common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. J Clin Invest 1998;102:1831-1840.
10. Kawano A, Shimoda S, Kamihira T, Ishikawa F, Niiro H, Soejima Y, et al. Peripheral tolerance and the qualitative characteristics of autoreactive T cell clones in primary biliary cirrhosis. J Immunol 2007;179: 3315-3324.
11. Shimoda S, Ishikawa F, Kamihira T, Komori A, Niiro H, Baba E, et al. Autoreactive T-cell responses in primary biliary cirrhosis are proinflammatory whereas those of controls are regulatory. Gastroenterology 2006;131:606-618.
12. Chuang YH, Lian ZX, Tsuneyama K, Chiang BL, Ansari AA, Coppel RL, et al. Increased killing activity and decreased cytokine production in NK cells in patients with primary biliary cirrhosis. J Autoimmun 2006;26:232-240.
13. Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: "l'union fait la force." Blood 2005;106: 2252-2258.
14. Goodnow CC. Multistep pathogenesis of autoimmune disease. Cell 2007;130:25-35.
15. Palmer MT, Weaver CT. Autoimmunity: increasing suspects in the CD4+ T cell lineup. Nat Immunol 2010;11:36-40.
16. Gershwin ME, Mackay IR. The causes of primary biliary cirrhosis: convenient and inconvenient truths. HEPATOLOGY 2008;47:737-745.
17. Strutt TM, McKinstry KK, Dibble JP, Winchell C, Kuang Y, Curtis JD, et al. Memory CD4+ T cells induce innate responses independently of pathogen. Nat Med 2010;16:558-564.
18. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 2008;9:495-502.
19. Tu Z, Bozorgzadeh A, Pierce RH, Kurtis J, Crispe IN, Orloff MS. TLR-dependent cross talk between human Kupffer cells and NK cells. J Exp Med 2008;205:233-244.
20. Hart OM, Athie-Morales V, O'Connor GM, Gardiner CM. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. J Immunol 2005;175:1636-1642.
21. Stegmann KA, Bjorkstrom NK, Veber H, Ciesek S, Riese P, Wiegand J, et al. Interferon-alpha-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. Gastroenterology 2010; 138:1885-1897.
22. Oliviero B, Varchetta S, Paudice E, Michelone G, Zaramella M, Mavilio D, F, et al. Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. Gastroenterology 2009; 137:1151-1160.
23. Takayama T, Kamada N, Chinen H, Okamoto S, Kitazume M, Chang J, et al. Imbalance of NKp44+NKp46- and NKp44-NKp46+ natural killer cells in the intestinal mucosa of patients with Crohn's disease. Gastroenterology 2010;139:882-892.
24. Gur C, Porgador A, Elboim M, Gazit R, Mizrahi S, Stern-Ginossar N, et al. The activating receptor NKp46 is essential for the development of type 1 diabetes. Nat Immunol 2010;11:121-128.
25. Gao B. Natural killer group 2 member D, its ligands, and liver disease: good or bad? HEPATOLOGY 2010;51:8-11.
26. Takeda K, Kojima Y, Ikejima K, Harada K, Yamashina S, Okumura K, et al. Death receptor 5 mediated-apoptosis contributes to cholestatic liver disease. Proc Natl Acad Sci U S A 2008;105:10895-10900.
27. Takii Y, Nakamura M, Ito M, Yokoyama T, Komori A, Shimizu-Yoshida Y, et al. Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. Lab Invest 2005;85: 908-920.
28. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005;23: 275-306.
29. Hopf U, Moller B, Stemerowicz R, Lobeck H, Rodloff A, Freudenberg M, et al. Relation between Escherichia coli R(rough)-forms in gut, lipid A in liver, and primary biliary cirrhosis. Lancet 1989;2:1419-1422.
30. Butler P, Valle F, Hamilton-Miller JM, Brumfitt W, Baum H, Burroughs AK. M2 mitochondrial antibodies and urinary rough mutant bacteria in patients with primary biliary cirrhosis and in patients with recurrent bacteriuria. J Hepatol 1993;17:408-414.
31. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity 2006;25:383-392.
32. Croft M. The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol 2009;9:271-285.
33. Luger D, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, et al. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. J Exp Med 2008;205:799-810.
34. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 2007;13:543-551.
35. Walter U, Santamaria P. CD8+ T cells in autoimmunity. Curr Opin Immunol 2005;17:624-631.
36. Rajagopal D, Paturel C, Morel Y, Uematsu S, Akira S, Diebold SS. Plasmacytoid dendritic cell-derived type I interferon is crucial for the adjuvant activity of Toll-like receptor 7 agonists. Blood 2010;115: 1949-1957.
37. Stary G, Klein I, Kohlhofer S, Koszik F, Scherzer T, Mullauer L, et al. Plasmacytoid dendritic cells express TRAIL and induce CD4+ T-cell apoptosis in HIV-1 viremic patients. Blood 2009;114:3854-3863.
38. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 2004;4:762-774.
39. Sozzani S, Vermi W, Del Prete A, Facchetti F. Trafficking properties of plasmacytoid dendritic cells in health and disease. Trends Immunol 2010;31:270-277.
40. Issè K, Harada K, Zen Y, Kamihira T, Shimoda S, Harada M, et al. Fractalkine and CX3CR1 are involved in the recruitment of intraepithelial lymphocytes of intrahepatic bile ducts. HEPATOLOGY 2005;41: 506-516.
41. Pallandre JR, Krzewski K, Bedel R, Ryffel B, Caignard A, Rohrlich PS, et al. Dendritic cell and natural killer cell cross-talk: a pivotal role of CX3CL1 in NK cytoskeleton organization and activation. Blood 2008; 112:4420-4424.

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Original contribution

Frequent overlap of active hepatitis in recurrent primary sclerosing cholangitis after living-donor liver transplantation relates to its rapidly progressive course

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Summary Recurrence of primary sclerosing cholangitis after liver transplantation is a challenging issue. Liver pathologies of recurrent primary sclerosing cholangitis after living-donor liver transplantation have not been reported. Here, liver pathologies of explanted grafts and biopsies of 9 patients who underwent retransplantation for recurrent primary sclerosing cholangitis were compared with those of native livers. Recurrence was diagnosed in 13 of 36 patients for primary sclerosing cholangitis post–living-donor liver transplantation, and 9 of them underwent retransplantation. All explanted grafts revealed biliary cirrhosis with sclerosing cholangitis, and 6 patients had additional features of active hepatitis. Liver biopsies showed that 3 had active hepatitis in addition to fibrous cholangitis at recurrence of primary sclerosing cholangitis. Two developed active hepatitis later after the diagnosis of recurrence. In explanted grafts, in addition to extensive hilar lymphoplasmacytic cholangitis, 4 cases showed hilar xanthogranulomatous cholangitis. The latter was not evident in 7 native livers. Ductopenia was extensive in all native livers, although such changes were relatively mild in explanted grafts at retransplantation. Patients with recurrent primary sclerosing cholangitis developed progressive graft failure, and the interval between diagnosis of recurrence and retransplantation (mean, 3.2 years) was shorter than that between diagnosis of primary sclerosing cholangitis and first transplantation (mean, 7.7 years). The rather rapid deterioration of recurrent primary sclerosing cholangitis after liver transplantation may be related to the frequent overlap of active hepatitis.

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1. Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by long-term inflammation and fibrosis of the intrahepatic and/or extrahepatic bile ducts. Progressive obliteration of the biliary tree eventually leads to biliary cirrhosis, and liver transplantation (LT) is still the only therapeutic option for end-stage patients with PSC [1]. Nonanastomotic strictures from various causes frequently occur in PSC groups in comparison with control groups [2]. Although PSC reportedly recurs after orthotopic LT with an incidence of 5% to 20% [2], cadaveric LT provides excellent long-term graft survival (5-year, 86%; and 10-year, 70%) for patients with end-stage PSC [3]. However, in the case of living-donor LT (LDLT), PSC recurrence has been higher, with an incidence of nearly 50%, and allograft loss rate is high [4,5].

The diagnosis of PSC recurrence after LT should be based on cholangiographic and histologic findings such as fibrous cholangitis and/or fibro-obliterative lesions [2], and almost all reported series describe the histology of PSC recurrence as compatible with the criteria [3].

Active hepatitis characterized by distinct interface hepatitis, prominent lobular and perivenular necroinflammation, and portal inflammation are infrequently found in PSC livers, and such cases are called PSC-autoimmune hepatitis (AIH) overlap [6]. This overlap is more often found in children than in adults [7]. To date, there have been no detailed histologic descriptions of the association of active hepatitis in recurrent PSC, and only a few brief reports on this issue are available: Demetris et al [6] reported that an association with active hepatitis is unusual. Khettry et al found lymphoplasmacytic lobular hepatitis in one of 6 definite cases of recurrent PSC, and they called mild necroinflammatory changes in posttransplanted biopsies in the absence of recurrent PSC "autoimmune liver disease," although its exact clinicopathologic features remain unspecified [8].

Recently, we reported that the recurrence rate of PSC is high in LDLT cases and the posttransplantation course of patients with recurrent PSC has been poor; the 5- and 10-year graft survival rates were 69% and 40%, respectively [4,5]. Therefore, to overcome this imminent and important challenge in LDLT for PSC, we tried to characterize the histologic features of recurrent PSC after LDLT.

2. Materials and methods

2.1. Classification of the biliary tree

The biliary tree is dividable into the extrahepatic and intrahepatic bile ducts. The right and left hepatic duct and their first to third branches are collectively called "hilar bile ducts." The intrahepatic bile ducts, proximal to the right or left hepatic duct, are classified as the intrahepatic large and small bile ducts [9]. The former are visible grossly and consist of the first to third branches of right or left hepatic bile ducts. The latter are recognizable microscopically and consist of septal and interlobular bile ducts, and the latter are connected to bile ductules.

2.2. Study population

From June 1990 to July 2009, a total of 1387 patients underwent LDLT at Kyoto University Hospital. Of these, 1309 patients received primary LDLT, and the remaining 78 patients received a second or third LDLT. Among them, 34 patients underwent primary LDLT for end-stage PSC. We added to the study population 2 patients with PSC who received primary LDLT at other hospitals and were included in the second LDLT population at Kyoto University Hospital (totally, 36 patients). During LDLT, the biliary system was reconstructed with a Roux-en-Y choledochojejunostomy for all but one patient with PSC [5], and one with recurrent PSC underwent duct-to-duct reconstruction.

2.3. Definition of recurrent PSC posttransplantation

Recurrent PSC was diagnosed based on the following criteria defined by Graziadei et al [2,3]: (1) a confirmed diagnosis of PSC before liver transplantation; (2) a cholangiogram showing nonanastomotic biliary strictures of the intrahepatic and/or extrahepatic biliary tree with beading and irregularity occurring >90 days posttransplantation; and/or (3) a liver biopsy showing fibrous cholangitis and/or fibro-obliterative lesions with or without ductopenia, biliary fibrosis, or biliary cirrhosis. The presence of other causes of biliary stricturing such as hepatic artery thrombosis/stenosis, long-term rejection, and ABO blood type incompatibility were excluded [2].

2.4. Laboratory data and cholangiography

The following variables were evaluated: serum aspartate aminotransferase (AST; normal, 13-29 IU/L), alanine aminotransferase (ALT, 8-28 IU/L), γ-glutamyltranspeptidase (GGT, 9-54 IU/L), alkaline phosphatase (ALP, 118-335 IU/L), total bilirubin (T-Bil, 0.2-1.0 mg/dL), and IgG (788-1841 mg/dL). Autoantibodies were analyzed by indirect immunofluorescence. Cholangiography was performed by percutaneous transhepatic cholangiography and/or magnetic resonance cholangiography. The latter was applied routinely in recent cases.

2.5. Histologic assessment

Liver biopsies during the post-LDLT course and explanted livers at primary LDLT and at retransplantation were reviewed by 2 pathologists (AM-H and YN). These

specimens were fixed in 10% buffered formalin and embedded in paraffin. About 20 thin sections were cut from these specimens, and they were used for routine staining such as hematoxylin and eosin (H&E), Gomori's reticulin, and Masson's trichrome stainings. Routine immunostaining of cytokeratin 7 was done to identify biliary elements.

Interface activity and lobular necroinflammation were graded on a scale of 0 to 3: (0), (1), (2), and (3) represent no, mild, moderate, and severe necroinflammatory reactions, respectively [10]. Fibrosis was also quantified on a scale of 0 to 4: no fibrosis (0), fibrous expansion of portal tracts (1), bridging fibrosis (2), bridging fibrosis with lobular distortion (3), and cirrhosis (4) [10]. Periductal fibrosis (fibrous cholangitis), ductal proliferation, ductopenia, and cholestasis were scored using a scoring system with some modifications [11] as not present (0), mild (1), moderate (2), or severe (3). A fibrous core reflecting a bile duct obliterative lesion in PSC [12] and xanthogranulomatous cholangitis [13] were also examined. Lymphoplasmacytic inflammation of hilar bile ducts was also scored from none (0) to severe (3). To determine the presence of AIH features, the revised International Autoimmune Hepatitis group (IAHG) scoring system was used as an objective tool for evaluation. A score >15 indicates definite AIH, and a score of 10 to 15 probable AIH [14].

2.6. Treatment

The primary immunosuppressive regimen was a combination of tacrolimus and prednisolone. The maintenance immunosuppressive regimen consisted of tacrolimus monotherapy. Patients who did not respond to the treatment received prednisolone and/or antimetabolites (mycophenolate mofetil or mizoribine). All patients with PSC received ursodeoxycholic acid therapy.

2.7. Statistics

The paired Student t test was used to compare continuous variables. The χ^2 test or Fisher exact test were used to compare frequencies of categorical variables. Statistical analysis was performed using StataSE 9.0 (Stata Corporation, College Station, TX). $P < .05$ was regarded as statistically significant.

3. Results

3.1. Clinical features

Thirty-three of 36 in the original patient population had survived more than 90 days after LDLT. Two of 33 who received ABO blood type incompatible transplantations were excluded. Of the remaining 31, 23 were blood-related donors (15 parents, 6 siblings, and 2 offsprings) and 8 non–blood-related donors (4 spouses and 4 domino transplantation donors).

Among these 31 patients, 2 had an anastomotic biliary stricture and one showed long-term rejection, and these 3 were not considered as having PSC recurrence. Fourteen (4 male and 10 female) of the remaining 28 patients fulfilled the criteria for PSC recurrence after LDLT; 11 were of blood-related donors and 3 non–blood-related donors, and the recurrence rate was higher in the former (11 of 23 cases; 47.8%) than in the latter (3 of 8 cases; 37.5%), although not statistically significant. One female patient revealed histologic evidence of PSC recurrence on failed grafts at retransplantation, but her deteriorated clinical course after LDLT was mainly because of acquiring hepatitis C virus (HCV) infection leading to cirrhosis. Thus, the patient was excluded from further analysis.

The clinical features for the remaining 13 patients with recurrent PSC are shown in Table 1. Nine had concomitant ulcerative colitis. The evidence of PSC recurrence after

Table 1 Clinical characteristics of the patients with recurrence of PSC

Patient	Sex	Time interval between PSC diagnosis and LT (y)	Age at LT	UC	Donor	PSC recurrence after LT (y)	Outcome	Time interval between recurrent PSC and re-LT (y)	Follow-up after re-LT or LT
1	Female	3.6	5	−	BRD	3.3	re-LT	0.7	Alive, 9 y
2	Female	4.4	19	+	BRD	2.1	re-LT	3.6	Alive, 6.6 y
3	Female	1.2	20	+	BRD	1.9	re-LT	7.7	Alive, 2 y
4	Female	9	34	+	BRD	5.2	re-LT	4.1	Alive, 1.6 y
5	Male	20	24	+	BRD	5.3	re-LT	2.7	Alive, 2 y
6	Male	3	25	+	BRD	4	re-LT	2.4	Alive, 2.8 y
7*	Female	8	47	−	NBRD	1.7	re-LT	1.3	Dead, 2 wk
8*	Male	8	21	−	BRD	3	re-LT	4	Alive, 6.9 y
9	Female	12	14	+	BRD	2.2	re-LT	2.2	Alive, 4.4 y
10	Female	4.8	30	+	BRD	5.5	cirrhosis	–	Alive, 11 y
11	Female	5	45	+	NBRD	5.9	cirrhosis	–	Alive, 11 y
12	Female	16	36	+	BRD	2.4	mild fibrosis	–	Alive, 6.3 y
13	Male	8	67	−	NBRD	1.2	mild fibrosis	–	Alive, 1.7 y

BRD indicates blood-related donor; NBRD, non–blood-related donor; re-LT, retransplantation; UC, ulcerative colitis.
* Primary LT at other hospitals.

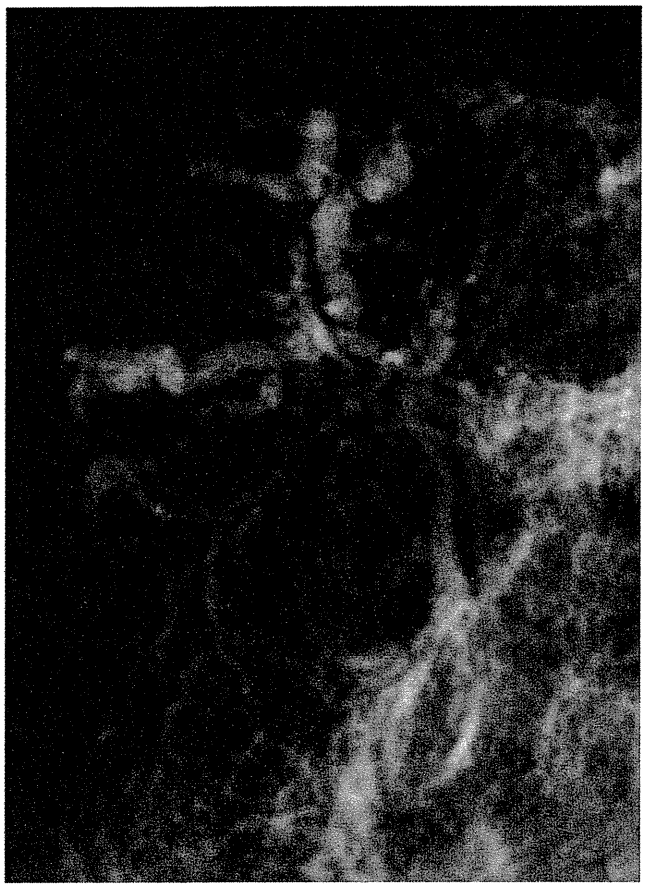


Fig. 1 Magnetic resonance cholangiopancreatography (MRCP) from patient 6 (5.9 years after LT) demonstrates irregular narrowing and dilatation of intrahepatic and extrahepatic bile ducts, compatible with recurrence of PSC.

LDLT was found at a mean of 3.4 years (range, 1.2-5.9 years). Cholangiographically, all patients had multiple strictures involving both the extrahepatic and intrahepatic bile ducts (Fig. 1). Nine required retransplantation, whereas 4 were alive after a mean follow-up period of 7.5 years (range, 1.7-11 years) post-LDLT with 2 progressing to cirrhosis and waiting for retransplantation.

For 9 retransplanted patients, the mean time from PSC diagnosis to primary LDLT was 7.7 years (range, 1.2-20 years), whereas the mean time interval between the diagnosis of PSC recurrence and retransplantation was 3.2 years (range, 0.7-7.7 years) ($P < .05$). One patient died 15 days after retransplantation because of sepsis. The remaining 8 patients were alive, and the mean follow-up after retransplantation was 4.4 years (range, 1.6-9 years). Two patients (cases 2 and 7) had a history of hepatic vein stenosis after LDLT, but the stenosis had resolved at the time of diagnosis of PSC recurrence.

3.2. Laboratory findings and liver histology

Laboratory data at the time of diagnosis of PSC recurrence for 13 patients are summarized in Table 2. The mean data were as follows: AST (97 ± 51 IU/L), ALT (104 ± 61 IU/L), ALP (1445 ± 675 IU/L), GGT (376 ± 273 IU/L), T-Bil (1.7 ± 0.9 mg/dL), and IgG (2530 ± 932 mg/dL). Autoantibodies were detectable in 5 patients (antinuclear antibodies, 4 cases; anti–smooth muscle antibody, one case).

Liver biopsies after primary LDLT were available in 12 cases at the time of PSC recurrence. No biopsy after LDLT was available in one (case 7). Among them, 3 cases had features of active hepatitis in addition to fibrous cholangitis consistent with PSC (cases 1, 2, and 4) (Fig. 2A and B), and

Table 2 Laboratory findings at the time of recurrence of PSC and treatment

Patient no.	PSC recurrence after LT (y)	AST	ALT	ALP	GGT	T-Bil	IgG	Autoantibody	Histologic overlap hepatitis (>moderate degree)	AIH score	Treatment
1	3.3	200	228	2980	845	2.1	4492	Sm+	+	13	FK, PSL
2	2.1	166	216	1097	292	2.8	2483	ANA (×160)	+	8	FK, PSL
3	1.9 (pure PSC)	74	88	1557	269	1.5					FK, MZR
3[b]	8.4 (overlap AIH)	81	66	827	293	2.5	1716	ANA+(53.7)[a]	+	11	FK, MZR, PSL
4	5.2	52	69	1388	263	1.1	2677	ANA (×40)	+	10	FK, MMF, PSL
5	5.3	77	72	1656	132	2.3	3498	–	–	8	FK, MMF, PSL
6	4	73	104	740	249	2	1686	ANA (×160)	–	5	FK, MMF, PSL
7	1.7	NA	NA	NA	NA	NA	NA	NA	+	NA	FK, MMF, PSL
8	3 (pure PSC), 5.6 (overlap AIH)	NA	NA	NA	NA	NA	NA	NA	+	NA	NA
9	2.2	163	130	2368	938	3.2	1425	–	–	4	FK, PSL
10	5.5	90	57	1373	441	0.7	1960	–	–	5	FK
11	5.6	53	63	1323	196	0.7	2837	–	–	6	FK, PSL
12	2.4	45	34	695	21	0.5	NA	NA	–	NA	FK
13	1.2	73	83	713	495	2.1	NA	NA	–	NA	FK, PSL, MMF

ANA indicates antinuclear antibody; FK, tacrolimus; PSL, prednisolone; MMF, mycophenolate mofetil; MZR, mizoribine; Sm, anti–smooth muscle antibody; NA, not available.

[a] Enzyme-linked immunosorbent assay.

[b] Patient 3 at the diagnosis of overlap hepatitis with PSC.

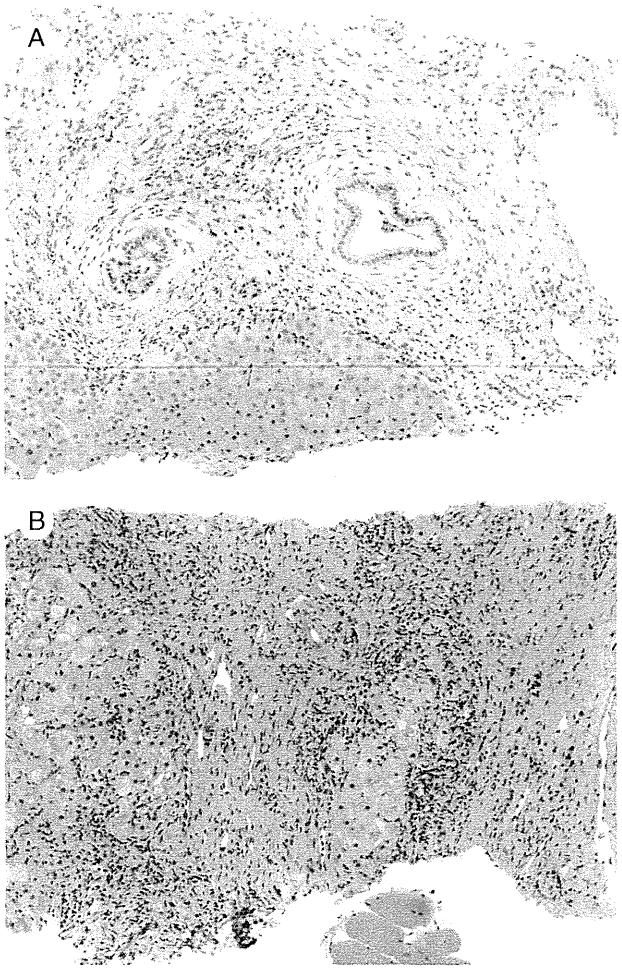


Fig. 2 Biopsy liver of recurrent PSC after LDLT. A, Posttransplant liver biopsy 5.9 years posttransplant from patient 3. Small bile ducts show an onion-skin appearance, compatible with recurrence of PSC. Interface is focally disrupted with mild lymphocytic infiltration. HE. B, Posttransplant liver biopsy 8.4 years posttransplant from patient 3. Dense lymphoplasmacytic infiltrate in fibrous septa and interface hepatitis is also evident. Well-formed interlobular bile ducts are not discernible. HE.

they satisfied the criteria for a probable diagnosis of AIH using the IAHG scoring system (Table 2). In the remaining 9 cases, periductal fibrosis and variable biliary fibrosis were found, but interface and lobular hepatitis were not significant at the time of PSC recurrence. Two of these 9 cases developed additional histologies of active hepatitis at 6.5 years and 2.6 years after the diagnosis of pure PSC recurrence, respectively (cases 3 and 8). In the other case, active hepatitis was found in the explanted liver at the time of retransplantation (case 7). In summary, 6 of 9 patients who required retransplantation developed active hepatitis features, whereas none of the 4 nonretransplanted patients showed active hepatitis ($P < .05$).

Before primary LDLT, PSC was diagnosed 7 years after the diagnosis of AIH in case 8 in whom the native liver was not available, and none of the remaining patients had evidence of AIH/PSC overlap syndrome.

3.3. Histologic features of recurrent PSC livers in comparison with native PSC livers

Histologic assessment of the explanted livers both at the time of primary LDLT (native livers) and retransplantation (explanted grafts) for 9 retransplanted patients with PSC recurrence are shown in Table 3.

The native livers were available in 7 of the 9 patients, and they showed biliary cirrhosis with prominent cholestasis and ductular proliferation, consistent with end-stage PSC. In all livers, there was extensive bile duct loss with frequent fibrous core (Fig. 3A), and the remaining bile ducts frequently showed periductal fibrosis (onion-skin fibrosis) with variable epithelial damage. These changes were found from intrahepatic large bile ducts to small bile ducts. The lymphoplasmacytic inflammation involving the hilar bile ducts and intrahepatic bile ducts were variable: moderate in 3 cases, mild in 3 cases, and almost absent in the remaining cases. Biliary sludges were also deposited in such affected bile ducts (Fig. 3B). Xanthogranulomatous changes were not evident in all livers. In addition, parenchymal necroinflammation with interface hepatitis was mild in 3 cases and almost absent in the remaining 4 cases.

Explanted livers were available in 8 of 9 patients. One underwent retransplantation at another hospital, and a failed

Table 3 Histologic scores in native livers and in liver allografts from patients with recurrence of PSC

Patient no.	Native liver							Graft							
	1	2	3	4	5	6	9	1	2	3	4	5	6	7	8
Lymphoplasmacytic inflammation of hilar and intrahepatic large bile ducts	2	2	1	2	0	1	1	2	2	3	1	3	2	2	3
Hilar xanthogranulomatous inflammation of hilar and intrahepatic large bile ducts	0	0	0	0	0	0	0	0	0	3	0	3	2	0	3
Ductopenia	3	2	1	3	3	3	3	1	1	1	2	1	1	1	1
Bile ductular proliferation	2	2	2	3	3	3	2	1	1	2	2	3	1	3	1
Cholestatis	1	2	1	2	3	3	2	3	3	1	3	3	3	2	3
Fibrous cholangitis including periductal fibrosis	1	3	3	2	1	2	3	3	2	3	3	3	1	2	3
Liver fibrosis	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Parenchymal necroinflammation with interface hepatitis	0	0	1	1	0	1	0	3	2	3	3	1	1	2	2
Submassive to massive necrosis	0	0	0	0	0	0	0	yes	0	0	0	0	0	0	0

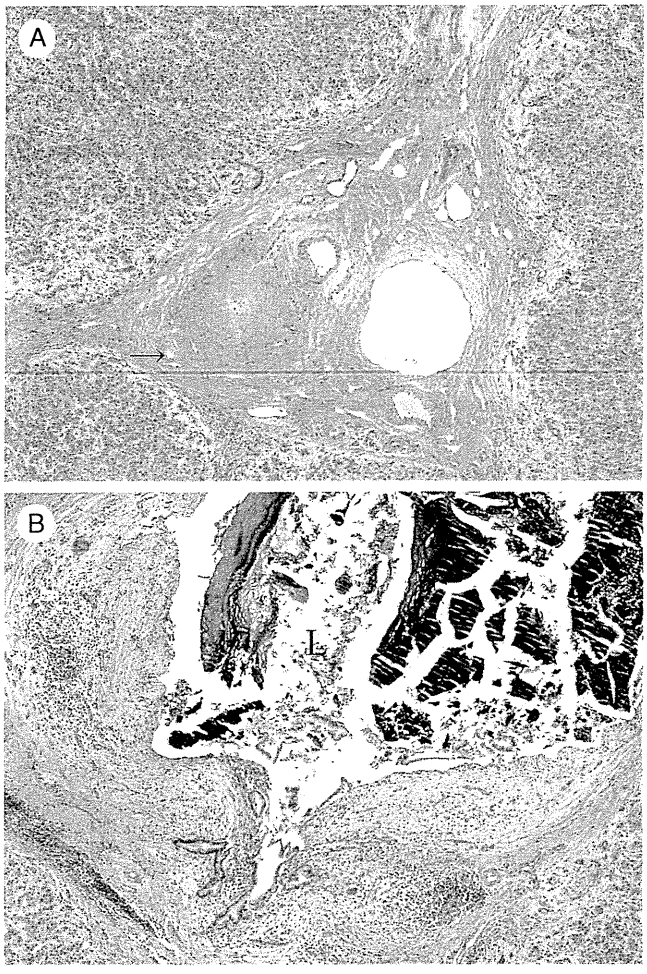


Fig. 3 Native liver from patient 2. A, A septal bile duct (arrow) shows fibro-obliterative lesions replaced by a fibrous scar with minimal inflammation. HE. The liver parenchyma shows advanced biliary cirrhosis. B, A large intrahepatic hilar duct shows mild to moderate lymphoplasmacytic inflammation and fibrosis with microstone formation in the duct lumen (L). HE.

allograft was not available (case 9). All explanted grafts showed biliary cirrhosis with prominent cholestasis and ductular proliferation. The fibrous core lesion was not common in comparison with native livers. Bile duct loss was mild in 7 cases and moderate in the remaining one. The remaining bile ducts consistently showed fibrous cholangitis (onion-skin fibrosis) (Fig. 4A). Hilar bile ducts of all cases showed lymphoplasmacytic cholangitis: 3 cases were marked (Fig. 4B and C), 4 cases were moderate, and the remaining one was mild. Interestingly, xanthogranulomatous cholangitis was evident in 4 cases (Fig. 4D and E): 3 cases showed marked changes and one case, moderate changes. Lobular necroinflammation with dense lymphoplasmacytic infiltration (active hepatitis) was evident (>moderate degree) in 6 explanted grafts (Fig. 4F). One of these 6 cases showed submassive to massive necrosis (case 1), and the patient deteriorated rapidly resulting in retransplantation 0.7 years after a diagnosis of PSC recurrence (Fig. 4G). Vascular complications were not detected.

The hilar hepatic artery did not show foam cell arteriopathy or vascular sclerosis associated with long-term rejection.

3.4. Immunosuppressive therapy

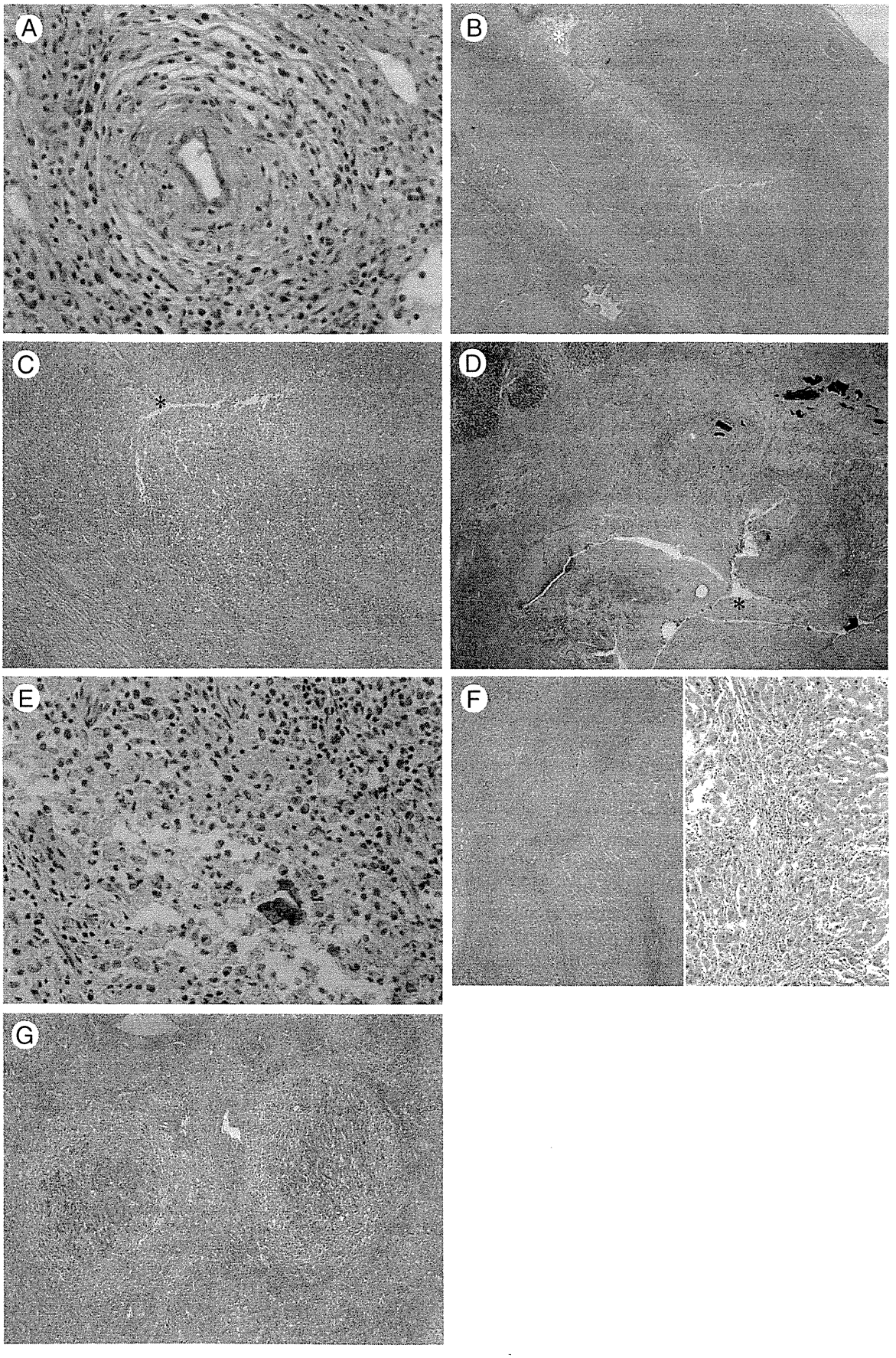
Triple immunosuppression was used at the diagnosis of PSC recurrence in 5 of 13 patients, and the other 4 were treated with prednisolone and tacrolimus. In one, prednisolone was added when overlap of active hepatitis developed in addition to the PSC features. Two were on FK monotherapy. Despite biochemical improvement with immunosuppressive therapy, 11 patients eventually developed liver cirrhosis, followed by retransplantation in 9 patients.

4. Discussion

It was found in this study that the degree of ductopenia reflecting fibro-obliterative cholangitis was relatively mild in recurrent PSC after LDLT in comparison with native livers. Instead, 6 of 13 patients with recurrent PSC had additional features of moderate to marked active hepatitis. It was also found in this study that recurrent PSC post-LDLT course was more rapid than the clinical course of PSC before LDLT. Recently, Luth et al [11] reported that, in AIH/PSC overlapping syndrome, ductopenia was relatively mild and necroinflammation of the hepatic parenchyma was prominent in comparison with pure PSC without active hepatitis, suggesting that recurrent PSC after LDLT has a tendency to develop AIH/PSC more than pure PSC without active hepatitis and also implicating that frequent overlap of active hepatitis related to AIH may explain the relatively deteriorated and rapid course for patients with PSC recurrence after LDLT. Interestingly, one case of recurrent PSC with active hepatitis underwent retransplantation due to submassive to massive hepatic necrosis, probably reflecting an overlap of severe AIH.

The overlap of AIH in PSC is more commonly seen in children than in adults [7]. Interestingly, in this study, 9 of 13 recurrent patients were female and 3 patients were younger

Fig. 4 Explant liver after recurrence of PSC after LDLT. A, Explanted liver allograft from patient 4. An interlobular bile duct shows a fibro-obliterative duct lesion with an onion-skin appearance. HE. B, A large intrahepatic large bile duct shows marked lymphoplasmacytic inflammation and ulceration of biliary epithelium (patient 5). HE. C, Higher magnification of B. The bile duct lumen (*) is *collapsed*. HE. D, Hilar bile duct shows marked thickening of the duct wall and inflammation. The bile duct lumen (*) is stenotic. Xanthogranulomatous cholangitis. Azan-Mallory stain. E, Higher magnification of D. Xanthogranulomatous changes with bile fragment. HE. F, Marked lymphoplasmacytic infiltration with interface hepatitis is evident in biliary type cirrhosis (left, lower magnification; right, higher magnification). HE. G, Cirrhotic nodules with an irregular interface infiltrated by lymphocytes with submassive hepatocyte necrosis (patient 1). HE.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than 20 years old, whereas 4 patients were between 20 and 30 years old at the time of the first liver transplantation. The female predominance and the rather younger age of our cases may be one of the factors for the frequent overlap of active hepatitis in recurrent PSC, and similar features are also known in AIH [14]. All but one patient with PSC showed pure PSC with no histologic or serologic evidence of AIH in native livers, suggesting that patients with recurrent PSC with active hepatitis were newly experiencing PSC/AIH overlap syndrome after LDLT. Only one patient in our study showed features of AIH and then developed bile duct abnormalities diagnostic of PSC on cholangiography over a 7-year period in native liver.

When the IAHG system for the diagnosis of AIH was applied, 3 of 6 cases who showed histologically active hepatitis were evaluated as "probably" AIH and none is "definite" AIH in this study. It is reported that the IAHG system for the diagnosis of AIH does not work well for post-LT AIH,[8] this may be responsible for this apparently low evaluation of AIH in this study.

The present study showed that lymphoplasmacytic cholangitis of the hilar bile ducts of recurrent PSC was rather prominent in comparison with native PSC livers. This is compatible with the above finding that necroinflammation of the hepatic parenchyma and interface is stronger in recurrent PSC than in native PSC livers. In native PSC livers, as the disease advances, the inflammation tends to subside, leaving a combination of portal-septal fibrosis and cholestasis.[12] This suggests that the disease progression in recurrent PSC after LDLT may be different from native PSC. Another possibility is that post-LDLT retransplantation may have been performed earlier in the disease course, so recurrent PSC may have more inflammation.

Xanthogranulomatous cholangitis was evident in 4 of 8 recurrent PSC livers at retransplantation, although such changes were absent in native livers. Keaveny et al [13] reported that 16 of 51 native PSC livers showed xanthogranulomatous cholangitis at LT, and PSC cases with xanthogranulomatous cholangitis were associated with a higher rate of early post-LT mortality or retransplantation. These findings suggest that the frequent occurrence of xanthogranulomatous cholangitis in recurrent PSC after LDLT may be partly responsible for the rather rapid progression of recurrent PSC in comparison with native PSC. Although the mechanisms of xanthogranulomatous cholangitis in recurrent PSC remain speculative, Roux loop reconstruction and superimposed biliary infection might have played some role in the formation of xanthogranulomatous cholangitis.

Recent Japanese experience showed that in LDLT, PSC recurrence and resultant graft loss occurred with an incidence of nearly 50% [4,5]. Blood-relative donors can be at risk for recurrent autoimmune disease. The recurrence rate among patients with PSC in our study was higher in those with blood-related donors, although not statistically significant. Interestingly, it was found in this study that frequent overlap of active hepatitis and frequent occurrence of xanthogranulomatous cholangitis of the hilar bile duct may be responsible for the rapid progression and deteriorating course of recurrent PSC post LDLT. This raises the possibility that steroids or other immunosuppressive drugs may be beneficial for patients with recurrent PSC with overlap hepatitis post LDLT. However, much care must be taken in drawing any therapeutic conclusions. However, this study was a retrospective study with a small number of cases, so a large and preferably prospective trial is necessary to evaluate the efficacy of treatment and histologic features of recurrent PSC after LT.

In conclusion, although native liver explants of PSC showed features of extensive ductopenia and more biliary cirrhosis, the explanted grafts of recurrent PSC after LDLT additionally showed features of active hepatitis consistent with overlap of AIH. This overlap appears to relate to a more rapid deterioration of recurrent PSC after LDLT.

References

[1] Angulo P, Lindor KD. Primary sclerosing cholangitis. Hepatology 1999;30:325-32.

[2] Graziadei IW, Wiesner RH, Batts KP, et al. Recurrence of primary sclerosing cholangitis following liver transplantation. Hepatology 1999;29:1050-6.

[3] Graziadei IW, Wiesner RH, Marotta PJ, et al. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. Hepatology 1999;30:1121-7.

[4] Haga H, Miyagawa-Hayashino A, Taira K, et al. Histological recurrence of autoimmune liver diseases after living-donor liver transplantation. Hepatol Res 2007;37(Suppl 3):S463-9.

[5] Egawa H, Taira K, Teramukai S, et al. Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation: a single center experience. Dig Dis Sci 2009;54:1347-54.

[6] Demetris AJ. Distinguishing between recurrent primary sclerosing cholangitis and chronic rejection. Liver Transpl 2006;12:S68-72.

[7] Abdalian R, Dhar P, Jhaveri K, Haider M, Guindi M, Heathcote EJ. Prevalence of sclerosing cholangitis in adults with autoimmune hepatitis: evaluating the role of routine magnetic resonance imaging. Hepatology 2008;47:949-57.

[8] Khettry U, Keaveny A, Goldar-Najafi A, et al. Liver transplantation for primary sclerosing cholangitis: a long-term clinicopathologic study. Hum Pathol 2003;34:1127-36.

[9] Nakanuma Y, Hoso M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. Microsc Res Tech 1997;38:552-70.

[10] 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.

[11] Luth S, Kanzler S, Frenzel C, et al. Characteristics and long-term prognosis of the autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. J Clin Gastroenterol 2009;43:75-80.

[12] Portmann B, Nakanuma Y. Diseases of the bile ducts. in: Burt AD, Portman BC, Ferrell LD, editors. Pathology of the Liver. 5th ed. New York: Churchill Livingstone; 2006. p. 517-81.

[13] Keaveny AP, Gordon FD, Goldar-Najafi A, et al. Native liver xanthogranulomatous cholangiopathy in primary sclerosing cholangitis: impact on posttransplant outcome. Liver Transpl 2004;10: 115-22.

[14] Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929-38.

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Risk Factors for Recurrence of Primary Sclerosing Cholangitis after Living Donor Liver Transplantation in Japanese Registry

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The outcomes of primary sclerosing cholangitis (PSC) after living donor liver transplantation (LDLT) in a large series have not been reported. We aimed to determine long-term patient and graft survival, risk factors for PSC recurrence, and the significance of recurrence after LDLT in a Japanese registry. Questionnaires concerning patient characteristics, treatments, and clinical courses were used. Data of 114 patients undergoing primary LDLT for PSC from July 1996 to December 2008 in 29 institutions were evaluated. For strict diagnoses of recurrence, patients with hepatic artery thrombosis (n = 8), ABO-blood-type-incompatible transplantation (n = 8), and established ductopenic rejection (n = 2) were excluded and 96 patients were analyzed for risk factors. Recurrence was diagnosed in 26 patients (27%) at 8 to 79 months after transplantation. Patient, graft, and recurrence-free survivals were 78, 74 and 57% at 5 years after LDLT, respectively. The graft loss rate was 69 versus 23% in patients with versus without recurrence, respectively. Multivariate analysis revealed that high MELD scores, first-degree-relative donors, postoperative CMV infection, and early biliary anastomotic complications were significant risk factors for recurrence. PSC recurrence was a significant risk factor of graft loss but not patient death. PSC recurrence was frequent and had significant impacts on outcomes after LDLT.

Key words: biliary complication, cytomegalovirus (CMV), graft loss, living donor transplantation, MELD score, primary sclerosing cholangitis, recurrent disease, related donation

Abbreviations: PSC, primary sclerosing cholangitis; LDLT, living donor liver transplantation; ALP, alkaline phosphatase; r-GTP, gamma-glutamyl transferase; CMV, cytomegalovirus; MELD, model for end-stage liver disease; HR, hazard ratio; CI, confidence interval; CNI, CALCINEURIN inhibitors.

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Introduction

Primary sclerosing cholangitis (PSC) recurs in 11–37% of patients within 5 years after liver transplantation (LT), and a handful of studies have tried to identify risk factors for recurrence after LT (1–8). Gordon and Gautman failed to analyze risk factors for recurrence in their meta-analysis (9, 10). Both Gordon and Demetris mentioned that living donor liver transplantation (LDLT) could be a risk factor for recurrence, but there have been no large-scale studies (9, 11). Tokyo and Kyoto groups reported their single center experiences showing higher incidences of recurrence (4 in 9 and 11 in 20 patients, respectively) after LDLT in Japanese populations (12,13). Interestingly, a related donor, especially parents, was one of the independent risk factors for recurrence in the Kyoto series (13).

Berquist reported that first-degree relatives and siblings are associated with a PSC prevalence approximately 100-fold that of nonrelatives (14). As easily imagined, the chance of organ donation by first-degree relatives is very high in LDLT, and it could be a reason for the frequent recurrence. To confirm this hypothesis in a multicenter study group, we collected data on patients undergoing LDLT for PSC in a Japanese registry organized by the Japanese Liver Transplantation Society.

Emphasis was placed on examining long-term patient and graft survival, and the incidence and outcome of recurrence of PSC (rPSC) after LDLT in a Japanese population. Risk factors for recurrence and graft loss were also evaluated.

Patients and Methods

Questionnaires were sent to 32 institutions in which a total of 139 LTs for PSC were registered with the Japan Liver Transplant Society up to December 2008. The questionnaires referred to patient characteristics, treatments, and clinical courses. Patient characteristics included disease, age, sex, blood types, HLA of the recipient and donor, the model of end-stage liver disease (MELD) score, new Mayo risk score (15), inflammatory bowel disease, cholangiocarcinoma, and relationship to the donor. Treatment data included graft type, manner of biliary reconstruction, number of biliary anastomoses, immunosuppressants in the initiation phase and at one year, and steroid pulse treatment for acute cellular rejection (ACR). Clinical courses included hepatic artery complications, cytomegalovirus (CMV) antigenemia, CMV diseases, numbers of ACR and steroid-resistant ACR, biliary anastomotic complications, PSC recurrence, graft loss, and survival. Data on mortality and causes of death were collected.

This retrospective multicenter study was approved by the Ethics Committee of Kyoto University Hospital as the site of data collection and analysis, according to the Declaration of Helsinki of 1975 as revised in 1996.

Patients

Data on 132 patients at 29 centers were collected. Eleven patients with second transplantations, four patients with domino transplantations, two patients with deceased donor LTs and one patient with a disease other than PSC were excluded (Figure 1). One hundred and fourteen patients (58 males and 56 females) underwent primary standard LDLT for PSC from July 1996 to December 2008 (Figure 1). Ages ranged from 1 to 66 years, with a median of 31 years. Fifty-three patients also had ulcerative colitis and four had cholangiocarcinoma. The MELD score ranged from 6 to 36, with a median of 19 in the 106 adult patients. The new Mayo score for PSC ranged from −0.7355 to 5.099, with a median of 2.7092. The follow-up period ranged from 1 to 153 months, with a median length of 42 months.

Donor selection and graft type (114 patients)

The donors were parents in 54 cases, siblings in 27, sons (no daughters) in 13, spouses in 19 and an uncle in 1 case. Ages ranged from 19 to 67 years, with a median age of 45 years. The patients were male in 64 and female in 50 cases. The blood-type combination was incompatible in eight cases.

Surgery and immunosuppression regimen

The graft type was the right lobe in 52 cases, the left lobe or lateral segments in 59, the right lateral sector graft in 2 and unknown in 1 case. The manner of biliary reconstruction was hepatico-jejunostomy in 101 cases, duct-to-duct in 12 cases and unknown in 1 case.

Initial immunosuppression protocols were tacrolimus and steroid in 82, cyclosporine and steroid in 5, tacrolimus, steroid and mycophenolate mofetil (MMF) in 15, cyclosporine, steroid, and MMF in 1 and others in 11 patients, including anti-IL2 receptor antibody in 3, OKT-3 in 2 and deoxyspagarine in 1. Immunosuppression therapy was not altered because of PSC.

Pathological diagnoses, such as ACR or chronic rejection, were made according to Banff criteria (16). When ACR was confirmed, patients were treated with a high-dose corticosteroid. Steroid-resistant rejection was treated with lymphocyte antibodies.

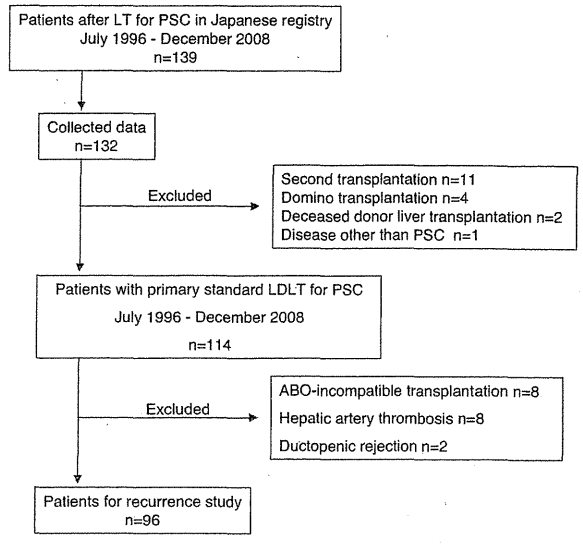

Figure 1. Patient enrolment. Questionnaires were sent to 32 institutions in which a total of 139 liver transplantations for PSC were registered with the Japan Liver Transplant Society up to December 2008. Data on 132 patients at 29 centers were collected. Eleven patients with second transplantations, four patients with domino transplantations, two patients with deceased donor liver transplantations and one patient with a disease other than PSC were excluded. One hundred and fourteen patients underwent primary standard LDLT for PSC from July 1996 to December 2008. Eight patients with ABO-incompatible transplantation, eight patients with hepatic artery thrombosis and two patients with ductopenic rejection were excluded from analysis for recurrence according to Graziadei.

Definition of recurrence

If indicated by abnormal liver tests including the cholestatic biochemical profile, further investigations were carried out with biopsies and/or cholangiography by percutaneous transhepatic cholangiography, endoscopic retrograde cholangiography or magnetic resonance cholangiography which recently became a standard.

PSC recurrence was strictly defined using both positive and negative criteria according to Graziadei in all centers participating in this study (17). Criteria included a confirmed diagnosis of PSC before transplantation and intrahepatic multiple biliary strictures confirmed by cholangiography occurring more than 90 days after transplantation, or biopsy findings showing fibrous cholangitis and/or fibro-obliterative lesions.

Exclusion criteria included the presence of other causes of multiple biliary stricture (secondary sclerosing cholangitis) such as hepatic artery thrombosis or stenosis, ductopenic rejection, and ABO-blood-type incompatibility. When a biliary anastomotic complication was not treated successfully or a patient had no biliary-related-disease free period without any treatment until the development of biliary sclerosis, multiple nonanastomotic stricture was diagnosed as secondary sclerosing cholangitis.

Prophylaxis for viral infection

CMV serology prior to surgery was available in 87 donors and 97 recipients. CMV IgG was positive in 77 of 87 donors (88.5%) and in 77 of 97

recipients (79.4%). The combination of sero-negative recipients and sero-positive donors was observed in 13 of 95 cases (13.7%) in which data to evaluate the combinations were available. Ganciclovir was administered intravenously as a prophylaxis for CMV infection in 8 patients. The decision to conduct preemptive treatment according to CMV antigenemia was made by individual centers. CMV diseases were diagnosed when clinical manifestations concomitant with positive CMV antigenemia developed or positive findings, such as microabscess in the liver and inclusion body in the intestinal wall, were observed on pathological examination of biopsy specimens (18).

Tissue typing

Tissue typing was performed in patients and donors for HLA-A, HLA-B (Bw) and HLA-DR for class I and II loci according to Terasaki's methods in each institution or commercial laboratories.

Statistical analysis

Survival and time-to-recurrence curves were calculated using the Kaplan–Meier method. The log-rank test was used to evaluate the effects of characteristics on recurrence and graft loss. Graft loss was defined as death or retransplantation. Factors before and during surgery and initial immunosuppression were analyzed in all patients (n = 96). Then, landmark analyses were performed to evaluate the association between recurrence or graft loss and postoperative factors at 3 and 12 months after transplantation as well as patient characteristics (19). The postoperative factors within 3 months were analyzed in patients who survived 3 months or longer (n = 92), and postoperative factors within 12 months were analyzed in patients who survived 12 months or longer (n = 79). Multivariate Cox regression analysis with backward elimination was used to evaluate the association between recurrence or graft loss and patient characteristics, and estimate the hazard ratio (HR) and its 95% confidence interval (CI). To assess the effect of PSC recurrence on graft loss or death, we performed Cox regression analysis with recurrence as the time-dependent covariate. A p-value of 0.05 was used for variable selection and was regarded as significant. SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Overall outcome of total population of primary standard LDLT for PSC in Japanese registry

The 5-year patient-survival and graft-survival rates of the 114 patients were 74 and 70%, and 10-year patient-survival and graft-survival rates were 57 and 34%, respectively (Figure 2A). Seventeen patients died of hepatic failure (7 rPSC, 2 chronic rejection, 1 multiple hepatic infarction, 1 *de novo* autoimmune hepatitis, 1 ABO-incompatibility-related cholangitis, 1 chronic cholangitis, 4 unknown), eight died of infection, four of multiple organ failure, three of cancer (2 cholangiocarcinoma and 1 carotid gland cancer) and four of unknown causes. Sixteen patients underwent retransplantation. Thirteen underwent retransplantation due to rPSC (2 with rPSC clinically diagnosed were excluded from the subsequent recurrence study because of ABO incompatibility), one due to rejection and two due to unknown graft failure (2 were excluded from the subsequent recurrence study because of ABO incompatibility). CMV antigenemia was positive in 36 patients, and 14 had symptoms (CMV syndrome in 9, hepatitis in 2, peritonitis in 1, pleuritis in 1, colitis in 1).

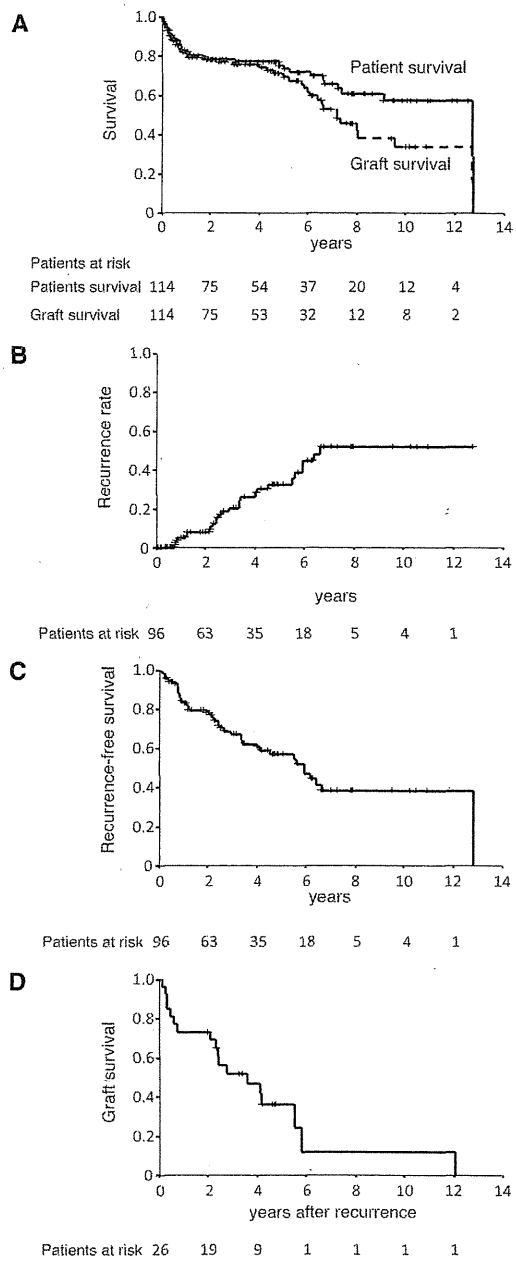

Figure 2. Outcomes. (A) Patient and graft survival after LDLT for PSC (n = 114). (B) Recurrence rate of PSC after LDLT (n = 96). (C) Recurrence-free survival after LDLT for PSC (n = 96). (D) Graft survival after PSC recurrence (n = 26).

Recurrence

Eight patients with ABO-incompatible transplantation, eight with hepatic artery thrombosis and two with ductopenic rejection were excluded from analysis for recurrence according to Graziadei (Figure 1) (17). In the 96 patients enrolled for rPSC analysis, 26 showed recurrence

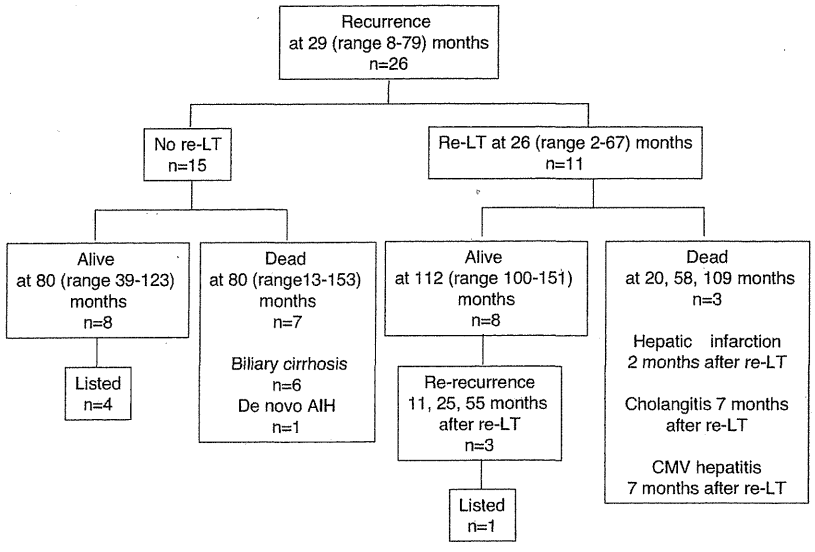

Figure 3. Outcomes of patients with PSC recurrence. Re-LT, liver retransplantation; listed = listed for next transplantation; AIH = autoimmune hepatitis and CMV = cytomegalovirus.

(27%). Patient and graft survival rate of the 96 patients were 88% and 87% at 1 year, 82% and 81% at 3 years, 78 and 74% at 5 years, 71 and 59% at 7 years and 61 and 41% at 10 years after LDLT, respectively. The diagnosis of recurrence was made by only pathological findings in 3, and by both pathological and radiological findings in 18 (endoscopic cholangiography in 2, magnetic resonance cholangiography in 8, percutaneous transhepatic cholangiography in 7, ultrasonography in 1). The diagnosis of recurrence was made by only radiological findings in five patients (endoscopic cholangiography in 1, magnetic resonance cholangiography in 1, percutaneous transhepatic cholangiography in 2, CAT scan cholangiography in 1). The time to recurrence ranged from 8 to 79 months after LDLT. The recurrence rates were 32% at 5 years and 52% at 10 years, as shown in Figure 2. Recurrence-free survival was 57% at 5 years and 38% at 10 years, as shown in Figure 2.

Hepatic chemistries at the diagnosis of recurrence were as follows (minimal–maximal and median values): gamma-glutamyl transferase (γ-GTP): 60 – 2558 and 348 IU/L, alkaline phosphatase (ALP): 317 – 2558 IU/L and 1048 IU/L, asparate aminotransferase (AST): 18–710 and 74 IU/L, and total bilirubin: 0.3–31 and 2.0 mg/dL. In comparison, hepatic chemistries of patients without rPSC diagnoses at the last follow-up were as follows: AST: 10–114 and 25 IU/L and total bilirubin: 0.9–2.30 and 1.1 mg/dL.

Figure 2 shows graft survival after recurrence. Graft survival was 54% at 3 years, 39% at 5 years and 15% at 7 years after recurrence. Figure 3 shows clinical courses after recurrences in the 26 patients. The interval from recurrence to retransplantation ranged from 2 to 67 months with a median of 26 months in 11 patients. Three of these died after retransplantation. Among eight patients that survived retransplantation, three patients developed rerecurrence and one of them was listed for a third transplantation. Among 15 patients without retransplantation, 7 patients died between 4 and 144 months after recurrence, with a median of 33 months, owing to biliary cirrhosis in 6 and autoimmune hepatitis in 1. Among another eight patients alive without retransplantation, four patients were listed for retransplantation because of biliary cirrhosis.

Risk factors for recurrence and graft loss

The characteristics of donors and recipients, HLA match, operative data, initial immunosuppression and clinical courses are shown in Table 1.

The ages of the 96 patients in this study ranged from 1 to 66 years, and the median age was 31 years. The patients were divided into three groups: those aged under 18 years, from 18 to 29 years and 30 years or older. The donors were divided into two groups: those aged under 39 years and 40 years or older. Donors consisted of 42 parents, 12 siblings, 25 sons, 1 uncle and 16 spouses. For analysis, patients were divided into unrelated versus related or first-degree-related donors versus others. HLA match was divided into three groups (no match, '0'; one match, '1'; two matches, '2'). In 44 patients with inflammatory bowel disease (IBD), four patients had undergone colon resection prior to LDLT. Four patients developed *de novo* IBD. Antinuclear antibody (ANA) was positive in 31, negative in 34 and unknown in 31 patients before transplantation. Seven patients had other autoimmune diseases, such as pancreatitis in four, hepatitis in two, and dermatitis.

In 92 patients followed for 3 months or longer, the impacts of CMV antigenemia within 3 months, CMV disease within 3 months, ACR within 3 months and usage of corticosteroid pulse for ACR within 3 months were analyzed.

Table 1: Prognostic factors for recurrence and graft loss: Univariate analysis (n = 96)

		Recurrence (n = 26)		Graft loss (n = 34)	
Characteristics	No. (%)	%	p-Value*	%	p-Value*
Age					
Under 18 years old	13 (14)	31	0.379	38	0.328
18–29 years old	26 (27)	42		54	
30 years old –	57 (59)	19		26	
Sex					
Female	48 (50)	31	0.833	33	0.415
Male	48 (50)	23		38	
MELD score					
–23	66 (75)	18	<0.001	24	0.013
24–	22 (25)	50		64	
Unknown	8				
New Mayo					
<1.3	23 (24)	22	0.181	35	0.679
<2.7	25 (26)	32		44	
<3.6	24 (25)	25		25	
3.6≤	24 (25)	29		38	
Inflammatory bowel disease					
Without	49 (53)	22	0.099	29	0.161
With	44 (47)	34		39	
Unknown	3				
Age (donor)					
–39 years old	37 (39)	22	0.293	35	0.948
40 years old–	58 (61)	31		36	
Unknown	1				
Sex (donor)					
Female	38 (40)	29	0.821	34	0.731
Male	58 (60)	26		36	
Gender mismatch					
Match	44 (46)	23	0.251	30	0.514
Mismatch	52 (54)	31		40	
Donor					
Unrelated	16 (17)	13	0.198	25	0.701
Related	80 (83)	30		38	
Donor					
Unrelated/related (sibling/uncle)	42 (44)	14	0.015	19	0.018
Related (parent/son)	54 (56)	37		48	
HLA-A matched number					
0	8 (9)	25	0.374	0	0.086
1	48 (56)	27		38	
2	30 (35)	30		37	
Unknown	10				
HLA-B matched number					
0	12 (14)	17	0.721	8	0.398
1	62 (72)	31		39	
2	12 (14)	25		33	
Unknown	10				
HLA-DR matched number					
0	15 (18)	7	0.033	20	0.157
1	53 (62)	32		36	
2	17 (20)	35		41	
Unknown	11				
Graft type					
Left lobe	50 (53)	26	0.678	32	0.182
Right lobe	43 (45)	28		40	
Right lateral	2 (2)	0		0	
Unknown	1				

Continued.

Table 1: Continued

Characteristics	No. (%)	Recurrence (n = 26) %	Recurrence (n = 26) p-Value*	Graft loss (n = 34) %	Graft loss (n = 34) p-Value*
Biliary reconstruction					
Choledocho-choledochostomy	10 (11)	40	0.176	50	0.315
Choledocho-jejunostomy	85 (89)	25		33	
Unknown	1				
Number of biliary anastomoses					
1	73 (80)	27	0.465	37	0.473
2 or more	18 (20)	22		22	
Unknown	5				
Immunosuppressants at initiation phase					
CNI	2 (2)	0	0.504	0	0.536
CNI and steroid	69 (73)	30		42	
CNI and steroid and antimetabolites (and antibody)	23 (24)	17		17	
Unknown	2				
Cholangiocarcinoma					
Without	92 (96)	27	0.723	35	0.525
With	4 (4)	25		50	
Number of ACR					
0	57 (59)	25	0.617	33	0.6342
1	35 (36)	31		40	
2	2 (2)	0		0	
3 or more	2 (2)	50		50	
Patients followed for longer than 3 months (n = 92)					
CMV antigenemia within 3 months					
Negative	66 (72)	23	0.012	27	0.405
Positive	26 (28)	42		46	
CMV disease within 3 months					
Without	80 (87)	25	0.041	29	0.131
With	12 (13)	50		58	
Acute rejection within 3 months					
Without	52 (57)	27	0.601	29	0.919
With	40 (43)	30		38	
Corticosteroid pulse for ACR within 3 months					
No	37 (46)	22	0.954	30	0.693
Once or more	44 (54)	32		32	
Unknown	11				
Patients followed for longer than 12 months (n = 79)					
Biliary anastomotic complications within 1 year					
Without	64 (81)	27	0.039	23	0.202
With	15 (19)	60		53	
Immunosuppressants at 1 year after transplant					
CNI or none	13 (17)	31	0.367	31	0.109
CNI and steroid or antimetabolites	46 (60)	41		37	
CNI and steroid and antimetabolites	18 (23)	17		0	
Unknown	2				
Corticosteroid at 1 year after transplant					
Without	23 (30)	36	0.649	35	0.143
With	54 (70)	34		24	
Unknown	2				
Antimetabolites at 1 year after transplant					
Without	50 (65)	38	0.498	34	0.404
With	27 (35)	26		15	
Unknown	2				

MELD = model for end-stage liver disease; HLA = human leukocyte antigen; CMV = cytomegalovirus; ACR = acute cellular rejection; CNI: calcineurin inhibitors.
*Log-rank test.

In 79 patients followed for 12 months or longer, impacts of biliary anastomotic complications within 1 year, immunosuppressants at 1 year and usage of corticosteroid and antimetabolites at 1 year were analyzed. The immunosuppression protocol one year after transplantation was merely calcineurin inhibitors (CNI) in 11, CNI and steroids in 36 and CNI and antimetabolites in 10, CNI, steroids and antimetabolites in 18. Two patients received no immunosuppression at 1 year. One patient received a graft from his identical twin sibling, and another was too sick for immunosuppression.

Analysis of risk factors for recurrence

A univariate log-rank test of risk factors for recurrence (Table 1) showed that high MELD scores ($p < 0.001$), first-degree-related donors ($p = 0.015$), HLA-DR match ($p = 0.033$), CMV antigenemia ($p = 0.012$) and CMV diseases ($p = 0.041$) within 3 months, as well as biliary anastomotic complications within 1 year ($p = 0.039$), were significantly associated with a greater incidence of recurrence after transplant. Incidences of recurrence were 43% (18/42), 16% (4/25), 17% (2/12), 0% (0/1) and 13% (2/16) when donors were parents, sons, siblings, an uncle and nonrelatives, respectively. Multivariate landmark analysis at 12 months showed that independent recurrence risk factors were MELD scores greater than 24 (HR, 3.16; 95% CI, 1.32–7.57; $p = 0.010$), first-degree-related donors (HR, 3.12; 95% CI, 1.14–8.53; $p = 0.026$), CMV antigenemia within 3 months (HR, 3.32; 95% CI, 1.44–7.68; $p = 0.005$) and biliary anastomotic complications within 1 year (HR, 4.19; 95% CI, 1.64–10.7; $p = 0.003$, Table 2).

Number of ACR (Table 1), early ACR and steroid-resistant ACR (data not shown) had no significant impact. Posttransplant IBD and its activity (data not shown), as well as pretransplant IBD, had no significant impact. Four patients undergoing colon resection prior to LDLT had no rPSC, but the number was small (8). One of four patients with de novo IBD had rPSC.

Analysis of risk factors for graft loss

The univariate log-rank test of risk factors for graft loss (Table 1) showed that high MELD scores ($p = 0.013$) and first-degree-related donors ($p = 0.018$) were significantly associated with a greater incidence of graft loss. Multivariate analysis showed that independent risk factors for graft loss were MELD scores greater than 24 (HR, 2.34; 95% CI, 1.12–4.88; $p = 0.024$) and first-degree-related donors (HR, 2.48; 95% CI, 1.05–5.85; $p = 0.038$), as shown in Table 3. Graft loss was 69% (18/26) versus 23% (16/70) in patients with versus without recurrence, respectively. Time-dependent covariate analysis showed a significant relationship between PSC recurrence and graft loss after MELD score and donor adjustments (HR, 8.34; 95% CI, 2.78–25.1; $p < 0.001$).

Table 2: Prognostic factors for recurrence: Multivariate analysis

Pretransplantation factors (n = 96)			
Factors	Hazard ratio	95%CI	p-Value
MELD score			
−23	1		
24−	3.69	1.61–8.46	0.002
Donor			
Unrelated/related (sibling/uncle)	1		
Related (parent/son)	2.61	1.02–6.71	0.046
Landmark analysis (3 months) for patients followed for longer than 3 months (n = 92)			
MELD score			
−23	1		
24−	4.04	1.73–9.46	0.001
Donor			
Unrelated/related (sibling/uncle)	1		
Related (parent/son)	2.35	0.91–6.06	0.076
CMV antigenemia within 3 months			
Negative	1		
Positive	2.78	1.25–6.20	0.012
Landmark analysis (12 months) for patients followed for longer than 12 months (n = 79)			
MELD score			
−23	1		
24−	3.16	1.32–7.57	0.010
Donor			
Unrelated/related (sibling/uncle)	1		
Related (parent/son)	3.12	1.14–8.53	0.026
CMV antigenemia within 3 months			
Negative	1		
Positive	3.32	1.44–7.68	0.005
Biliary anastomotic complications within 1 year			
Without	1		
With	4.19	1.64–10.7	0.003

CI = confidence interval; MELD = model for end-stage liver disease; MELD = model for end-stage liver disease; CMV = cytomegalovirus.

Among 26 patients with PSC recurrence, risks for graft loss were analyzed employing the log-rank test. First-degree-related donors and right lobe grafts were significant risks ($p = 0.033$, and 0.006, respectively) for the early development of graft loss after recurrence.

Analysis of prognostic factors for death

There were no significant prognostic factors for death. Time-dependent covariate analysis show no relationship between PSC recurrence and death after MELD score and donor adjustments ($p = 0.200$).

Cholangiocarcinoma

Four patients had cholangiocarcinoma at LDLT and two of them died due to cholangiocarcinoma within 1 year. A patient with an early cholangiocarcinoma diagnosed before transplantation survived. Another three patients

Table 3: Prognostic factors for graft loss: Multivariate analysis

Pretransplantation factors (n = 96)			
Factors	Hazard ratio	95%CI	p-Value
MELD score			
–23	1		
24–	2.34	1.12–4.88	0.024
Donor			
Unrelated/related (sibling/uncle)	1		
Related (parent/son)	2.48	1.05–5.85	0.038

MELD = model for end-stage liver disease.

had normal tumor markers and no abnormal findings on radiological examination prior to transplantation, and postoperative histological examination showed advanced cholangiocarcinoma with lymph node metastasis.

Discussion

Graziadei included hepatic artery thrombosis/stenosis, established chronic rejection, preservation injury and ABO incompatibility between donors and recipients in their exclusion criteria because they cause radiologically and/or histologically similar lesions to PSC recurrence (17). In Japan, similar phenomena after LDLT were reported and ischemic insult, immunological reaction or both were discussed for the etiology of sclerosing bile duct changes (13,20–22). Weismuller reported that PSC appears to be caused by a complex interaction between deregulated immune mechanisms in genetically predisposed persons and environmental factors like infectious agents, and that this multitude of possible pathogenetic processes should resolve the issue regarding whether PSC is perhaps just a common clinical end-stage syndrome of a number of similar diseases with different etiologies (23). This study showed that first-degree-related donors, high MELD scores, early CMV infections and early biliary anastomotic complications were significant independent risks for recurrence after LDLT in a Japanese population, strictly according to Graziadei's criteria (17). According to Weismuller, a parental donor was a genetically pathogenetic factor, and high MELD scores, early CMV infections and early biliary anastomotic complications were environmental factors after LDLT in a Japanese population.

Donor-relatedness is an issue specific to LDLT. We found that first-degree-relatives, especially parents, were the most risk-associated donors. The incidence of recurrence in recipients with grafts from related donors other than parents as well as nonrelated donors was similar to those reported for deceased donor LT (1–8,17).

HLA-DR8 was reported as one of the risk factors for recurrence in Caucasian populations (6,8). In this study, the incidence of HLA-DR8 in patients was similar at 16% (15 in 96 patients) to Tamura's series (18% 2/9)(12). Conversely, the incidences of HLA-DR15 were very high in PSC recipients in the Kyoto series (45%) and Tamura's series (44%) but only 30% in this study (12,13). The reported incidence of DR 15 was 14.8% in a Japanese population (24). Tamura reported that 3 of 4 patients with HLA-DR15 showed recurrence (75%). In the Kyoto series, a donor with HLA-DR15 was found to be a significant risk factor with the log-rank test, although not with Cox regression analysis. They separately analyzed the effects of HLA-DR1501 and HLA-DR1502 on recurrence, and found no significance (17). Since the quality of the data of HLA varied among centers in this study, we employed HLA typing with the level of serological analysis, namely a two-digit number, to assess HLA matching. To clarify the significance of HLA in PSC recurrence, we need to reanalyze HLA of all patients.

CMV infection has been reported as a risk factor for biliary anastomotic complications, although the mechanism has not yet been clarified (20,22,25,26). The Kyoto group reported that ABO-blood-type incompatibility, hepatic artery complication, and CMV disease were significant risk factors for nonanastomotic biliary complications in pediatric LDLT (20). They mentioned that small artery occlusion caused by CMV might lead to biliary complications through ischemic insult, referring to a report of Melrick (27). Recently, Bolovan-Fritts reported a significant role of fractalkine in endothelial damage in CMV infection (28). Cytomegalovirus-mediated up-regulation of chemokine expression, including fractalkine, was correlated with the acceleration of chronic rejection in a rat heart transplantation model (29). Furthermore, Miyagawa reported a role of arteriopathy in chronic rejection even in LT (30). Altogether, CMV could provoke inflammation leading to biliary damage though ischemic insults or immune reaction activation. Valganciclovir, an oral prodrug of ganciclovir, is an attractive agent for both antiviral prophylaxis and the preemptive treatment of CMV viremia (31), which was approved in 2009 in Japan. Its contribution to the recurrence prevention is expected.

Regarding the effects of biliary anastomotic complications on rPSC, all these complications were treated successfully once, and there was no continuing or recurrent cholangitis between early anastomotic complications and late recurrence. The anastomotic complications could provoke inflammation along the proximal hepatic ducts, leading to ischemic insults or immune reaction activation. Again, one can conclude that efforts to reduce anastomotic complications could reduce PSC recurrence.

A MELD score greater than 24 was one of the independent recurrence risk. It is very difficult to explain the relationship between high MELD scores and PSC recurrence. Each MELD score parameter was compared between patients with scores under and over 24. Bilirubin (9.08 ± 0.84 vs. 21.9 ± 1.70 mg/dL; MELD < 24 vs. MELD > 24), INR (1.25 ± 0.06 vs. 2.43 ± 2.21; MELD < 24 vs. MELD > 24) and creatinine (0.55 ± 0.04 vs. 0.72 ± 0.07 mg/dL;

MELD < 24 vs. MELD > 24) were significantly ($p < 0.0001$, $p < 0.0001$ and $p = 0.079$, respectively) greater in patients with MELD scores greater than 24 than under 24. The major factors contributing to high MELD scores were hepatic dysfunction. Patients with long-term PSC might have had an immunologic potential to develop recurrence, or immune systems exposed to markedly damaged livers might realize immunologic potential. The duration from transplantation to recurrence became shorter in the second than in the first grafts (17). One can conclude that once patients with PSC begin to exhibit deteriorated hepatic functions, earlier referrals to transplant centers might be recommended.

Cholangiocarcinoma in the remnant bile duct is a concern, although duct-to-duct anastomosis in LT for patients with PSC was not a recurrence risk. The UCLA group undergoes choledochojejunostomy when inflammation is present in frozen-section biopsy specimens of the common bile duct (32). In our series, four patients (3%) had cholangiocarcinoma. Intraoperative consultation had not been performed to evaluate the presence of cholangiocarcino in three patients. Intraoperative histological examination for cholangiocarcinoma in frozen-section biopsy specimens of the common bile duct is mandatory in transplantation for PSC.

The immunosuppressants one year after transplantation had no significant impact on recurrence, although triple therapy seemed effective (8). This suggests that the current immunosuppressive regimen available in Japan cannot prevent PSC recurrence. B-cell-targeted treatment for recurrence has not yet been performed.

PSC recurrence was a significant risk for graft loss, as well as MELD scores and first-degree-related donors. In particular, there was a significant relationship between recurrence and graft loss even after MELD score and donor adjustments in this study, although Goss reported that PSC recurrence had no effect on patient or graft survival in deceased donor LT (32). Interestingly, first-degree-related donors and right lobe grafts were significant risks for the rapid development of graft loss after recurrence. A possible mechanism contributing to the effect of first-degree-related donors was discussed above, but it is difficult to discuss the role of right lobe grafts. We might need to study more cases to clarify this. On the other hand, recurrence was not a significant risk factor for patient survival. The reason for this is possibly related to retransplantation for patients with recurrence. Hence, to improve outcomes of LDLT for PSC, the prevention of recurrence is the most important.

We could not collect further data such as severity (mild/moderate, severe) of ACR, the duration of steroids after ACR, extent of IBD, duration before LT, courses and duration of steroid therapy for IBD before LT. This is a limitation of the study. A protocol biopsy and magnetic resonance cholangiography could disclose early pathology of rPSC in future.

In conclusion, LT using a graft from a first-degree-related donor, especially a parent, should be avoided. Earlier referral to transplant centers, CMV prophylaxis and efforts to decrease biliary anastomotic complications are required to reduce rPSC.

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Authors Contributions

Hiroto Egawa designed this study and wrote the entire paper. Yoshihide Ueda and Takafumi Ichida collected data and discussed with Hiroto Egawa, Satoshi Teramukai performed statistical analysis, and Yasuni Nakanuma, Saburo Onishi and Hirohito Tsubouchi made critical revisions of the manuscript for important intellectual content.

Disclosure

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

References

1. Vera A, Moledina S, Gunson B et al. Risk factors for recurrence of primary sclerosing cholangitis of liver allograft. Lancet 2002; 360: 1943–1944.

2. Khettry U, Keaveny A, Goldar-Najafi A et al. Liver transplantation for primary sclerosing cholangitis: A long-term clinico-pathologic study. Mum Pathol 2003; 34: 1127–1136.
3. Kugelmas M, Spiegelman P, Osgood MJ et al. Different immunosuppressive regimens and recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl 2003; 9: 727–732.
4. Brandsaeter B, Schrumpf E, Bentdal O et al. Recurrent primary sclerosing cholangitis after liver transplantation: A magnetic resonance cholangiography study with analyses of predictive factors. Liver Transpl 2005; 11: 1361–1369.
5. Cholongitas E, Shusang V, Papatheodoridis GV et al. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl 2008; 14: 138–143.
6. Alexander J, Lord JD, Yeh MM et al. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl 2008; 14: 245–251.
7. Campsen J, Zimmerman MA, Trotter JF et al. Clinically recurrent primary sclerosing cholangitis following liver transplantation: A time course. Liver Transpl 2008; 14: 181–185.
8. Alabraba E, Nightingale P, Gunson B et al. A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. Liver Transpl 2009; 15: 330–340.
9. Gordon F. Recurrent primary sclerosing cholangitis: Clinical diagnosis and long-term management issues. Liver Transpl 2006; 12: S73–S75.
10. Gautam M, Cheruvattath R, Balan V. Recurrence of autoimmune liver disease after liver transplantation: A systemic review. Liver Transpl 2006; 12: 1813–1824.
11. Demetris AJ. Distinguishing between recurrent primary sclerosing cholangitis and chronic rejection. Liver Transpl 2006; 12: S68–S72.
12. Tamura S, Sugawara Y, Kaneko J et al. Recurrence of primary sclerosing cholangitis after living donor liver transplantation. Liver Int 2007; 27: 86–94.
13. Egawa H, Taira K, Teramukai S et al. Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation: A single center experience. Dig Dis Sci 2009 Jun; 54: 1347–1354.
14. Berquist A, Lindberg G, Saarinen S et al. Increased prevalence of primary sclerosing cholangitis among first-degree relatives. J Hepatol 2005; 42: 252–256.
15. Kim WR, Therneau TM, Wiesner RH, et al. A revised natural history model for primary sclerosing cholangitis. Mayo Clin Proc 2000 Jul; 75: 688–694.
16. Banff Working Group;Demetris AJ, Adeyi O, Bellamy CO et al. Liver biopsy interpretation for causes of late liver allograft dysfunction. Hepatology 2006; 44: 489–501.
17. Graziadei IW, Wiesner RH, Marotta PJ et al. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. Hepatology 1999; 30: 1121–1127.
18. Ljungman P, Griffiths P, Paya C. Definiions of Cytomegalovirus infection and disease in transplant recipients. Immunocomprom Hosts 2002; 34: 1094–1097.
19. Anderson JR, Cain KC, Gelber RD. Analysis of survival by tumor response. J Clin Oncol 1983; 1: 710–719.
20. Egawa H, Uemoto S, Inomata Y et al. Biliary complications in pediatric living related liver transplantation. Surgery 1998; 124: 901–910.
21. Egawa H, Oike F, Buhler L et al. Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. Transplantation 2004; 77: 403–411.
22. Kasahara M, Egawa H, Takada Y et al. Biliary reconstruction in right lobe living-donor liver transplantation: Comparison of different techniques in 321 recipients. Ann Surg 2006; 243: 559–566.
23. Weismuller TJ, Wedemeyer J, Kubicka S et al. The challenges in primary sclerosing cholangitis—Aetiopathogenesis, antoimmunity, managemant and malignancy. J Hepatol 2008; 48: 38–57.
24. Hashimoto M, Kinoshita T, Yamasaki M et al. Gene frequencies and haplotypic associations within the HLA region in 916 unrelated Japanese individuals. Tissue Antigens 1994; 44: 166–173.
25. Halme L, Hockerstedt K, Lautenschlager I. Cytomegalovirus infection and development of biliary complications after liver transplantation. Transplantation 2003; 75: 1853–1858.
26. Egawa H, Inomata Y, Uemoto S et al. Biliary anastomotic complications in 400 living related liver transplantations. World J Surg 2001; 25: 1300–1307.
27. Melnick JI, Adam E, DeBakey ME. Possible role of cytomegalovirus infection in atherosclerosis. JAMA 1990; 263: 2204–2207.
28. Bolovan-Fritts CA, Spector SA. Endothelial damage from cytomegalovirus-specific host immune response can be prevented by targeted disruption of fractalkine-CX3CR1 interaction. Blood 2008; 111: 175–182.
29. Streblow DN, Kreklywich C, Yin Q et al. Cytomegalovirus-mediated upregulation of chemokine expression correlates with the acceleration of chronic rejection in rat heart transplants. J Viro 2003; 77: 2182–2194.
30. Miyagawa-Hayashino A, Tsuruyama T, Haga H et al. Arteriopathy in chronic allograft rejection in liver transplantation. Liver Transpl 2004; 10: 513–519.
31. Levitsky J, Singh N, Wagener MM et al. A survey of CMV prevention strategies after liver transplantation. Am J Transplant 2008; 8: 158–161.
32. Goss JA, Shackleton CR, Farmer DG et al. Orthotopic liver transplantation for primary sclerosing cholangitis a 12-year single center experience. Ann Surg 1997; 225: 472–483.

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BASIC STUDIES

Significance of periductal Langerhans cells and biliary epithelial cell-derived macrophage inflammatory protein-3α in the pathogenesis of primary biliary cirrhosis

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Abstract

Background/aims: To clarify the primary biliary cirrhosis (PBC)-specific antigen-presenting mechanism, we examined the distribution and phenotypic characteristics of infiltrating dendritic cells (DCs) with respect to bile ducts and the mechanism of migration in terms of the periductal cytokine milieu and biliary innate immunity. *Methods and results:* Immunohistochemistry using liver sections from patients with PBC and controls revealed that blood dendritic cell antigen (BDCA)-2^+ plasmacytoid DCs were found mainly in the portal tracts in PBC and the controls, but their distribution was not related to bile ducts. BDCA-1^+ and $CD19^-$ myeloid DCs were also found in portal tracts in PBC and the controls and, in particular, Langerin+Langerhans cells (LCs) were dominantly scattered around or within biliary epithelial layers of the damaged bile ducts in PBC. Moreover, experiments with cultured human biliary epithelial cells (BECs) showed that an LC-attracting chemokine, macrophage inflammatory protein-3α, was produced by BECs in the response to cytokines [interleukin (IL)-1β, tumour necrosis factor-α and IL-17] and pathogen-associated molecular patterns. *Conclusions:* LCs existing around or within biliary epithelial layers are important as periductal antigen-presenting cells in PBC and the migration of LCs into bile ducts is closely associated with the periductal cytokine milieu and biliary innate immunity in PBC.

Primary biliary cirrhosis (PBC) is characterized histologically by cholangiopathy, which is marked by chronic non-suppurative destructive cholangitis (CNSDC) and bile duct loss, and is thought to be caused by bile duct-specific autoimmunity (1–3). Serologically, antimitochondrial antigen (AMA) is detected in the sera of most PBC patients, but its major autoepitope, pyruvate dehydrogenase complex-E2 (PDC-E2), is not specific to bile ducts (3). We showed that a cytokine network around bile ducts and biliary innate immunity involving Toll-like receptors (TLRs) are important for the pathogenesis of cholangiopathy in PBC (4, 5). Moreover, the presence and response to PDC-E2 of autoreactive T cells (6) is currently believed to require interaction with professional antigen-presenting cells (APCs), namely, dendritic cells (DCs). Therefore, the presence of APCs in biliary trees is likely important to the bile duct-specific autoimmunity in PBC. Although S-100-positive DCs and activated CD83-positive DCs were previously demonstrated to have a role in the initiation of inflammatory responses in cases of PBC (7, 8), the mechanism of their bile duct-specific distribution and the interaction between biliary epithelial cells (BECs) and DCs are still unknown.

The DCs are generally classified into myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) and reside in tissues of most organs under normal conditions. DCs function as immunological sensors for danger signals and are closely associated with the interaction between the innate and the adaptive immune systems. mDCs derived from myeloid progenitors are ontogenically related to the monocyte/macrophage lineage and have strong activity to capture antigens. Because Langerhans cell (LC) is differentiated from monocytes, LCs belong to mDC (9). In contrast, pDCs showing a plasmacytoidal morphology originate from pDC precursors in peripheral blood. Phenotypically, mDCs and pDCs express blood dendritic cell antigen (BDCA)-1 (also known as CD1c)