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Multivisceral transplantation in pigs: a clinicopathological analysis of tissue rejection.

Shintaro Mitsuoka* Noriaki Tanaka†

Kunzo Orita‡

*Okayama University,

†Okayama University,

‡Okayama University,

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Shintaro Mitsuoka, Noriaki Tanaka, and Kunzo Orita

Abstract

In this study, we established the surgical procedure and postoperative care of multivisceral transplantation (MVTX) in pigs, and examined the functional changes and rejection pattern of transplanted organs in MVTX. Twenty-two MVTXs were performed without immunosuppression, and nine cases (41%) that survived for 5 days or more after MVTX were used for evaluation. Rejection in grafts including the liver, pancreas, and gastrointestinal tract were assessed histopathologically. On day 5 after transplantation, the duodenum and small bowel already showed signs of mild rejection. On the other hand, in the liver, pancreas and stomach, rejection occurred later and was still mild on day 16. Hepatic rejection in MVTX appeared to occur later than in simple liver transplantation (LTX). These results showed that the susceptibility to rejection of individual visceral organs varies.

KEYWORDS: multivisceral transplantation, allograft, rejection, pigs

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Multivisceral Transplantation in Pigs: A Clinicopathological Analysis of Tissue Rejection

Shintaro MITSUOKA*, Noriaki TANAKA and Kunzo ORITA

First Department of Surgery, Okayama University Medical School, Okayama 700, Japan

In this study, we established the surgical procedure and postoperative care of multivisceral transplantation (MVTX) in pigs, and examined the functional changes and rejection pattern of transplanted organs in MVTX. Twenty-two MVTXs were performed without immunosuppression, and nine cases (41%) that survived for 5 days or more after MVTX were used for evaluation. Rejection in grafts including the liver, pancreas, and gastrointestinal tract were assessed histopathologically. On day 5 after transplantation, the duodenum and small bowel already showed signs of mild rejection. On the other hand, in the liver, pancreas and stomach, rejection occurred later and was still mild on day 16. Hepatic rejection in MVTX appeared to occur later than in simple liver transplantation (LTX). These results showed that the susceptibility to rejection of individual visceral organs varies.

Key words: multivisceral transplantation, allograft, rejection, pigs

Multivisceral transplantation (MVTX), in which the liver, pancreas, spleen, stomach, duodenum, small bowel, and colon are transplanted *en bloc*, was first successfully performed by Starzl *et al.* (1) in dogs in 1962. This experiment demonstrated the technical feasibility of MVTX and the possibility of functional recovery following the operation. MVTX was clinically applied for the first time in 1983 by Starzl *et al.* (2) to treat short bowel syndrome combined with end-stage liver failure.

MVTX, however, has presented new immunological problems that have not been encountered in the transplantation of single organs. Using pigs, we (3) previously studied abdominal organ cluster transplantation (AOCTX) including the liver, pancreas, and duodenum, and showed that the pancreas and duodenum were more susceptible to rejection than the liver. Thus, in MVTX, it was estimated that each organ also develops a different pattern of rejection following *en bloc* transplantation. Furthermore, since MVTX include the small bowel and colon, it was anticipated that abundant immunocompetent cells in the intestine may affect the outcome of transplantation.

Other problems in MVTX include difficulties with the surgical procedure and the complexity of postoperative care. In this study, we established the surgical procedure and postoperative care of MVTX in pigs as a large-animal experimental model. We thus studied the functional changes and rejection patterns of transplanted organs after MVTX in pigs.

Materials and Methods

Animals. Twenty-two Dulock-Jersey pigs weighing 20-25kg were used as donors. Twenty-two Large-White pigs were used as recipients and they were heavier than donors by about 3-5kg. The experimental animals underwent no special pretreatment except for fasting for 24h.

Donor operation. The donor operation was performed under general anesthesia. The aorta was dissected out from the diaphragm to the iliac bifurcation and its branches, except for the celiac axis and superior mesenteric artery (SMA), were ligated. The liver, pancreas, and small bowel were completely freed from their retroperitoneal attachments, and the esophagus and sigmoid colon were transected. The abdominal aorta was clamped above the celiac axis, and a cannula was inserted

* To whom correspondence should be addressed.
Present address: Shobara Red Cross Hospital, 2-7-10, Nishihonmachi, Shobara, Hiroshima 727, Japan

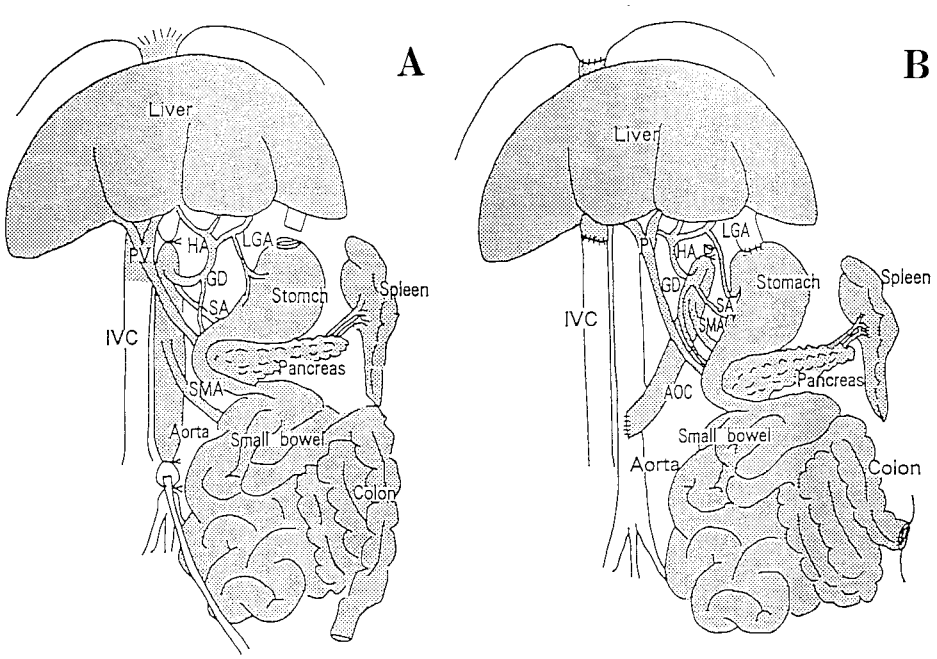

Fig. 1 The procedure for swine multivisceral transplantation (MVTX). (**A**) Harvesting procedure. (**B**) Transplantation procedure. The shaded area indicates the multivisceral grafts. AOC, aortic conduit; HA, hepatic artery; LGA, left gastric artery; IVC, inferior vena cava; SMA/V, superior mesenteric artery/vein; PV, portal vein; HA, hepatic artery; SA, splenic artery; GD, gastric artery.

into the distal portion of the aorta. The visceral organs including the liver, pancreas, spleen, stomach, duodenum, small bowel, and colon were perfused with 1000 to 1500 ml of Euro-Collins (EC) solution at 4 °C through the aorta. To permit free efflux of the blood and perfusate, the inferior vena cava was cut above and below the liver. Then, these organs were harvested *en bloc*, and immersed in cold EC solution during preparation of the diaphragmatic cuff and perivascular trimming. The total time for the donor operation was 1.8 to 2.5 h. The procedure is illustrated in Fig. 1A.

Recipient operation. Premedication consisted of ketamine (10 mg/kg IM) and atropine (0.5 mg/body) and animals were maintained using GOF anesthesia after endotracheal intubation. All of the intra-abdominal organs were freed from retroperitoneal attachments, and then the esophagus and sigmoid colon were transected. Veno-venous bypass from the distal potion of the vena cava to the external jugular vein was prepared. The celiac axis and SMA were ligated, and the supra- and infrahepatic vena cava were isolated. Then the liver, pancreas, spleen, stomach, duodenum, small bowel, and colon were removed *en bloc*. Vascular anastomosis was performed in the following order: (a) suprahepatic vena cava; (b) arterial anastomosis between the donor aorta and the infrarenal recipient aorta; and (c) infrahepatic vena cava. The gastrointestinal tract was reconstructed by esophagogastrostomy and artificial anus. The mean operation time was 6.2 h, anhepatic time was 57.4 min, and ischemic time was 128.4 min. The operation time (including the anhepatic and total ischemic times) was not related to the survival time. The transplantation procedure and the operation time for our MVTX model is summarised in Fig. 1B and Table 1. None of the animals received immunosuppressants, and all were treated with antibiotics (aztreonam 2.0 g/day) for the first 7 days after surgery.

Biochemical and histological examinations. Graft functions were monitored by blood chemistry tests including the serum total bilirubin (T.Bil.), glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH), serum amylase (sAMY), blood glucose, and blood urea nitrogen (BUN). Autopsy was

performed when each animal died, or by sacrifice. Tissue specimens were fixed with 10% buffered formalin and stained with hematoxylin and eosin for histological analyses.

Evaluation of the grade of allograft rejection in each organ. Allograft rejection in the liver and pancreas was assessed histopathologically using the modified criteria of Snover (4) and Sibley and Sutherland (5), respectively. The gastrointestinal tract including the stomach, duodenum, small bowel and colon were evaluated by the modified criteria of Rosemurgy and Schraut (6).

Rejection of the liver was graded as follows: (−) normal or mild portal inflammation; (+) moderate or strong portal inflammation without endotheliitis, (++) moderate or strong portal inflammation with endotheliitis, mild or moderate duct damage; (+++) strong or severe portal inflammation with endotheliitis, strong duct damage.

Rejection of the pancreas was graded as follows: (−) normal or moderate lymphocyte infiltration in the exocrine tissue; (+) moderate or strong lymphocyte infiltration with polymorphonuclear cells in the exocrine tissue; (++) findings in Grade +, plus eosinophil infiltration and parenchymal damage; (+++) findings in Grade ++, plus endotheliitis.

Rejection of the gastrointestinal tract was graded as follows: (−) normal or mild inflammatory infiltration; (+) moderate inflammatory infiltration or moderate villous damage; (++) strong inflammatory infiltration or moderate villous damage; (+++) strong inflammatory infiltration, or strong villous damage with cryptitis.

Results

Survivals and causes of death. Twenty-two MVTXs were performed in this study. Seven of the 22 recipients (32%) died within the first 24h after surgery; the causes of death were shock following reperfusion or bleeding after surgery. Six pigs (27%) died between the 2nd and 4th postoperative days; the causes were postoperative bleeding, severe infection, and dehydration. Table 1 shows a summary of the nine cases that survived for 5 days or more after MVTX (41%). The causes of death in the pigs which survived for 5-10 days were not rejection. Case No. 1 showed a large quantity of bleeding in the abdominal cavity; No. 2 showed inflammatory infiltration in the lungs; No. 5 showed decreased liver function and marked jaundice; and Nos. 3, 4, and 6 showed muddy ascites, combined with fever and tachycardia. The two pigs surviving for 14 days and 16 days died of small bowel rejection combined with severe diarrhea and emaciation.

Graft function following MVTX. Graft function of the recipients surviving for more than 5 days is summarized in Fig. 2. During the first 7 days after surgery, T.bil. levels in the recipient serum remained below 2.0 mg/dL, but began to increase rapidly from day 8. GOT and LDH levels increased rapidly just after surgery and reached a peak on day 1 or 2, then decreased and remained slightly elevated until the recipient died. The levels of sAMY and blood glucose rapidly increased on the 1st day after surgery, then gradually decreased and

Table 1 Summary of 9 pigs survived for more than 5 days after multivisceral transplantation (MVTX)

Pig. No.	AHT (min)	TIT (min)	OPT (h)	Survival time (days)	Causes of Death
1	60	170	5.4	5	Intraabdominal bleeding
2	52	147	6.2	5	Pneumonia
3	58	94	6.1	7	Peritonitis
4	69	74	5.8	7	Peritonitis
5	43	79	5.6	8	Liver failure
6	52	147	6.2	8	Peritonitis
7	62	87	5.8	10	(Sacrificed)
8	66	223	7.2	14	Small bowel rejection
9	55	135	7.8	16	Small bowel rejection
Average (Mean ± SD)	57.4 ± 7.5	128.4 ± 46.8	6.2 ± 0.7	8.9 ± 3.7	–

Abbreviations: AHT, anhepatic time; TIT, total ischemic time; OPT, operation time

Table 2 Histopathologic findings of allograft rejection in the visceral organs after MVTX

Visceral organs	Histopathologic findings								
	Pig number								
	1	2	3	4	5	6	7	8	9
Liver									
Portal inflammation									
LC	−	−	−	−	−	±	++	+	±
PMN	−	−	−	−	−	±	+	−	−
EOS	−	−	−	−	−	−	+	+	++
Endotheliitis									
PV	−	−	−	−	−	−	−	−	−
CV	−	−	−	−	−	−	−	−	−
Parenchymal change									
Cholestasis	−	−	−	−	−	++	−	+	++
Necrosis	−	−	−	−	+	++	+	+	+
Pancreas									
Inflammatory infiltrate									
LC	±	−	+	+	±	ND	+	+	+
PMN	−	−	−	−	−	ND	±	±	±
EOS	−	−	−	−	−	ND	−	±	±
Endotheliitis	−	−	−	−	−	ND	−	±	−
Parenchymal damage	+	±	+	±	−	ND	+	+	−
Stomach									
Inflammatory infiltrate									
LC	±	−	−	±	−	±	±	+	+
PMN	−	−	±	±	±	±	±	±	±
EOS	−	−	−	−	−	−	−	±	±
Villous damage	−	−	−	−	±	±	±	+	+
Cryptitis	−	±	−	−	±	±	±	±	±
Duodenum									
Inflammatory infiltrate									
LC	+	+	+	+	±	+	+	+	+
PMN	−	−	±	±	−	±	±	±	±
EOS	−	±	+	±	±	+	+	++	++
Villous damage	±	±	−	+	±	+	+	+	+
Cryptitis	−	±	±	±	±	±	+	+	+
Small bowel									
Inflammatory infiltrate									
LC	+	+	+	+	+	+	++	+	+
PMN	±	±	±	±	±	±	±	±	±
EOS	−	±	±	+	+	±	+	+	+
Villous damage	±	±	+	+	±	+	+	++	++
Cryptitis	±	±	+	+	+	±	±	+	+
Colon									
Inflammatory infiltrate									
LC	−	±	±	±	±	±	+	+	+
PMN	±	±	±	±	±	±	+	+	+
EOS	−	−	±	±	±	±	+	+	+
Villous damage	−	±	−	±	±	±	±	+	+
Cryptitis	−	±	±	±	±	±	+	±	+

Histopathologic findings were graded in five stages from − (none) to +++ (severe), as shown in Text.
ND, no diagnosis because of severe pancreatitis.
PMN, polymorphonuclear cell; LC, lymphocyte; EOS, eosinophil; PV/CV, portal/central vein.
MVTX: See Table I

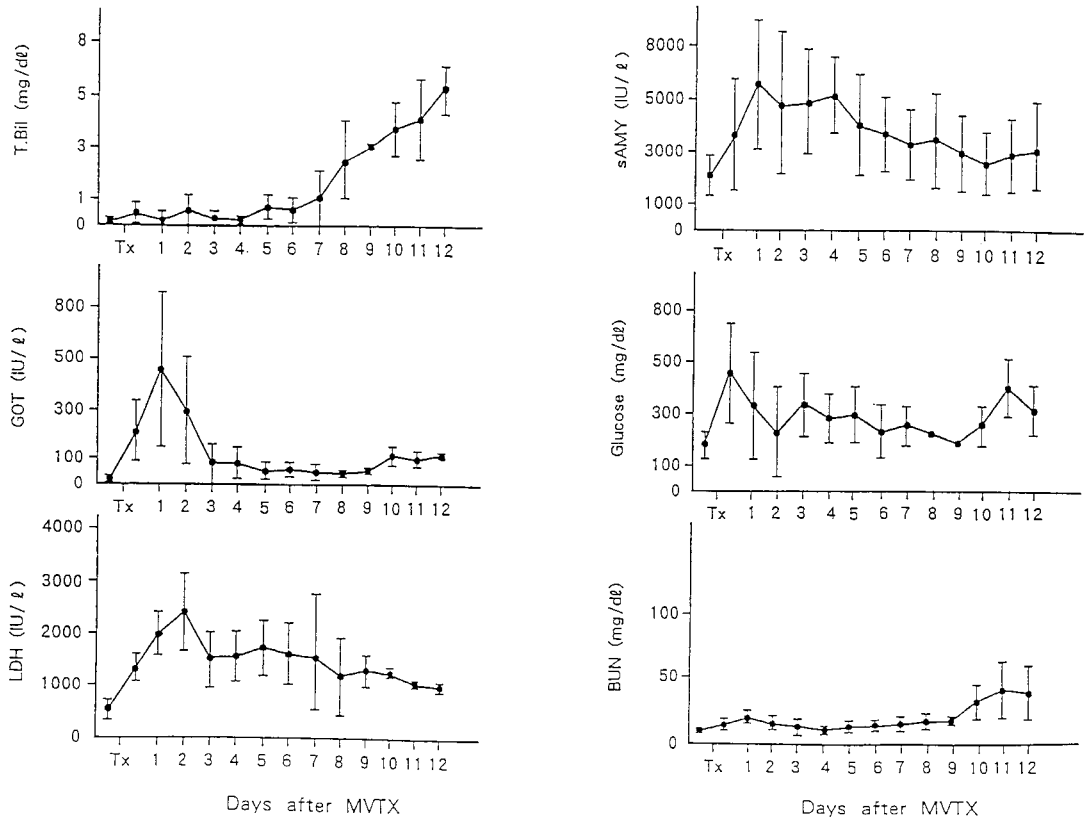

Fig. 2 Organ function after MVTX (liver, pancreas, and kidney). MVTX: See Fig. 1.

Table 3 Summary of allograft rejection in the visceral organs after MVTX

POD	Liver	Pancreas	Stomach	Duodenum	Small bowel	Colon
5th	−	−	−	+	+	−
7th	−	−	−	+	+	−
8th	−	−	−	+	+	−
10th	+	+	−	##	##	+
14th	+	##	+	##	##	##
16th	+	+	+	##	##	##

Severity of rejection was graded in four stages from − (none) to ## (severe) as shown in Text.
POD, postoperative day. MVTX: See Table 1.

recovered to within normal limits on day 9 or 10. BUN remained within the normal range until about 9 days after surgery when it rapidly increased until death.

Pattern of rejection after MVTX. Histopathologic changes of the liver were portal inflammation, endotheliitis, and parenchymal changes; those of the pancreas were inflammatory infiltrates, endotheliitis, and parenchymal change; and those of the stomach, duodenum, small bowel, and colon were inflammatory infiltration, villous damage, and cryptitis. These histopathologic findings were graded as follows: (−) none; (±) mild; (+) moderate; (++) strong; (+++)

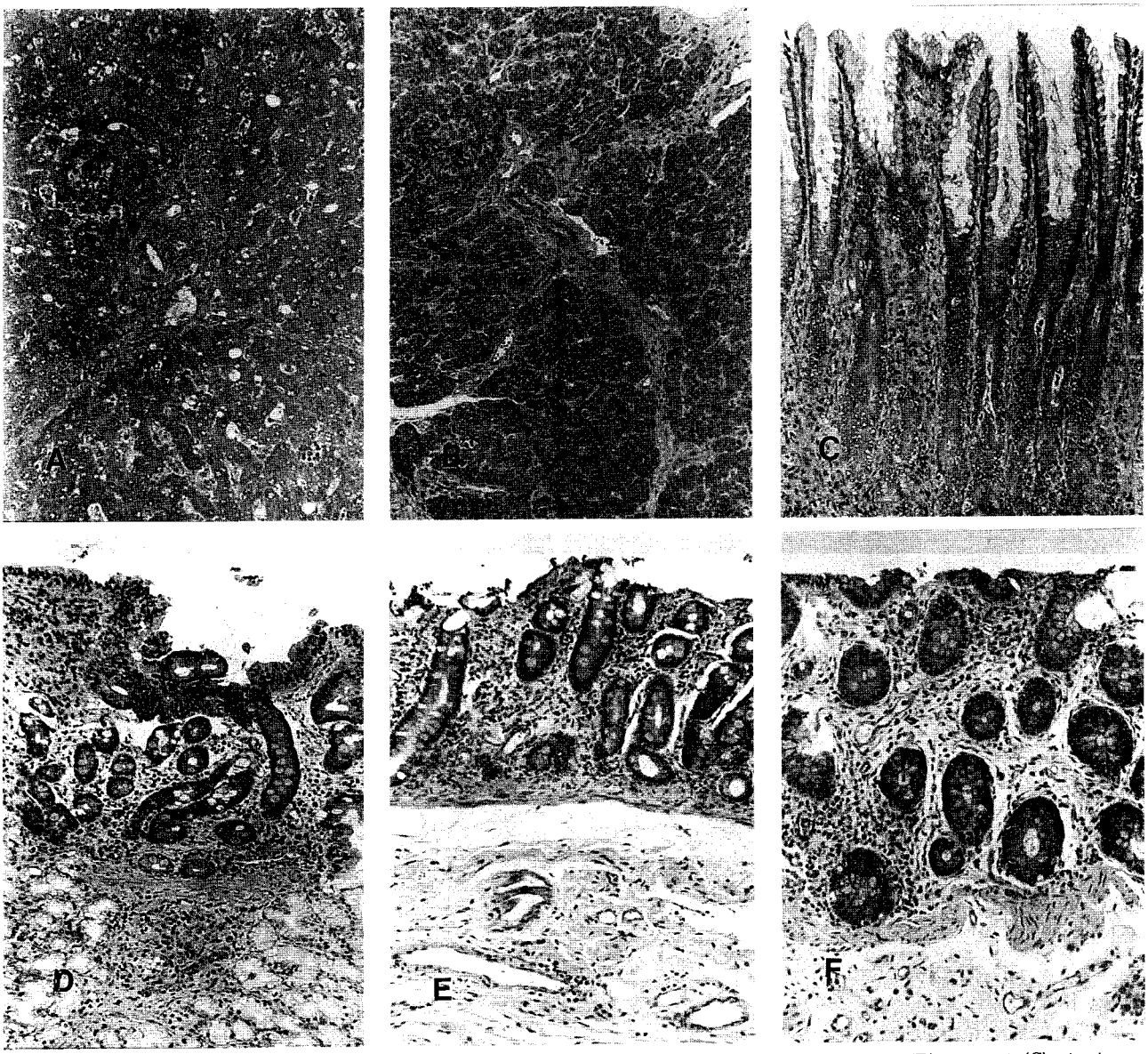

Fig. 3 Microscopic appearance of MVTX grafts on day 16 in the case of Pig No. 9. The liver (**A**), pancreas (**B**), stomach (**C**), duodenum (**D**), small bowel (**E**), and colon (**F**) on the 16th postoperative day (x100). Rejection is indicated by inflammatory infiltrates and fibrosis, severe in the duodenum and small bowel, moderate in the colon, and mild in the liver, pancreas, and stomach. MVTX: See Fig. 1.

severe, as shown in Table 2. Summing up these pathological findings of each organ, we evaluated allograft rejection in the liver, pancreas, stomach, duodenum, small bowel, and colon. As shown in Table 3, the duodenum and small bowel already showed mild rejection on day 5 after transplantation, but no rejection was observed in any of the other organs. On day 10, the liver, pancreas, and colon showed mild rejection, and the duodenum and small bowel showed moderate rejection, but no rejection was observed in the stomach. On day 16, all organs demonstrated rejection, but the degree of rejection differed among transplanted organs. Severe rejection was observed in the duodenum and small bowel. Moderate rejection was observed in the colon, but the liver, pancreas, and stomach still showed only mild rejection. Recipient tissues including the heart, lung, and skin were

also examined. No specific abnormalities were observed except for bronchopneumonia in the lungs. No skin lesions related to graft-versus-host disease (GVHD) were found in any animal. Fig. 3 shows the histology of specimens obtained from each visceral organ on day 16 in MVTX.

Discussion

We established a swine MVTX model to study the biochemical and immunological aspects of MVTX. It has been reported that the individual organs in MVTX exhibit different features of rejection (8). We also examined, using the swine model, the features of rejection in each transplanted organ when multiple visceral organs were transplanted simultaneously.

In this study, hepatic rejection was observed histologically on day 10, but it was estimated to have started earlier, because T.Bil. had already increased (beginning on day 8). The liver showed necrosis of hepatocytes and cholestasis on day 8 and inflammatory infiltration in Glisson's capsules on day 10. The increase in T.Bil. was considered to be due to cholangitis. Pancreatic rejection was also demonstrated by round cell infiltration in fibrosing stroma histologically on day 10. The serum levels of glucose and sAMY peaked on the 1st day after surgery, and the glucose level showed a second peak on day 11, which was coincident with rejection of the pancreas. The increased BUN on day 9 was estimated to be due to acute tubular necrosis (ATN), because many cases showed degeneration and necrosis of the renal tubules. Monitoring of small bowel rejection is difficult. Intestinal perforation due to rejection has been reported on MVTX in dogs (1), rats (8), and pigs (2), but it was not observed in the present study. Functional tests have proved to be of little value in detecting small bowel rejection. Only deep biopsies provide sufficient information for rejection. In this study, rejection of the small bowel was first confirmed by biopsy on day 5. Recipient pigs developed severe diarrhea from day 2 or 3, but it could not be determined whether it was induced by small bowel rejection or not.

The question of whether or not rejection in multivisceral grafts occurs at the same time and with the same intensity is an interesting one. Our findings suggested that the duodenum and small bowel is more susceptible to rejection than the liver, pancreas, and stomach. Yamauchi *et al.* (7) suggested that the cause of organ-specific susceptibility to rejection may be due to the differences in expression of major histocompatibility complex antigens among transplanted organs. Oberhuber *et al.* (9) examined the pattern of rejection after transplantation of the stomach, small bowel, and pancreas in rats. They reported that the small bowel was more susceptible to rejection than either the stomach or the pancreas. Therefore, mucosal biopsies of the stomach were unlikely to provide a reliable guide to rejection in the small bowel. Our study also indicated that the small bowel was more susceptible than the stomach. This phenomenon may be explained by the fact that lymphoid tissue in the stomach is relatively poor quantity in compared with that in the intestine.

Early reports in rats (8) and pigs (10) indicated that the liver in MVTX was rejected later or less severely than that in LTX. Furthermore, in our studies, the rejection of the liver in MVTX was detected about 3 days later than that in AOCTX (3). The delayed appearance of hepatic rejection in MVTX is considered to be mainly due to the presence of the small bowel allograft, which is replete with mucosa-associated-lymphoid tissue. It is also likely that the liver in MVTX escapes from the attack of allogeneic leukocytes, since the small bowel is the first and main target of leukocyte attack.

To our knowledge, MVTX has been performed in 7 patients by 1991 (2, 11–14), four of whom survived for more than 3 months. Starzl *et al.* (2) reported a 3-year-old girl who survived for 192 days after MVTX under cyclosporine-based immunosuppression, and died of Epstein-Barr virus (EBV)-associated lymphoproliferative disorder (LPD). The authors indicated that over-immunosuppression was implicit in the development of LPD. Williams *et al.* (11) also reported a patient who underwent MVTX and died of EBV-associated LPD. They suggested that MVTX itself might have conferred an immunosuppressive influence on the host. Recently, the new immunosuppressive drug, FK-506 has been used to achieve clinical improvement in difficult cases of small bowel and MVTX. Todo *et al.* (14) reported a 32-year-old man who underwent MVTX under FK-506-based immunosuppression, and survived for more than 5.6 months. They demonstrated that FK-506 was effective for MVTX, small bowel transplantation, and liver-intestinal transplantation. They did not report severe side effects of FK-506 and EBV infection. In our study of AOCTX (3), we observed that daily administration of FK-506 0.1 mg/kg effectively prevented cluster allograft rejection. In the present study, we have experienced only

one case of swine MVTX with FK-506. The recipient showed a good postoperative course with amelioration of severe diarrhea and survived for 3 weeks, and no side effects of FK-506 were observed. Histological examination indicated that mild rejection was observed only in the small bowel, and that rejection was prevented in other transplanted organs.

When any large organ containing lymphoid tissue is engrafted, fatal graft-versus-host disease (GVHD) may occur. Starzl *et al.* (2) reported that pigs which underwent MVTX developed skin lesions caused by GVHD. In this study, no GVHD-related skin lesions were observed, although the mesenteric lymph nodes in the graft was showed generalized swelling in all cases. Histological examination of transplanted lymph nodes and spleen was performed in only one case (No. 3). The mesenteric lymph nodes showed marked lymphoid hyperplasia in the paracortical area and the spleen showed atrophy of the lympatic follicles. It was not clear whether these findings depend on rejection or on other causes.

In conclusion, the present study indicates that individual organs in MVTX are not rejected at the same time and with the same intensity even if transplanted simultaneously. The duodenum and small bowel were rejected more severely than the liver, pancreas, and stomach, showing that the individual organs have different susceptibility to rejection.

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