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Colon vs small bowel rejection after total bowel transplantation in a pig model

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Abstract With the advent of FK506, small bowel transplantation has become clinically feasible. Both clinically and experimentally, jejunal and ileal biopsies are used for early diagnosis of rejection. More recently, the colon, in addition to the small bowel, has been transplanted to decrease the high incidence of diarrhea after small bowel transplantation. A Bishop-Koop ileostomy allows biopsies on a regular basis, but the diagnosis of rejection remains a problem after takedown of the ileostomy. Rejection of the ileum is more frequent and more severe than rejection of the jejunum or the colon. Colon biopsy after ileostomy takedown would not rule out rejection of the ileum.

Key words Small bowel · Rejection · Transplantation · Colon · FK 506

Introduction

The advent of FK506 has made small bowel transplantation a clinical reality [1]. Nevertheless, immunologic problems (e.g., rejection, graft-vs-host disease [GvHD], lymphoma) and infections continue to complicate the posttransplant course of small bowel recipients. An additional difficulty is the development of posttransplant diarrhea, frequently requiring hospitalization secondary to dehydration. In an attempt to decrease stomal output and prolong intestinal transit time, the ileocecal valve and the colon have been added to the small bowel graft [2]. However, the increased lymphatic mass associated with a total bowel (vs small bowel alone) transplant might increase the risk of rejection.

Diagnosing rejection in bowel recipients has been difficult due to the lack of suitable laboratory parameters. In most bowel transplant recipients, an ileostomy is created at the time of transplant, in order to do posttransplant biopsies to monitor rejection. But very little is known about the hierarchy of rejection between the jejunum, ileum, and colon after total bowel transplantation. We studied in a preclinical model the incidence of rejection in all three grafts to determine: (1) which portion of the intestine was most prone to rejection and (2) whether reliable monitoring of rejection after ileostomy takedown was possible by obtaining endoscopic biopsies from the jejunum or colon.

Material and methods

For this experiment, 70 outbred Yorkshire Landrace pigs were randomized as donors or recipients. Five total bowel transplant recipient groups were included: cyclosporin A (CsA) treated pigs ($n = 7$), FK506 treated pigs ($n = 5$), combined bone marrow and total bowel transplant pigs ($n = 8$), bone marrow and total bowel transplant pigs treated with FK506 pigs ($n = 11$), and combined liver and total bowel transplant ($n = 4$). For premedication, atropine (0.2 mg/kg intramuscularly) and thiopental sodium (30 mg/kg intravenously) were used. General anesthesia was maintained with 3 % isoflurane. Donor pigs were fasted for 72 h before procurement. Magnesium citrate was given for bowel preparation, and bowel decontamination was not done. Recipient pigs were fasted for 24 h pretransplant.

Donor operation

Through a midline incision, the portal vein was dissected free from below the uncinate process up to the level of the liver hilum. The pancreas was divided anterior to the portal vein, the splenic vein was ligated and cut, and all pancreaticoduodenal veins were di-

vided. The small bowel was divided proximally (third portion of the duodenum) and the large bowel was divided at the sigmoid colon. The superior mesenteric artery was then identified at its takeoff from the aorta. An aortic tube was dissected free that contained the celiac trunk, which was then ligated. The distal aorta was looped and made ready for perfusion with University of Wisconsin organ preservation solution. Venous drainage was through the distal vena cava. Benchwork preparation of the graft consisted of ligation of the posterior lumbar arteries, then closure with Prolene 5/0 continuous suture of the aortic stump distal to the takeoff of the superior mesenteric artery.

Recipient operation

Total bowel transplantation

The native small and the native large bowel were completely resected, from the third portion of the duodenum up to the sigmoid colon. A Kocher maneuver exposed the recipient portal vein. After systemic heparinization (3000 units), the infrarenal aorta was clamped and the end-to-side anastomosis of the donor aortic tube to the recipient aorta was done using Prolene 5/0 continuous suture. The recipient portal vein was clamped longitudinally and the donor portal vein was anastomosed to the recipient portal vein, in a piggyback fashion, with Prolene 6/0 continuous suture. Mannitol, bicarbonate, and complementary intravenous fluid were given immediately before graft reperfusion. Graft cold ischemic time never exceeded 45 min. An end-to-end duodenoduodenostomy was followed by an end-to-side anastomosis between the donor sigmoid colon and the recipient sigmoid colon. Finally, a Bishop-Koop ileostomy was constructed about 20 cm proximal to the ileocecal valve. All intestinal anastomoses were done with a two-layer technique [3].

Combined liver and total bowel transplantation

After resection of the native small and the native large bowel, the common bile duct and the left and right hepatic arteries were ligated and divided. Before the recipient hepatectomy was begun, a veno-venous bypass was begun under systemic heparinization (100 U/kg). The inferior vena cava was cannulated via the right external iliac vein, the portal vein was ligated at the hepatic hilum and cannulated distally, and the venous return cannula was inserted into the right internal jugular vein. The hepatectomy was done, and the en bloc liver-bowel graft was brought into the field. The suprahepatic followed by the infrahepatic caval anastomoses were done first and a 3 cm segment of the infrahepatic caval anastomosis was left open as a vent. The donor aortic conduit was anastomosed end-to-side to the infrarenal aorta and the recipient portal vein was anastomosed in a piggyback fashion to the donor portal vein. To decrease portal clamping time, most of this latter anastomosis was done with the portal cannula still in place. The aortic conduit and the portal vein were unclamped and the graft was revascularized. The donor common bile duct was ligated and the proximal end of the donor small bowel was anastomosed side-to-side to the recipient gall bladder. The recipient duodenum was anastomosed end-to-side to the donor jejunum 60 cm distal to the biliary anastomosis [4].

Immunosuppression

CsA pigs received quadruple immunosuppression for induction: horse antipig thymocyte globulin (ATG, 10 mg/kg for 10 days), CsA (3 mg/kg per day), prednisone (2 mg/kg per day), and azathioprine (2.5 mg/kg per day). Prednisone and azathioprine were reduced by 50% at 8 days and again at 15 days posttransplant. CsA whole blood concentrations, as determined by high-pressure liquid chromatography (HPLC), were maintained > 400 ng/ml for the first 7 days posttransplant, then between 200 and 400 ng/ml thereafter.

FK506 pigs received FK506 (0.2 mg/kg per day) and prednisone (2 mg/kg per day) for induction and maintenance. Prednisone was reduced by 50% at 8 days and again at 15 days posttransplant. FK506 trough levels, as determined by a microparticle enzyme immunoassay (ABBOTT IMX®), were maintained between 10 and 35 ng/ml posttransplant.

All immunosuppressive regimens were given intravenously. ATG and FK506 were infused daily over a 3-h period; CsA, prednisone, and azathioprine were given daily as single injections. Rejection episodes were not treated in any group.

Preparation of immunosuppressants

ATG

Polyclonal horse ATG was produced by immunizing a horse with 3.4×10^9 fresh pig thymocytes in complete Freund's adjuvant on days 0 and 14. The horse underwent plasmapheresis starting at 21 days, on a 3-day schedule for a total of 15 bleeds. Plasma from each plasmapheresis was stored at −20°C until pooled for final fractionation. An equal volume of each bleed was then pooled, adsorbed with human red blood cell membranes (stroma), then adsorbed with pig red blood cell stroma. The plasma was stabilized with SiO_2, and the biologically active horse IgG was isolated by OAE chromatography. The final product was filtered and bottled at a protein concentration of 50 mg/ml. Horse ATG (POT 100) was stored at −20°C until used [5].

CsA

The CsA (100 mg/ml) for intravenous injection was prepared from CsA powder (50 g) added to ethanol (250 ml). Then, a sufficient quantity of sterile water was added to obtain a final volume of 500 ml for injection. The final solution was passed through a 0.22 μm filter placed in sterile vials.

Bone marrow preparation

Fresh donor bone marrow was obtained from the exsanguinated donor at the time of procurement. Bilateral long bones (tibiae, femora, and humeri) served as donor bones for marrow collection. Bone marrow cells were immediately processed, using a method identical to our clinical bone marrow transplant program. First, bone debris and fragments were removed and single cell suspensions were prepared. Stroma cells were removed and bone marrow cells were then isolated from neutrophils and red blood cells by density gradient separations. Trypan blue exclusion tests, done on the final cell preparation, indicated more than 95% cell viability. Fresh bone marrow cells were then infused intravenously a few hours posttransplant. Two doses of DSBMT were tested, low dose (5×10^7 bone marrow cells/kg) and high dose (5×10^8 bone marrow cells/kg).

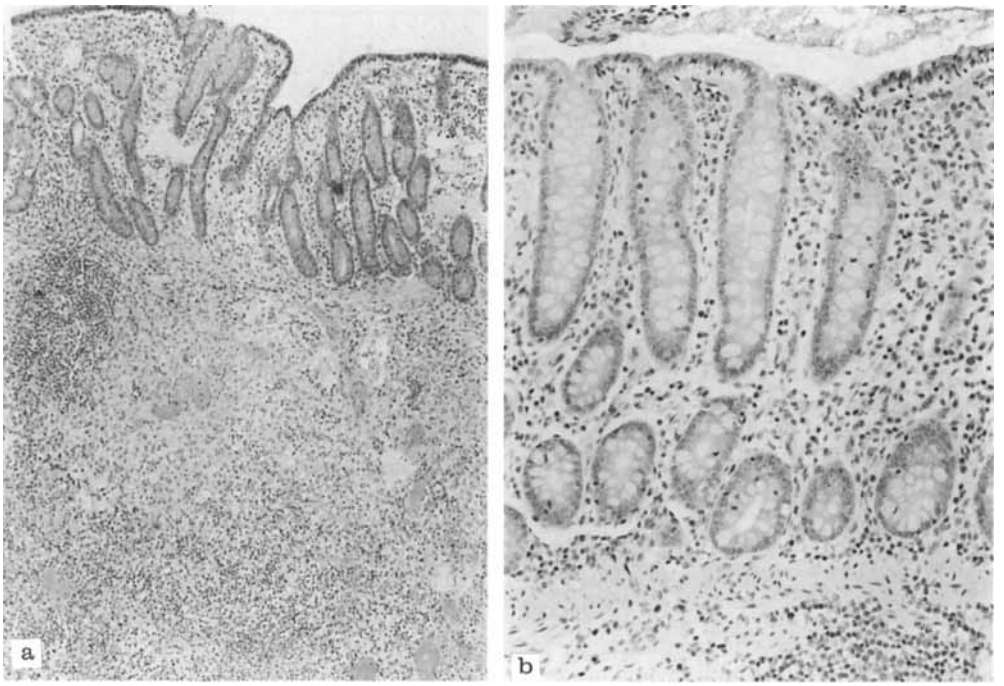

Fig. 1a Mild to moderate rejection of the colon; there is an increase of inflammatory infiltrate in the mucosa and submucosa (H&E). **b** A higher magnification shows prominents intraepithelial lymphocytes and occasional epithelial cell necrosis (H&E)

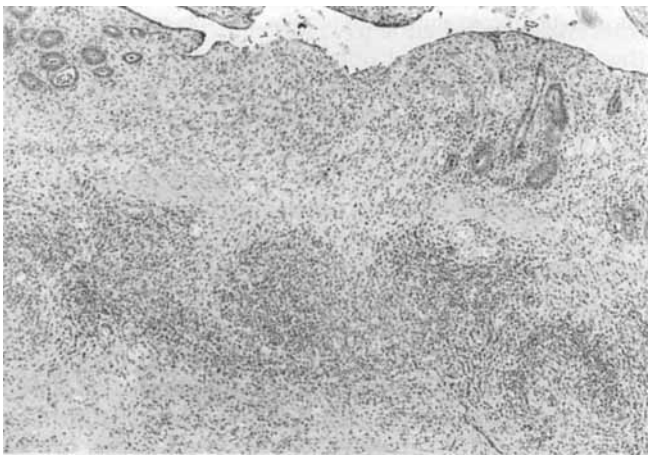

Fig. 2 Severe rejection of the colon shows extensive epithelial loss with increased lamina propria and submucosal inflammation (H&E)

Postoperative care

Recipient pigs received Ringer's lactate with D5W for the first 2 days posttransplant, via a central line placed in the left internal jugular vein. Clear liquids were started on day 3 and normal diet was resumed on day 4. Buprenorphine hydrochloride was given for analgesia (0.3 mg/ml q 6 h). Pigs received the following antibiotic coverage for 7 days: cephalothin (500 mg/ml qd), ticarcillin (1 g b.i.d), and metronidazole (250 mg qd). After 7 days, we did not attempt to treat infectious episodes. Pigs losing more than 30 % of their initial body weight were killed according to the guidelines of the University of Minnesota's Research Animal Resources Committee.

Pathologic evaluation

Pigs survived 3–81 days posttransplant. Of the 35 pigs, 3 % died before 7 days posttransplant, 46 % between 7 and 14 days, 3 % between 14 and 28 days, and 48 % after 28 days. Deaths were due to three main reasons: rejection, GvHD, and infection. At the time of death, tissue sections were taken from the jejunum, ileum, and colon. The sections were fixed in 10 % buffered formalin, paraffin embedded, sectioned at 4 μm intervals, and stained with hematoxylin and eosin. One pathologist (REN) reviewed all the material without knowledge at the experimental groups.

Tissue rejection was evaluated using a previously published grading scheme for interstitial and vascular rejection of small bowel [5]. This grading scheme was modified slightly for colonic mucosa since the colon does not have a villous architecture. Mild interstitial rejection of the colon was defined as mildly increased lymphoplasma cellular infiltrate within the lamina propria and increased intraepithelial lymphocytes with goblet cell loss and occasional epithelial cell necrosis (Fig. 1 a, b). Moderate interstitial rejection of the colon was defined as moderately increased lymphoplasma cellular infiltrate within the lamina propria and increased intraepithelial lymphocytes with goblet cell loss and easily identifiable epithelial cell necrosis. Severe interstitial rejection of the colon was defined as extensive epithelial necrosis with increased lymphoplasma cellular lamina propria infiltrate (Fig. 2). Interstitial rejection of the small bowel was defined very similarly, but with the added feature of mild, moderate, and severe villous blunting (Figs. 3, 4). Vascular rejection was defined as mild, moderate, and severe with the presence of endothelialitis, vasculitis, and vasculitis with fibrinoid necrosis, respectively.

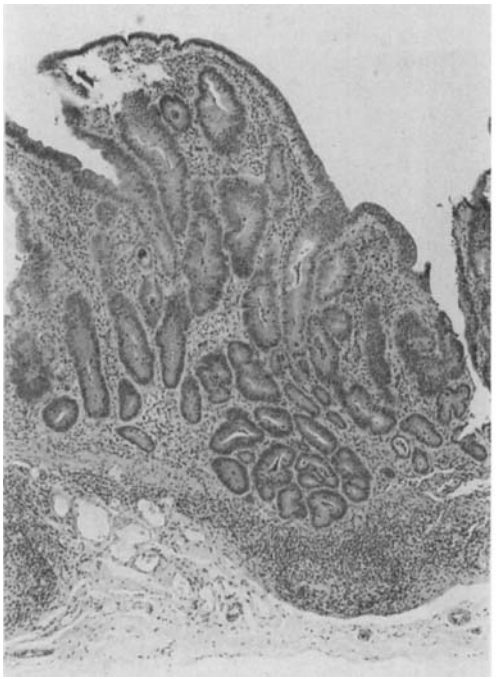

Fig. 3 Moderate rejection of the ileum. There is moderate villous blunting with increased lamina propria inflammation and epithelial cell necrosis (H&E)

Fig. 4 Severe rejection of the ileum. There is extensive epithelial necrosis with complete blunting of the villi. The infiltrate involves the mucosa, submucosa, muscularis propria, and subserosa (H&E)

Results

Ileum vs colon (Table 1)

Of the 35 pigs, 16 (46 %) had morphologically normal tissue in the ileum and colon, 4 (12 %) had necrotic bowel wall in both grafts, and 6 (18 %) had rejection in both grafts. Five pigs (14 %) had rejection of the ileum, but normal colon morphology. None of the pigs had rejection of the colon without rejection of the ileum, but 1 pig (3 %) showed rejection of the ileum with pseudomembranous colitis of the colon, 1 (3 %) showed fungal infection of the colon with normal ileal morphology, 1 (3 %) showed necrosis of the ileum with a normal colon, and 1 (3 %) showed necrosis of the ileum with rejection of the colon.

Of those pigs with discrepant diagnoses, 2 showed moderate interstitial rejection and 3 showed mild interstitial rejection, while the colons were normal. Of the 6 pigs with rejection in both grafts, 3 had identical rejection grades; in the other 3, rejection was more severe in the ileum than in the colon.

Ileum vs jejunum (Table 2)

Of the 35 pigs described above, 32 also had jejunal tissue sections taken. Of those, 16 (49 %) pigs had normal jejunal and ileal tissue. However, 5 pigs (15 %) showed rejection of the jejunum and the ileum and another 5 (15 %) showed necrosis of the jejunum and ileum. In 4 (12 %) pigs rejection was seen in the ileum but not the jejunum, in 1 (3 %) pig the ileum showed necrosis with rejection of the jejunum, and in 1 (3 %) pig the ileum was normal with necrosis of the jejunum. None of the pigs had rejection of the jejunum without rejection of the ileum.

In pigs with rejection of both the jejunum and the ileum, interstitial rejection was worse in the ileum than in the jejunum. In 2 pigs endothelialitis was seen in the jejunum but not in the ileum; in another 2 pigs endothelialitis was seen in the ileum but not in the jejunum.

Jejunum vs colon (Table 1)

Of the 32 pigs from which tissue sections of the jejunum and colon were taken, 17 (51 %) were normal, 5 (15 %) showed rejection of both grafts, and 4 (12 %) showed necrosis of both grafts. In 2 (6 %) pigs there were discrepancies with regard to rejection; one each of the grafts showed rejection while the other did not. In 4 (12 %) pigs four other discrepancies were noted in the following jejunum/colon combinations: one necrosis/rejection, one necrosis/normal, one normal/fungal infection, one normal/pseudomembraneous colitis.

Table 1 Findings in the colon vs the ileum and the jejunum

		Colon				
		Normal	Rejection	Necrosis	Fungal infection	Pseudomembranous colitis
Ileum ($n = 35$)	Normal	16	–	–	1	–
	Rejection	5	6	–	–	1
	Necrosis	1	1	4	–	–
Jejunum ($n = 32$)	Normal	17	1	–	1	1
	Rejection	1	5	–	–	–
	Necrosis	1	1	4	–	–

Table 2 Findings in the jejunum vs the ileum

Ileum	Jejunum		
	Normal	Rejection	Necrosis
Normal	16	–1	
Rejection	4	5	–
Necrosis	–	1	5

Discussion

Total bowel transplantation offers the opportunity when the ileostomy is eventually taken down to biopsy the colon to monitor for rejection. An abundance of literature addresses small bowel rejection in animal models with an expanding body of literature on human small bowel transplantation. However, little information exists on colon transplantation. The colon is functionally, architecturally, and morphologically different from the small bowel. Therefore, we can expect that the rate and susceptibility of colon rejection may be different from the small bowel. From prior experience with multiple organ transplantation, we know that different organs reject at different rates [6, 7]. Moreover, transplantation of multiple organs may show a different rate of rejection than if the same organs were transplanted individually [7].

In this study, we examined the occurrence of rejection in the colon vs the small bowel. Although five different treatment groups were included, our comparisons were not made in aggregate, but rather for each recipient. The efficacy of the five different treatments will be commented on elsewhere.

While 46 % of pigs did not show rejection of either graft, five more pigs showed rejection in the ileum than in the colon. Furthermore, in several pigs, rejection was more severe in the ileum than in the colon. Therefore, the morphologic manifestations of rejection either occur earlier or are more prominent in the ileum. Either way, the small bowel is a better source of biopsy tissue or specimens for detection of rejection. Similar findings have been reported in a mouse model and in a small series of human small bowel and colon transplants. Simeoni et al. [8] noted that small intestinal biopsy specimens were more informative than colon specimens. Plapler and Cohen [9] suggested that in rats colon allograft rejection was not as severe as small bowel rejection. Clinical incidents of normal colon tissue with advanced ileal rejection have also been reported [2]. From a practical perspective, the ileum seems to be the better organ to biopsy, since colon rejection was not seen without rejection of the small bowel. Therefore, ileostomy takedown should not be done until the risk of rejection is minimal. We found that rejection also occurs at a higher rate in the ileum than in the jejunum. In several pigs, rejection was more severe in the ileum than in the jejunum. These findings justify the use of a Bishop-Koop ileostomy for monitoring rejection.

The features of rejection are similar in the colon and in the small bowel. A notable difference is based on the microanatomy of the small bowel in the presence of villi. Villous blunting, although nonspecific, may provide a very early sign of rejection or be the clue to look for other features. One of the earliest detectable features of rejection in the colon is an increase in intraepithelial lymphocytes with goblet cell loss and possible epithelial cell necrosis. Intraepithelial lymphocytes stand out more notably in the colon and more notably than increased lamina propria inflammation. Rejection progresses with further increases in lamina propria inflammation and increases in epithelial necrosis. Vascular changes, such as endothelialitis and vasculitis, are also identified in the colon. While the primary reason for colon transplantation is to prevent or help control diarrhea, other severe diseases are also seen in the colon (as demonstrated by this study) such as pseudomembranous colitis and fungal infection.

In summary, our study shows that: (1) rejection affects the small bowel more frequently than the colon, (2) colon rejection correlates with small bowel rejection, but a normal colon biopsy may not indicate a normal small bowel, and (3) rejection is more frequent and more advanced in the ileum than in the jejunum. Thus, if only one bowel segment is to be biopsied, the ileum should be chosen. Consequently, ileostomy takedown should not be done unless the risk of rejection appears to be minimal, since biopsies of the jejunum or colon that are obtained via upper or lower endoscopy might miss rejection of the ileum 10–15 % of the time.

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