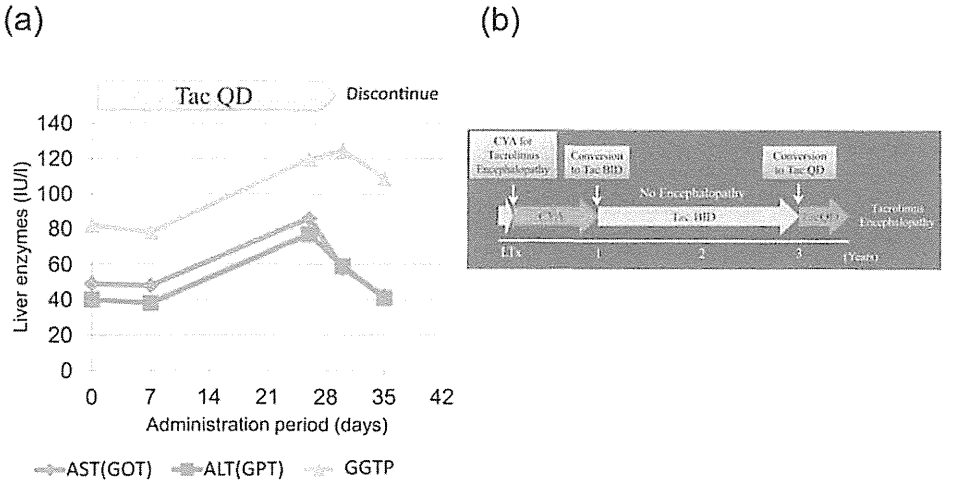

Fig 4. Clinical courses of the patients who discontinued prolonged-release tacrolimus. (a) Patient 1 had abnormal liver function tests after switching to prolonged-release tacrolimus, which normalized after prolonged-release tacrolimus was switched back to standard tacrolimus. (b) In Patient 9, tacrolimus encephalopathy recurred after conversion to prolonged-release tacrolimus, although the patient did not experience neuropathy for more than 2 years on standard tacrolimus. Tac BID: standard tacrolimus; Tac QD: prolonged-release tacrolimus; CyA: cyclosporine; AST: aspartate aminotransferase; ALT: alanine transaminase; GGTP: gammaglutamyl transpeptidase; LTx: liver transplant.

daily dosing. A granule-type formulation is awaited for once-daily tacrolimus, as is available with standard tacrolimus.

In conclusion, prolonged-release tacrolimus was useful for pediatric patients after LRDLT. Trough levels after conversion were compatible with those before conversion. Most patients were satisfied with prolonged-release tacrolimus. However, some patients failed conversion because of unexpected responses. Close observation after conversion is required even if patients have had an uneventful course on standard tacrolimus.

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ELSEVIER

A National Survey of Patients With Intestinal Motility Disorders Who Are Potential Candidates for Intestinal Transplantation in Japan

T. Ueno, M. Wada, K. Hoshino, S. Sakamoto, H. Furukawa, and M. Fukuzawa

ABSTRACT

Intestinal motility disorders are a major cause of intestinal failure. Severe cases such as idiopathic pseudo-obstruction represent life-threatening illnessed. Intestinal transplantation is a treatment for severe motility disorders with irreversible intestinal failure. However, the prevalence of severe motility disorders is unknown. We performed a national survey to identify patients with intestinal motility disorders who require an intestinal transplant. The national survey of 302 institutions treating intestinal motility disorders identified 147 patients treated from 2006 to 2011 at 46 institutions. The mean patient age was 12.1 years (range, 0.3–77.5). The mean age of onset was 3.0 years (range, 0.0–68.8). Diagnoses included chronic idiopathic intestinal pseudo-obstruction (n = 96), Hirschsprung disease (n = 29), megacystis microcolon intestinal hypoperistalsis syndrome (n = 18), and other (n = 6). There were 126 survivors and 21 patients who died during the last 5 years. The mortality rate was 14.3%. Eighty-five percent of patients required parenteral nutrition for more than 6 months, which was defined as irreversible intestinal failure. Among surviving patients with irreversible intestinal failure, 8 (9.4 %) developed hepatic failure with jaundice and 27 (31.8%) 2 or more central vein thromboses. In all, at least 35 patients (41%) with irreversible failure due to intestinal motility disorders may be candidates for transplantation. The prevalence of severe intestinal motility disorders was elucidated in Japan. Severe cases should be referred to transplant centers.

INTESTINAL MOTILITY DISORDERS are a major cause of intestinal failure. Severe cases such as idiopathic pseudo-obstruction are life-threatening. Causes of intestinal motility disorders seem to be multifactorial, and only a few have been elucidated. The prognosis is poor for patients with severe illness. The outcome for intestinal failure has improved dramatically due to the development of parenteral nutrition (PN). However PN-related complications, such as central venous catheter infection, thrombosis of venous access points, and PN-associated cholestasis of the liver, are still major problems for patients with intestinal failure. Intestinal transplantation is a treatment for irreversible intestinal failure due to severe disorders of intestinal motility that can significantly improve the prognosis and quality of life for patients. Progress in intestinal transplantation has improved survival. However, the prevalence of severe intestinal motility disorder is unknown. The Therapeutic Guidelines for Intestinal Failure Study Group performed a national survey to identify patients with intestinal motility disorders requiring an intestinal transplant.

METHODS

This national survey was designed as a 5-year retrospective observation study involving 302 institutions that treat intestinal motility disorders. These institutions were members of the Japanese Society of Pediatric Surgeons, the Japanese Society for Small Bowel Transplantation, and the Japanese Study Group for Home Parenteral and Enteral Nutrition. After an initial survey, a questionnaire about each patient was sent to responding institutions from the data center based at Osaka University. Patients with intestinal

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failure treated at each institution from 2006 to 2011 were included. Exclusion criteria were: (1) final diagnosis other than intestinal failure, (2) intestinal failure ultimately resolved, (3) intestinal failure resulting from malignancy, and (4) intestinal failure secondary to diseases in other organs. There were 354 patients reported by 69 institutions. Irreversible intestinal failure was defined as dependence on PN for more than 6 months. Out of these 354 patients, patients with intestinal failure due to motility disorders were identified. The following factors were assessed for possible associations with indications for intestinal transplantation: diagnosis, patient age, age of onset, sex, patient outcome, PN status, liver function tests (LFTs), and central line access. This study was approved by the Osaka University Hospital institutional review board and was supported by Health Science Research Grants from the Ministry of Health, Labor and Welfare of Japan.

RESULTS

There were 147 patients with intestinal motility disorders identified from 46 institutions. The prevalence was approximately one in one million. There were 55 male and 92 female patients. The female-to-male ratio was about 2:1. The mean patient age was 12.1 years (range, 0.3–77.5 years). The mean age of onset was 3.0 years (range, 0.0–68.8 years). Causes of intestinal failure are shown in Fig 1. During the observation period, 126 patients survived and 21 patients died. The mortality rate was 14.3%.

Detailed analysis was added for survivors to determine indications for intestinal transplantation. Of the surviving patients, 91 (62.0%) needed PN at least once a week, and 85 (57.8%) required PN for more than 6 months. Those 85 patients were defined as having irreversible intestinal failure. The following analyses were carried out for patients with irreversible intestinal failure. Catheter-related complications were assessed. The site of central vascular access (internal jugular vein, subclavian vein, and femoral vein) was reported. The number of venous access failures is shown in Fig 2. Twenty-seven patients (31.9%) had 2 or more instances of central vascular access loss.

There were 61 patients (71.8%) who developed abnormal LFTs suggestive of liver injury from PN, including 8 patients (13%) with jaundice. They were considered to have severe liver injury resulting from PN.

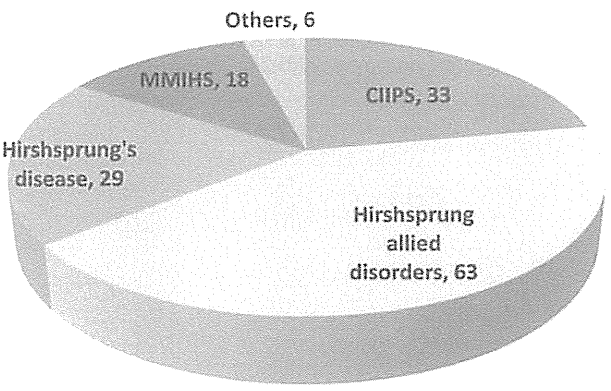

Fig 1. Causes of intestinal failure (n = 147). CIIPS, chronic idiopathic intestinal pseudo-obstruction; MMIHS: megacystis microcolon intestinal hypoperistalsis syndrome.

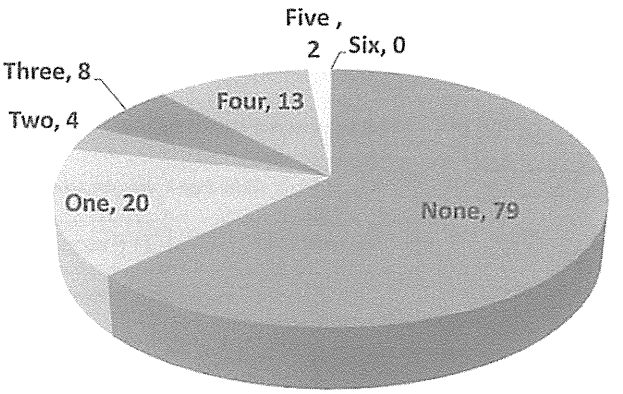

Fig 2. Number of central vascular access losses (n = 126). The number on the left indicates the number of vascular access losses.

Fifty-eight patients required at least 1 hospitalization in the previous year. Nineteen patients (22.4%) required hospitalization for more than 6 months over the previous year. Their quality of life was severely impaired.

A flowchart for identifying possible candidates for intestinal transplantation is shown in Fig 3. Patients dependent on PN for more than 6 months were defined as having irreversible intestinal failure. Those with more than 2 central vascular access losses, and abnormal LFTs with jaundice were considered for candidates for intestinal transplantation. Patients who died from liver failure or infection might be saved by intestinal transplant. They might be candidates for intestinal transplant too. In total, 45 patients were potential candidates for intestinal transplantation. Additionally, the 19 patients who were hospitalized for more than 6 months can be potential candidates given their poor quality of life.

DISCUSSION

Intestinal motility disorders include a wide range of diseases. Chronic intestinal pseudo-obstruction, the most common type of intestinal mobility disorder, is caused by ineffective intestinal contraction. It is characterized by symptoms and signs of intestinal obstruction.[1] Intestinal transplantation can significantly improve the prognosis and quality of life of patients with intestinal motility disorders in Japan.[1] Survival rates in Japan are comparable with rates from the international intestinal transplant registry.[2]

Previously, the prevalence of intestinal motility disorders in Japan was unknown. It was estimated that there were 100 severe cases nationwide. This study supports this figure because surveillance was of a large enough scale to cover the entire nation.

There were over 40 patients who may need intestinal transplantation. However, only 3–4 a year intestinal transplants are performed in Japan, even if 10 times as many patients may be cured by intestinal transplantation.

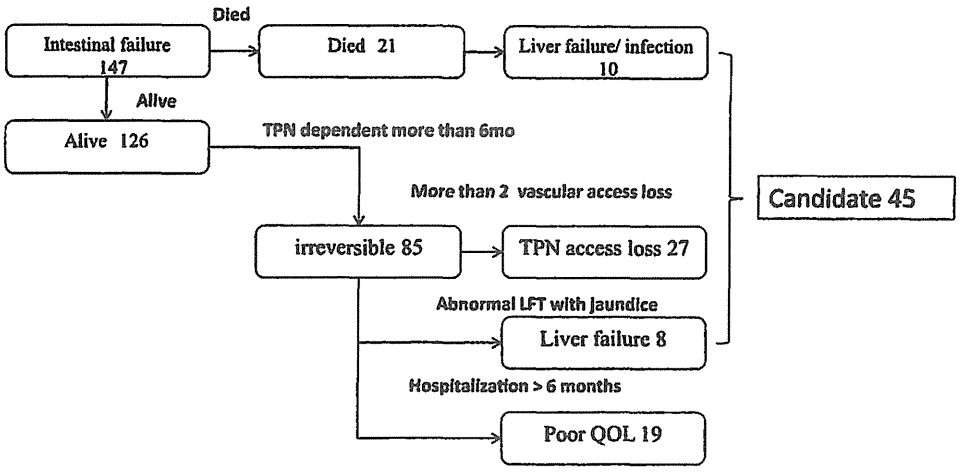

Fig 3. Candidates for intestinal transplantation. TPN, total parental nutrition; QoL, quality of life.

There were 2 major reasons for the relative paucity of intestinal transplants in Japan. One reason is the lack of available organs. For a long time, very few organs from deceased donors were obtainable in Japan. As with other solid organs, most intestinal transplants in Japan are performed with living donors. The shortage of organs has been alleviated due to a new act on organ transplantation that went into effect in 2010. However, the number of intestinal transplant has remained steady.

The financial barrier is the other, more profound reason preventing greater use of intestinal transplantation in Japan. Since the procedure is not covered by health insurance, either the patient or the transplant institution must pay the considerable costs out of pocket.

Some patients develop liver failure with intestinal motility disorders. These patients need simultaneous liver-intestine transplants. A combined liver-intestine transplant has less risk of acute rejection than an isolated intestinal transplant because the liver may have protective effects on the intestine. Current organ allocation guidelines do not allow for simultaneous combined liver-intestine organ retrieval; thus, a simultaneous liver-intestine transplant is impossible from deceased donor sources.

Previously, the laws on organ transplantation banned donors below 15 years of age. Intestinal transplants were not previously possible in infants because of organ size mismatch. Such patients will benefit from intestinal transplants in the future. Moreover, younger patients sometimes develop liver failure.[3] Multiorgan transplantation is a good option for such patients.[4]

It is difficult to determine the optimal timing for intestinal transplants to treat intestinal failure associated with intestinal motility disorders. Severe cases of intestinal motility disorders should be referred to institutions with expertise in transplantation.

In conclusion, the prevalence of severe motility disorders in Japan was elucidated. Patients with irreversible intestinal failure from intestinal motility disorders may be candidates for intestinal transplantation. Severe cases of motility disorder should be referred to transplant centers. Further investigation for patient details is required.

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Histology of Intestinal Allografts

Lymphocyte Apoptosis and Phagocytosis of Lymphocytic Apoptotic Bodies Are Diagnostic Findings of Acute Rejection in Addition to Crypt Apoptosis

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Abstract: Acute rejection of a small-bowel transplant is often difficult to diagnose due to complicated immune responses. The present study aimed to elucidate the specific immune responses involved in intestinal transplant rejection. We correlated immunohistologic findings with an increase in crypt apoptosis, which has been commonly accepted as a criterion for the diagnosis of acute cellular rejection (ACR). Of 8 patients who received an intestinal allograft at Kyoto University Hospital, biopsy specimens from 7 patients were assessed immunohistologically with antibodies against 20 types of lymphocytic antigens including CD3, CD4, CD8, CD79a, CD20, IgG, and T-cell receptor, along with assessment of the patients' clinical courses. It was revealed that, in addition to apoptotic crypts, T-lymphocyte apoptosis and phagocytosis of apoptotic bodies in the lamina propria of villi were findings of ACR; both were observed in all cases. Immunostaining of the Fas ligand, one of the apoptosis-inducing molecules, was useful for the identification of the apoptotic bodies in the lamina propria of villi. Apoptotic body phagocytosis may be a surrogate diagnostic finding of grafts undergoing ACR.

Key Words: intestinal transplantation, apoptosis, Fas ligand, lymphocyte, phagocytosis, acute cellular rejection

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BACKGROUND

Intestinal transplantation is a commonly accepted standard therapy for patients with irreversible parenteral nutrition complications associated with short-bowel syndrome,[1–8] Hirschsprung disease, and related diseases such as chronic idiopathic intestinal pseudo-obstruction syndrome or megacystis-microcolon-intestinal hypoperistalsis syndrome.[9,10] One-year patient survival rate after transplantation has improved, and more than half of the children who survive a transplant can now be weaned off parenteral nutrition.[1–8] However, acute cellular rejection (ACR), which may be detected by histopathologic methods, remains the major cause of intestinal graft failure after transplantation. Histopathologic diagnosis is useful in the detection of ACR.[11–15] In fact, for most patients who experience severe acute rejection, sufficient recovery of mucosal absorption function remains difficult, and bacterial infection is inevitable.[6] Therefore, the diagnosis and treatment of acute rejection in the early phase are critical for postoperative care.

The major histologic finding of acute rejection is a mixed cellular inflammation infiltrate including activated large lymphocytes and crypt apoptosis.[12,15] In the current study, we applied a grading system of acute rejection according to criteria defined at an international meeting.[12] Crypt apoptosis involves epithelial stem cell injury, and its development inhibits sufficient recovery of mucosa. The level of mucosal damage caused by ulceration due to apoptosis is therefore critical in terms of transplant success. The aim of this study was to precisely evaluate immune responses in an intestinal allograft before the appearance of crypt apoptosis in order to prevent the development of rejection.

MATERIALS AND METHODS

Case Selection

In the 15 years between May 1997 and February 2012, 8 patients underwent orthotopic intestinal transplantation at

TABLE 1. Clinical Profiles of Patients and Donors

Patient	Patient: Age (y)/Sex	Donor: Age (y)/Sex	ACR (POD)	Steroid Pulse	Immunosuppressants	Etiology	Induction
1	2/M	20-30/F	12	+	Tacro/azatio/steroid	SB	
2	0/F	20-30/F	12	+	Tacro/cyclo/steroid	SB	OKT3
			18	+			
			20	+			
			25	+			
3	4/M	20-30/F	66	+	Tacro/steroid	SB	
4	4/F	20-30/F	22	+	Tacro/steroid	SB	
5	12/F	20-30/F	27	+	Tacro/steroid	CIIP	Daclizumab
6	19/M	40-50/M	12	+	Tacro/cyclo/steroid	SB	Daclizumab
7	10/F	30-40/M	11	+	Tacro/cyclo/steroid	CIIP	Basiliximab
			19	+	Thymoglobulin		

Etiology: status of native intestine of the patient.
Steroid pulse: 10 to 20 mg/kg/d.
CIIP indicates chronic idiopathic intestinal pseudo-obstruction syndrome; F, female; M, male; SB, short-bowel syndrome.

Kyoto University Hospital. All of the protocols of this study were approved by the Committee of Medical Ethics of the Graduate School of Medicine, Kyoto University, and the study was performed with the informed consent of the patients' parents.

The patients commonly received immunosuppressant therapy consisting of tacrolimus for suppression of immune responses to the allograft. In brief, intravenous tacrolimus (baseline, 5 to 15 ng/mL) and methylprednisolone were commonly used as maintenance therapy for immunosuppression in patients 2 to 7.[16] Mycophenolate mofetil was transiently administrated for treatment of ACR in patients 5 to 7 after the present histologic examination.

To evaluate the degree of rejection and the effect of immunosuppressive therapy, daily endoscopic examinations were performed for the first 2 months. Specifically, endoscopic examination, including biopsy, was performed every day or every other day from posttransplantation operative day (POD) 7 to 20 (Table 1). From POD 21 to 50, biopsy examination was performed once or twice a week, and endoscopic examination was performed when patients complained of fever (> 37°C) and had an increased peripheral blood C-reactive protein level of > 1.0 mg/0.1 L. Frozen sections were prepared for immunohistochemical analysis, and hematoxylin and eosin (H&E)-stained sections of formaldehyde-fixed paraffin-embedded specimens were prepared from residual frozen samples. We examined the immunohistology of frozen biopsied specimens in addition to the H&E-stained paraffin-embedded specimens and investigated the early histologic features of ACR to determine the appearance of ACR before the development of crypt apoptosis. When ACR was diagnosed pathologically, the patients received steroid pulse therapy (15 to 20 mg/kg/d). The profiles of the patients and donors are shown in Table 1.

Immunohistochemistry

A total of 282 frozen biopsy specimens were taken from intact and damaged mucosa and used for histologic diagnosis. The immunohistochemistry protocol was previously reported.[17]

The following antibodies, obtained from the indicated sources, were used for immunostaining: anti-CD3 (Dako, Glostrup, Denmark); anti-CD4 (Dako); anti-CD8 (Dako); anti-CD20 (Dako); anti-CD79a (Dako); antileukocyte common antigen (Dako); anti-CD45RO (Dako); anti-Ki67 (Dako); anti-p53 (Dako); anti-PCNA (Dako); anti-cyclin D1(Dako); anti-Fas (Dako); anti-FasL (Nichirei, Tokyo, Japan); anti-IgG and anti-IgM, used to determine nonspecific binding (Dako); anti-C4d; and anti-T-cell receptor αβ chain (Immunotech Inc., Marseille, France). All antibodies were diluted to 1:100. 3,3'-Diaminobenzidine or phycoerythrin-labeled streptavidin and fluorescein isothiocyanate-labeled/phycoerythrin-labeled streptavidin (Vector Labs, Burlingame, CA) staining was used for visualization of the immunohistochemical signal. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL) staining of apoptosis was performed using an in situ apoptosis detection kit (Takara, Otsu, Japan).

Diagnosis of ACR

The histology of the intestinal graft was diagnosed according to previously reported criteria.[11,12,15] To identify ACR, the following histologic criteria were used: inflammatory infiltrate, increased crypt epithelial apoptosis (usually with > 6 apoptotic bodies/10 crypts), architectural distortion of villi, and mucosal ulceration changes. To accurately detect apoptotic bodies, TUNEL staining of the graft specimens was additionally performed.

Statistical Analysis

Interindividual differences in the responses to high-dose steroid therapy (> 15 mg/kg/d) were assessed using the Student *t* test (SPSS 10.0.5 from SPSS Inc., Chicago, IL), as this test provides the most conservative estimates. All *P* values were 2-sided and were considered to be statistically significant if < 0.01. *P* values were not adjusted for multiple testing.

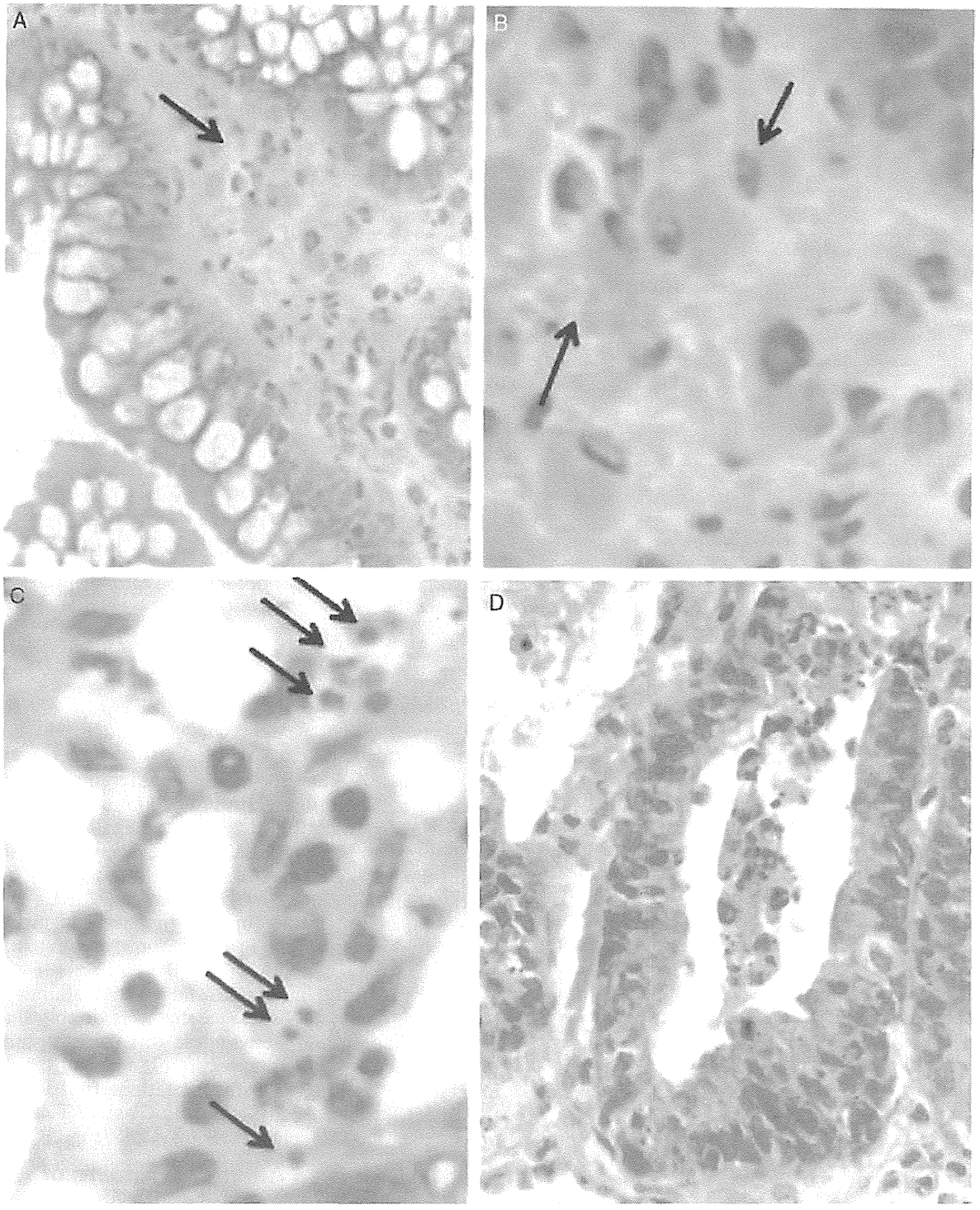

FIGURE 1. Collection of macrophages and apoptotic bodies in the LP of villi. H&E and immunohistochemical staining of biopsy specimens of intestinal transplants at POD 9 to 13 were analyzed. A–B, Collection of macrophages (indicated by arrows in A and B with apoptotic bodies in the LP. A, B, patient 7. C, Apoptotic bodies in the LP are indicated by arrows. Six apoptotic bodies were observed. D, Crypt apoptosis that appeared 2 days after the collections of LP macrophages containing apoptotic bodies appeared in patient 5.

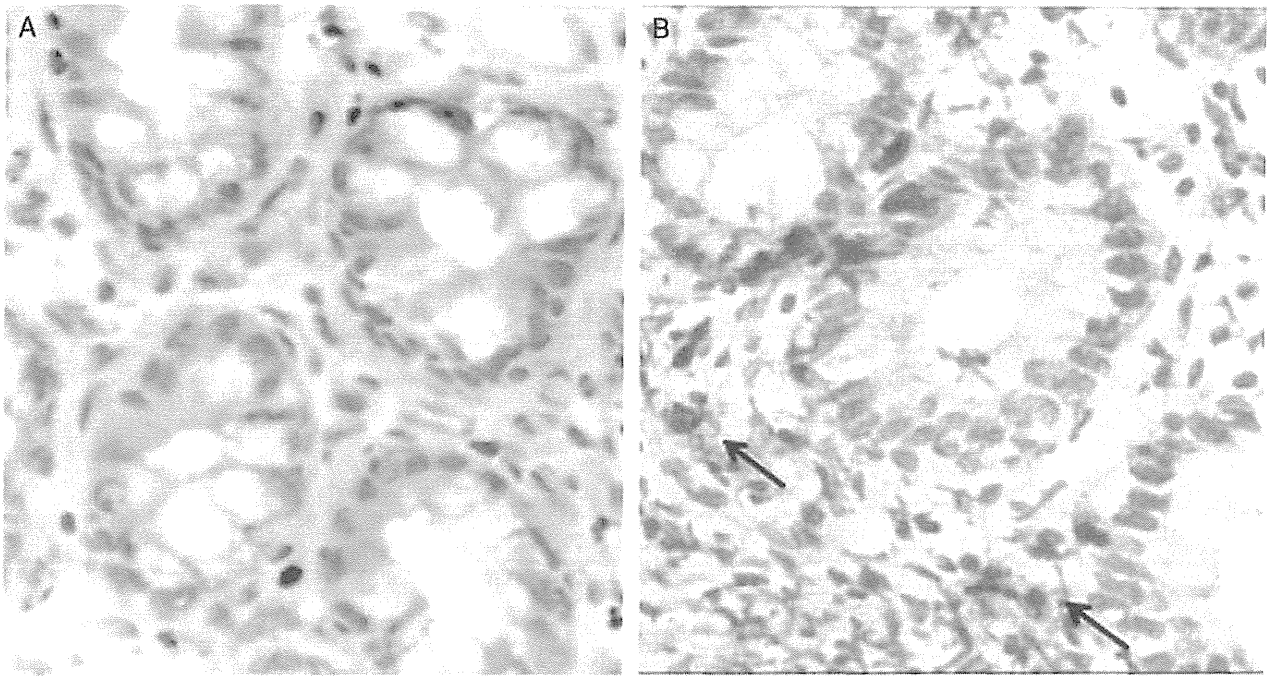

FIGURE 2. TUNEL staining of apoptotic bodies in the LP. TUNEL staining of the normal donor intestine for patient 2 (A) and transplanted intestine with ACR on POD 12 are shown. B, Arrows indicate the TUNEL-positive cells in the LP. The signal was visualized with 3,3′-diaminobenzidine. Counter staining was performed with H&E.

RESULTS

Apoptotic Bodies and Phagocytosis in the Lamina Propria of Villi

The transplanted intestines were assessed through H&E staining and immunohistochemical analysis of sections. We commonly observed apoptotic bodies when crypt apoptosis was present in the lamina propria (LP) of villi (Figs. 1A–C). These apoptotic bodies were localized around capillary veins and were frequently eosinophilic. In addition, the macrophages were weakly eosinophilic and their collections in the LP of villi contained the apoptotic bodies. The macrophage collections and apoptosis in the LP (LP apoptosis) was followed by the appearance of >6 crypt apoptoses per 10 crypts, which is diagnostic of ACR, in all 7 of the allografts studied (Fig. 1D).

Identification of Apoptotic Bodies

To confirm LP apoptosis, we stained the apoptotic bodies using the TUNEL method. The normal intestine, that is intestinal grafts before transplantation, was weakly stained at the crypt (range, 0.1 to 1.3 per 10 crypts; median, 0.4 per 10 crypts; n = 7) (Fig. 2A). In contrast, apoptotic crypts in the grafts undergoing ACR were intensely stained (range, 6.2 to 8.5 per 10 crypts; median, 6.3 per 10 crypts; n = 7) and apoptotic bodies in macrophages in the LP were additionally stained (Fig. 2B).

Apoptotic Bodies in the LP Contained T Cells

To identify the types of cells undergoing apoptosis in the LP of the graft during ACR, immunohistochemical analysis using an antibody against the Fas ligand (FasL), an apoptosis-related molecule, was carried out to detect apoptosis. In the normal control, that is grafts before transplantation, intact cells showed FasL signals (Fig. 3A); in contrast, apoptotic signals were positively stained by FasL antibody in the graft undergoing ACR in addition to the intact cells (Fig. 3B).

Subsequently, multicolor fluorescent staining was performed. FasL and T-cell receptor were doubly stained, and 4′,6-diamidino-2-phenylindole was used to stain the nuclei. Intact FasL+ T cells were observed in the donor normal intestine before transplantation. In addition to this intact FasL+ T cells, apoptotic FasL+ T cells were observed in the transplants during ACR (Fig. 3C, D).

Phagocytosis is an Additional Finding of ACR

In addition, macrophages phagocytosing the apoptotic FasL+ cells were frequently observed in the LP (Fig. 4A). Immunohistochemical analysis of a serial section of the same tissue using an anti-CD68 antibody also showed that $CD68^+$ macrophages phagocytosed FasL+ apoptotic bodies in the LP of villi in allografts ongoing ACR (Fig. 4B). Thus, FasL staining is useful for the identification of apoptotic bodies in allografts and TUNEL staining (Table 2).

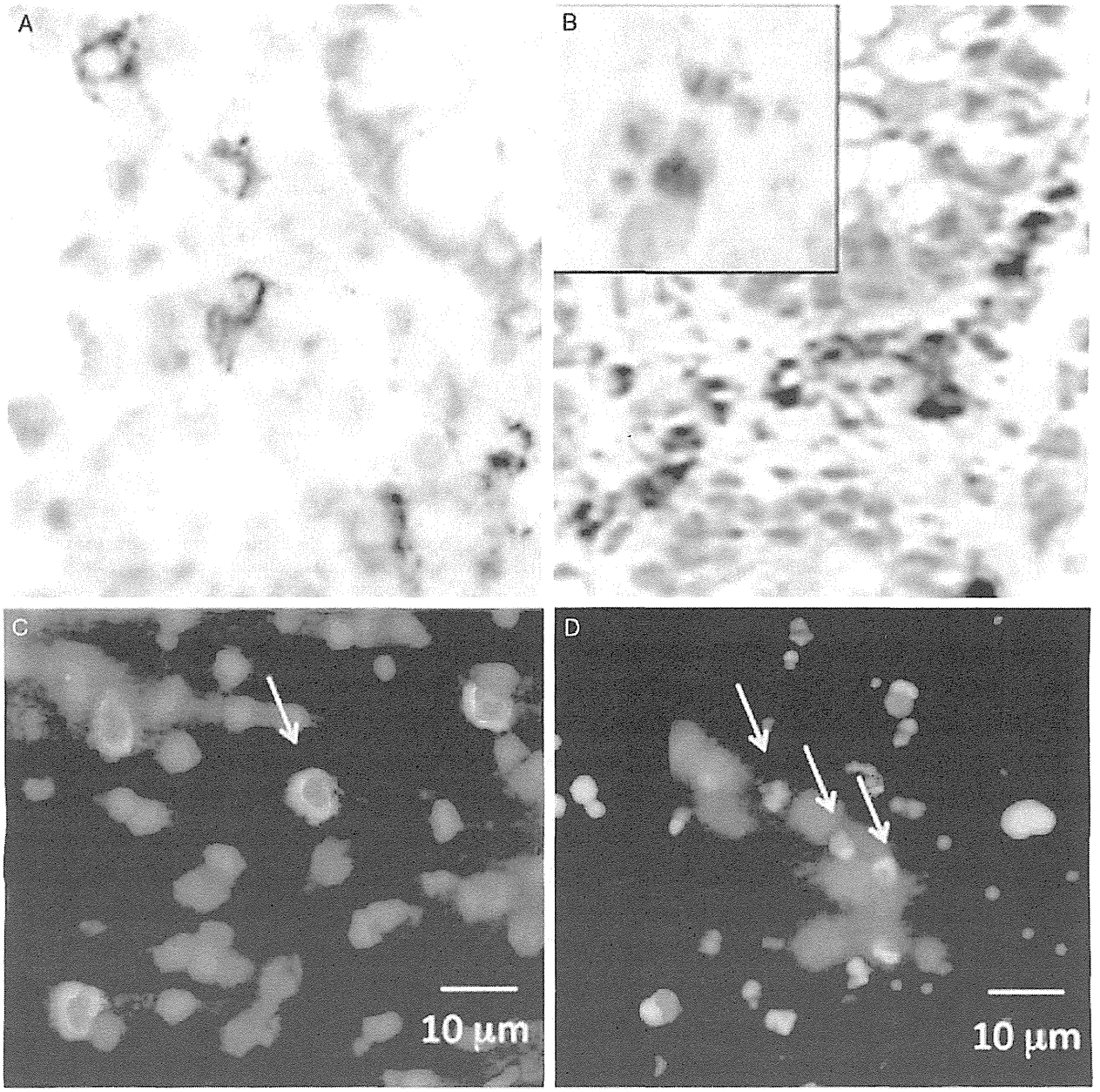

FIGURE 3. Apoptotic bodies containing T cells. (A, B) FasL immunostaining of donor intestine (A) and transplant tissues in ACR (B). The inset in (B) shows a high-power magnification of an apoptotic body aggregation. The FasL signal in apoptotic clusters was visualized with 3,3′-diaminobenzidine around the crypt. Counter staining was performed with hematoxylin. C and D, Co-staining of apoptotic clusters by fluorescent immunostaining of FasL and T-cell receptor. Phycoerythrin-streptavidin-stained FasL (red) and fluorescein isothiocyanate-streptavidin (green) (T-cell-receptor staining) can be seen. Nuclei were visualized using 4′,6-diamidino-2-phenylindole. C Triply stained intact FasL+ T cells in the donor intestine before transplantation (patient 7). The arrow indicates intact FasL+ T cells. D, Triply stained FasL+ apoptotic T cells in the donor intestine before transplantation (patient 7). Arrows in (D) indicate the apoptotic fragments. Scale bars are shown.

LP Apoptosis may be a Surrogate Finding of Crypt Apoptosis

On the basis of this staining, we semiquantitatively scored the apoptosis in the LP (score 0, no signals; score 1, scant and isolated signals; score 2, a few signal aggregations; score 3, signal aggregates surrounding the crypt). A total of 150 specimens did not contain the sufficient number of crypt (> 10) architecture for the diagnosis of ACR, and the LP apoptosis score closely correlated with the crypt apoptosis count in the remaining 132 sections ($R^2 = 0.87$, $P < 0.001$), indicating that LP apoptosis can be a surrogate finding of crypt apoptosis (Fig. 5).

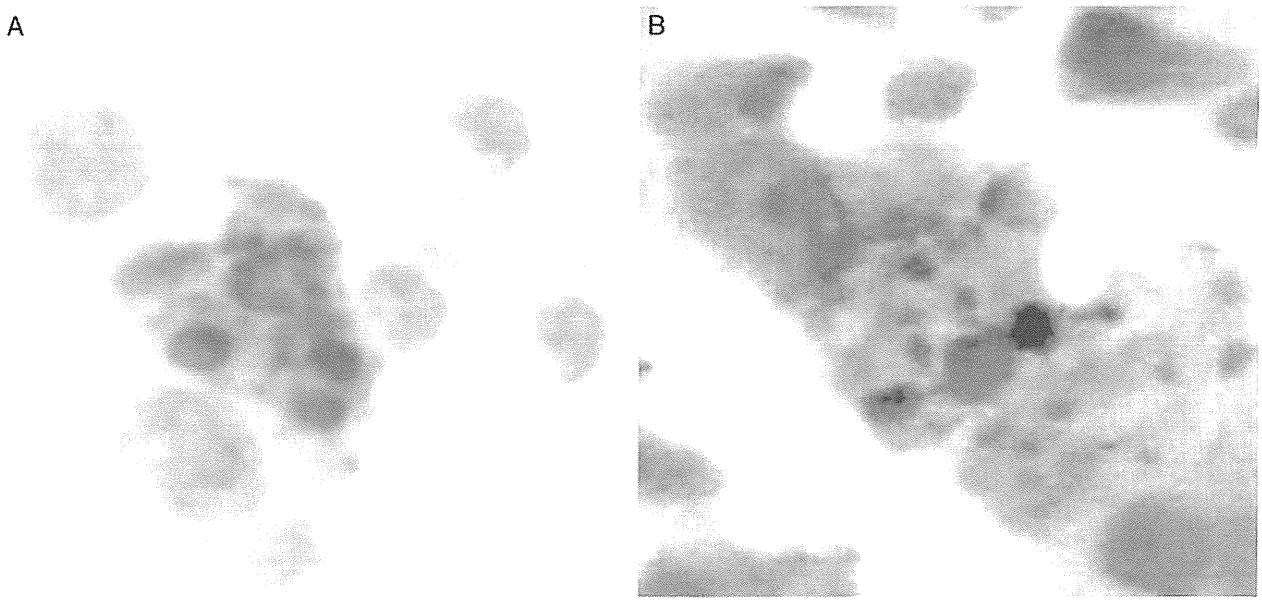

FIGURE 4. Phagocytosis of apoptotic bodies. A, FasL immunostaining of an apoptotic body cluster that was phagocytosed by a macrophage. The FasL signal was visualized with 3,3′-diaminobenzidine. Brown-stained FasL-containing apoptotic bodies surrounding the nucleus of the phagocytosing macrophage. B, Immunostaining of macrophages using anti-CD68, and visualization of the signal with 3,3′-diaminobenzidine. An apoptotic body cluster that was phagocytosed by a macrophage (brown stain) can be seen.

DISCUSSION

The present study includes only 7 transplants, but histologic assessment was performed using 20 types of immunohistochemical markers and was correlated with the clinical courses. FasL was selected as a marker of apoptosis in intestinal transplants on the basis of the immunohistochemical study.

For the long-term period, novel immunologic suppressants, such as mycophenolate mofetil, have been popularized. In the present cases, mycophenolate mofetil was transiently administrated after our histologic follow-up. The treatment protocol for the therapy and follow-up has been constitutively stable using tacrolimus and steroids. Although there have been previous reports regarding T-cell apoptosis in the intestine in ongoing ACR or graft-versus-host disease, its clinical significance has not been evident.[18–20] In the current study, FasL+ T-cell apoptosis was a useful indicator of evident transplant rejection.

TABLE 2. Histologic Profiles of Patients and Donors

Patient	ACR (POD)	Mixed Inflammatory Infiltrate	Apoptotic Crypt Counts	Villous Architecture	LP Apoptosis (Score)	Phagocytosis in LP	FasL+ T Cell in LP	Other Findings	ACR Grade
1	12	Marked	8.5	Distortion	3	+	Marked increase and apoptosis	Chronic rejection	3
2	12	Marked	6.3	Soughing	3	+	Marked increase and apoptosis	Neutrophilic infiltrates	2
	18		5.9		2	+	Modest increase		2
	20		6.1		2	+	Modest increase		2
	25		6.2		2	+	Modest increase		1
3	66	Mild	6.1		2	+	Modest increase		1
4	22	Moderate	7.5	Distortion	2	+	Modest increase		2
5	27	Marked	6.2	Distortion	2	+	Marked increase		2
6	12	Moderate	6.4	Soughing	2	+	Marked increase and apoptosis		2
7	11	Moderate	6.5	Ulceration	1	+	No significant increase	C4d immunostaining+	3
	19		7.6	Shortening	3	+	No significant increase		2

Apoptotic crypt count: per 10 crypts
ACR grades 0 to 3 according to the International grading scheme.[12]
LP apoptosis indicates lymphocytic apoptosis (in the LP) in ACR; phagocytosis in LP, phagocytosis (of the apoptotic bodies) in LP.