contrast, in the PG group, the 1-and 2-year survival rates were both 100%, a difference that was statistically significant ($P<0.05$; Fig. 3). The main causes of death in the non-PG group were graft dysfunction, rejection, and subsequent infection as well as bacterial sepsis after biliary stenosis. No patients in the PG group had a

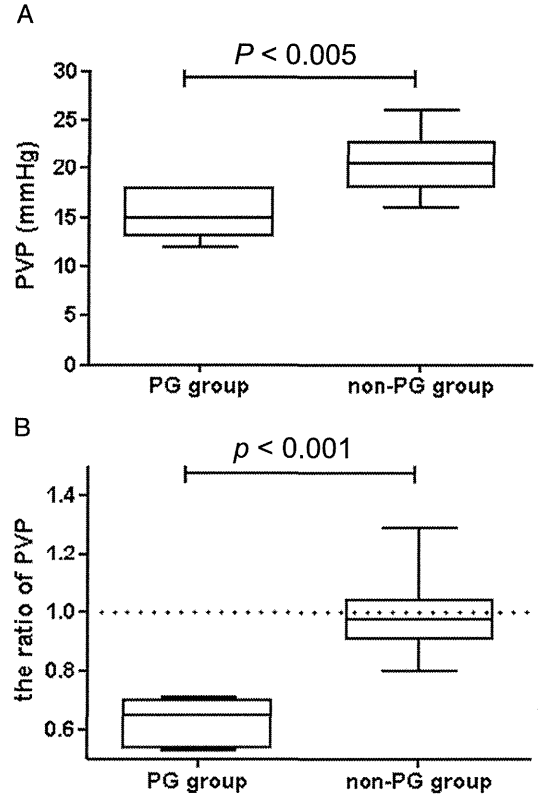

FIGURE 1. PVP value at the end of the operation (A) and ratio of PVP at the end of the operation to that at laparotomy (B) in the PG group and the non-PG group. An unpaired *t* test with Welch's correction was used to compare PVP and the ratio of PVP between the PG group and the non-PG group. The box plot represents the 25th to 75th percentiles, the dark line is the median, and the extended bars represent the 10th to the 90th percentiles. *$P<0.05$; ***$P<0.001$. PVP, portal vein pressure.

rejection episode. Rejection was diagnosed by liver biopsy and histologic findings showed features of SFSG and/or portal hypertension with rejection (see **Figure S1, SDC**, http://links.lww.com/TP/A807). The 2-year survival of SFSG patients (non-PG group) in era 1 (July 2003 to October 2007) was significantly worse than that of the non-SFSG patients in the same period (37.5% vs. 77.8%; $P<0.05$), whereas the 2-year survival of SFSG patients (PG group) in era 2 (November 2007 to December 2009) was not statistically different from that of the non-SFSG patients in the same period (100% vs. 77.1%). Of note, the 2-year survival of non-SFSG patients was similar between eras 1 and 2 (Fig. 4).

Estimation of Immunosuppressive Status After Surgery by Using the Carboxyfluorescein Diacetate Succinimidyl Ester-Mixed Lymphocyte Reaction Assay

Because the main cause of death in 3 patients in the non-PG group was related to rejection, we retrospectively analyzed the immunosuppressive postoperative status of both groups. All patients and their donors consented to be

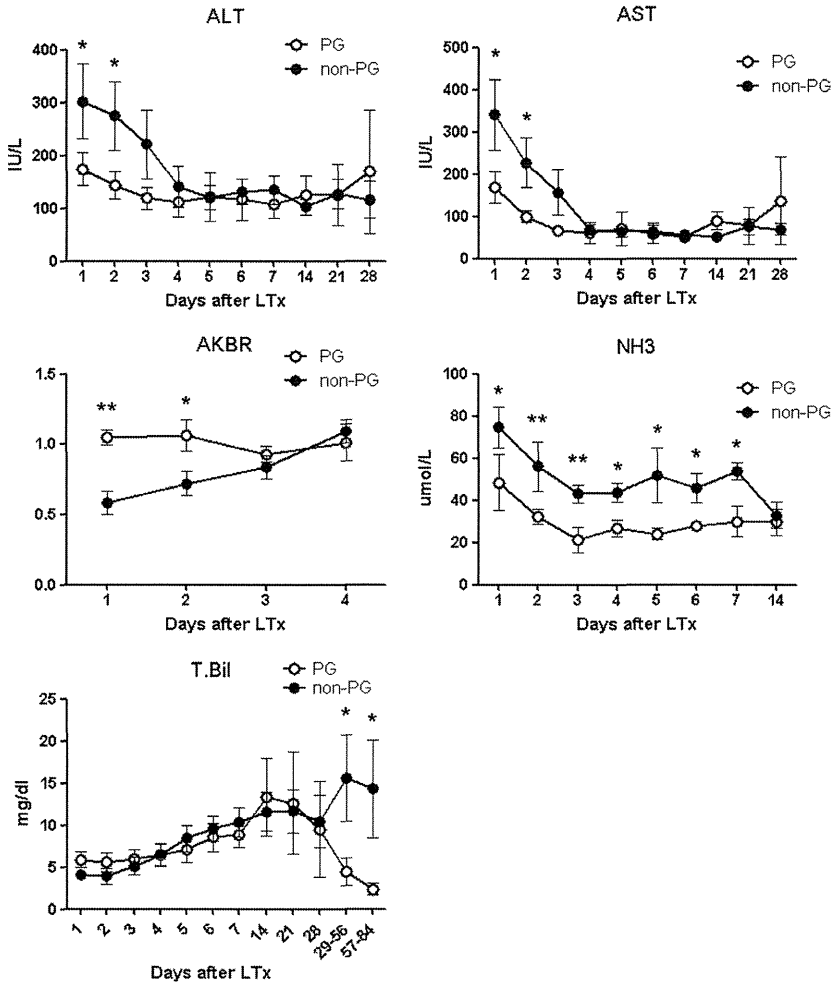

FIGURE 2. Liver function tests (ALT, AST, AKBR, NH_3, and T.Bil) of patients with (PG group; open circle) or without PGE1 portal infusion (non-PG group; closed circle) after LDLT. Data are mean±SEM for individual groups. An unpaired *t* test with Welch's correction was used to compare each of the indicated parameters between the PG group and the non-PG group. **P*<0.05; ****P*<0.01. AKBR, arterial ketone body ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDLT, living-donor liver transplantation; LTx, liver transplantation; NH_3, ammonia; T.Bil, total bilirubin.

subjected to a mixed lymphocyte reaction (MLR) assay with the carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling technique. In all five patients of the PG group, suppressed $CD8^+$ T-cell proliferation, which is defined as a stimulation index (SI)<2, was observed in the antidonor MLR assay (i.e., a hyporesponse to donor; mean SI, 1.10±0.13; Fig. 4A). The mean percentage of $CD25^+$ cells among the proliferating $CD8^+$ T cells, which are activated cytotoxic T cells, was 9.24±5.93 (Fig. 4B). In contrast, in five of the eight patients in the non-PG group, accelerated $CD8^+$ T-cell proliferation was observed in the antidonor MLR assay (i.e., a hyperresponse to donor; mean SI, 2.85±0.50; Fig. 4A). Furthermore, the mean percentage of $CD25^+$ cells among the proliferating $CD8^+$ T cells was 63.82±8.63 (Fig. 4B). These differences between the two groups were significant. Of note, three patients in the non-PG group who showed high antidonor response (i.e., SI of $CD8^+$ T cells>3) required steroid pulse treatment and died of graft dysfunction or infection after rejection. Two patients who showed a relatively high antidonor response (i.e., SI of $CD8^+$ T cells>2) required an increase in immunosuppressant doses. These results indicated that patients with SFSGs show accelerated antidonor immune responses and that continuous portal infusion of PGE1 suppressed this type of antidonor immune response.

DISCUSSION

Various approaches to controlling excessive portal flow and pressure have been proposed, such as dual grafting to increase graft volume (*11, 12*). Although this concept is simple, it requires two healthy living donors and involves increased risk to donors. Another approach is portal decompression with a portosystemic shunt (*13, 14*) or splenic artery manipulation, including splenectomy, embolization, and ligation (*15–17*). This method is more favored in terms of availability and donor risk. Nonetheless, there is

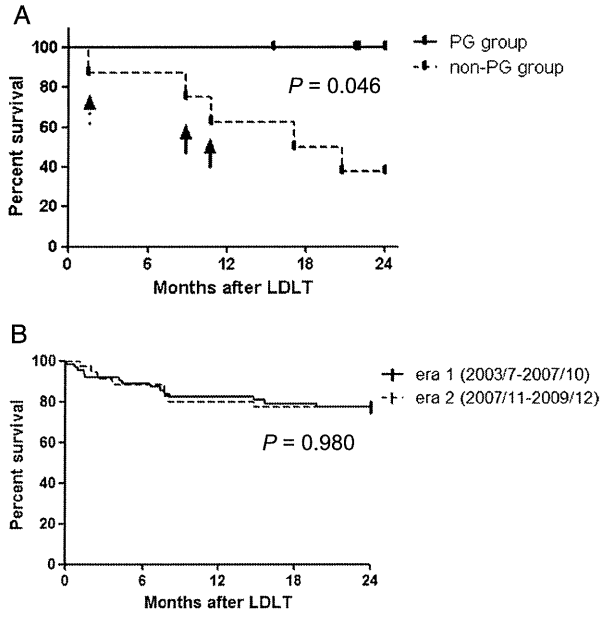

FIGURE 3. A, Kaplan–Meier patient survival curves of patients with (PG group; n=5; solid line) or without PGE1 portal infusion after LDLT (non-PG group; n=8; dotted line). In the non-PG group, the 1- and 2-year survival rates were 62.5% and 37.5%, respectively. In the PG group, the 1- and 2-year survival rates were both 100%, a difference that was statistically significant. *P<0.05. Dashed arrow represents a patient's death due to SFSS and rejection followed by infection, and solid arrows represent patients' death due to rejection-related reasons. B, Kaplan–Meier patient survival curves of non-SFSG patients in era 1 (from July 2003 to October 2007; n=62; solid line) or era 2 (from November 2007 to December 2009; n=35; dotted line). In the era 1 and era 2 groups, the 2-year survival rate was 77.4% and 77.1%, respectively, with no statistical difference (P=0.980). ABO-incompatible cases and splenectomy cases were excluded from analysis. LDLT, living-donor liver transplantation; PGE1, prostaglandin E1; SFSG, small-for-size graft; SFSS, small-for-size syndrome.

a considerable risk of infection in splenectomy or splenic artery ligation (*18*). Moreover, significantly higher mortality was observed in patients who had splenectomy mainly due to septic complications in liver transplantation (*19, 20*). In fact, we experienced one SFSG case in which the patient died of sudden sepsis without any primary focus 4 years after transplantation with splenectomy. Therefore, another method to control portal pressure and preserve the spleen is likely more preferable.

We have reported that portal administration of PGE1, a vasodilator of vessels containing smooth muscle (*21, 22*), prevented congestion of residual liver tissues in a rat extended hepatectomy model. In this study, we tried various vasodilators; however, residual liver congestion after hepatectomy was improved only by continuous portal infusion of PGE1. We also tried systemic continuous venous infusion of PGE1 at the same dose, but this was not effective. This suggests the therapeutic potential of portal PGE1 injection to prevent portal hypertension after LDLT with SFSGs.

We translated this method to adult LDLT with SFSGs, and portal infusion of PGE1 successfully reduced PVP, resulting in improved liver graft function in both early and late posttransplantation periods. This result was unexpected because the portal infusion of PGE1 was given for only the first week yet improved the long-term survival of recipients.

We used a CFSE-MLR assay to objectively evaluate the antidonor responses of the recipients (*23, 24*). The lack of $CD8^+$ and $CD25^+$ T-cell proliferation in antidonor MLR reflects the suppression of the antidonor response. In this immunologic investigation, all patients given the continuous portal infusion of PGE1 showed a well-suppressed response of the antidonor $CD8^+$ T cells (Fig. 4). In contrast, surprisingly, patients without the PGE1 treatment showed

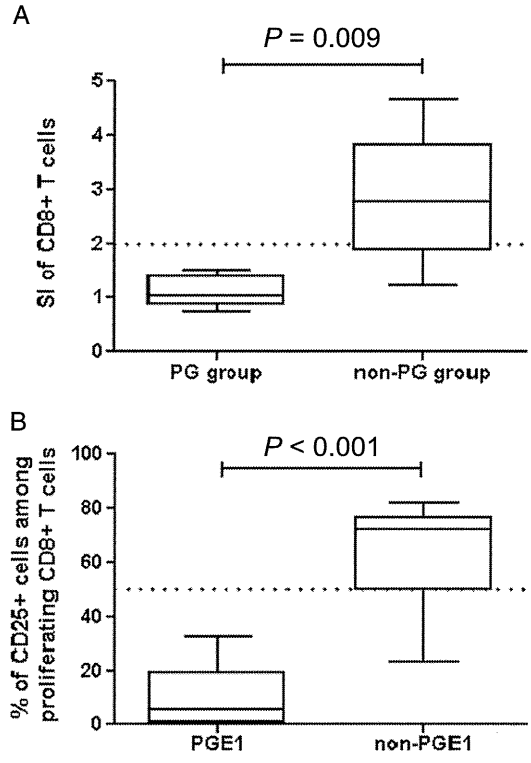

FIGURE 4. SIs of $CD8^+$ T-cell subsets in the antidonor MLR assay of patients in the PG group (n=5) and the non-PG group (n=8) on the third to fourth weeks after transplantation (A) and percentage of $CD25^+$ cells among proliferating $CD8^+$ T cells in patients of the PG group and the non-PG group (B). $CD8^+$ 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 the allogeneic combinations were calculated by dividing the mitotic index of a particular allogeneic (self to donor) combination by that of the self-control. An unpaired t test with Welch's correction was used to compare the SI and percentage of $CD25^+$ cells between the PG group and the non-PG group. The box plot represents the 25th to 75th percentiles, the dark line is the median, and the extended bars represent the 10th to the 90th percentiles. **P<0.01; ***P<0.001. MLR, mixed lymphocyte reaction; SI, stimulation index.