A chronic model of bowel allotransplantation is described. End-to-end microvascular anastomosis between superior mesenteric vessels was utilized. The recipient splenic vein was preserved to avoid postoperative pancreatitis. Euro-Collins solution was used to flush the vasculature in the lumen of the transplant. Low-dose cyclosporine was used for immunosuppression. With experience, 89% long-term survival was achieved.

MICROSURGERY 11:293–295 1990

A TRIBUTE TO THE LATE DR. RYO SHIMAZU: A CHRONIC BOWEL ALLOGRAFT MODEL IN THE RAT

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Dr. Ryo Shimazu was born in Sumoto, Japan, in 1952. He graduated from Tokyo University School of Medicine in 1977 and did postgraduate work at Tokyo University, Third Department of Surgery, Showa Hospital. He came to the United States in 1986 and worked in the surgical laboratories of the University of Mississippi Medical Center under the direction of the coauthors. Dr. Shimazu had been successful with total small bowel allograft (nonauxiliary) in an outbred rat model in Japan. However, he could not reproduce the model in an ACI-Lewis combination here. Dr. Shimazu was persistent and worked long hours to develop a successful model, but to no avail. After almost 6 months of continuous work, he was ready to abandon the field but was persuaded to test his technique in another rat strain combination. Fortunately, this model was successful and formed the basis of further studies that he undertook in small bowel transplantation. With conventional dosage of cyclosporine, he found long-term survival to be poor, however. Many animals died without signs of classical rejection. Dr. Shimazu thought that the high death rate was possibly due to overimmunosuppression and wanted to try a low-dose cyclosporine regimen. Even though the coauthors did not have confidence in the successful outcome of this strategy, he was encouraged to try. From this trial came the remarkable finding of indefinite allograft survival with low-dose cyclosporine regimen administered for 14 days and then completely stopped. It is not clear whether this finding can be extended to other strains and species. Nevertheless, it is clear that a powerful model of small bowel allotransplantation is now available for extensive study. Dr. Shimazu returned to Japan at the end of 1988 to set up a laboratory for the study of small bowel transplantation in Tokyo University. He died suddenly and unexpectedly in May, 1989, from an episode of dysarrhythmia. He is survived by his wife, Akemi, and two children, ages 6 and 8. His perserverance, meticulous attention to detail, and above all scientific brilliance provide an example worthy of emulation by aspiring young scientists. The chronic bowel allograft model that he developed is described below by the coauthors as a tribute to his memory.

INTRODUCTION

With the introduction of cyclosporine A, successful clinical application has extended to most solid organ transplants, with some notable exceptions. Despite several clinical attempts, long-term survival with small bowel transplantation has not yet been achieved. A large number of patients with short gut syndrome who are currently being maintained on total parenteral nutrition may benefit if the

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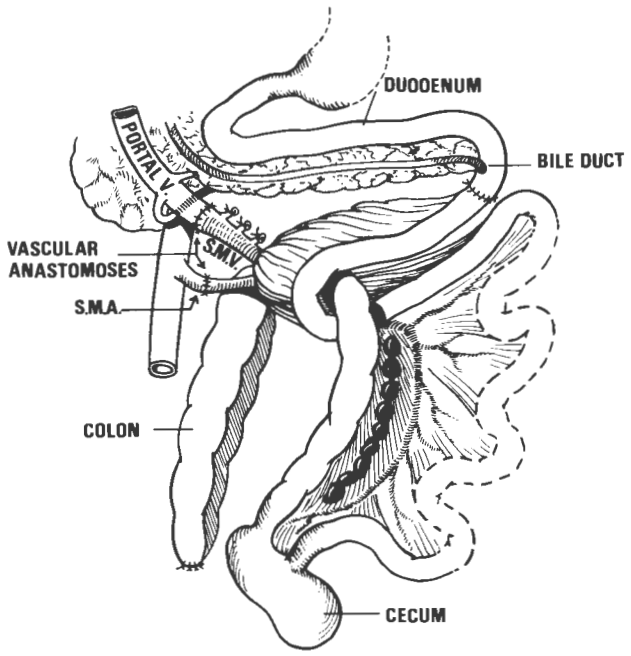

Figure 1. Construction of bowel allograft model.

small bowel can be transplanted successfully. Lack of a chronic allograft model for experimentation has impeded progress in this area. A successful long-term model was recently developed in our laboratories and is described below.

MATERIALS AND METHODS

Brown-Norway rats were used as donors and Lewis rats as recipients. These two strains differ at a major histocompatibility locus. Large rats (200–300 g body weight) were chosen, because vascular anatomoses in smaller animals were more difficult. Both donor and recipient were fasted for 24 hours but were allowed glucose or sucrose water (3–4%) ad libitum.

Operative Procedure (Fig. 1)

Phenobarbital (45 mg/kg) anesthesia was used for both the donor and the recipient.

Donor Procedure

Through a long midline incision, the right colon was retracted out of the abdomen, wrapped in a moist gauze, and kept out of the way. The small bowel itself was retained in the abdominal cavity but packed away from the area of dissection. The pancreas and omentum were freed from the colonic edge and mesentery, exposing the portal vein. The ligament of Treitz was divided and the segment of splenic vein between the splenoportal junction and the inferior mesenteric vein was exposed. It was necessary to ligate and divide a few small vessels around the pancreatic head during this procedure. The distal 1 cm of colon was resected and the remaining portion freed proximally to the splenic flexure. Heparin (100 U) was given intravenously, usually through the inferior vena cava, utilizing a fine hypodermic needle. The superior mesenteric artery was identified and partially freed at this point. The aorta was identified and clamped at the diaphragmatic level. The distal aorta just proximal to the bifurcation was cannulated and the bowel graft infused with Euro-Collins solution in situ. The solution contained heparin (1.5 U/ml) and was kept at 4°C until used. Approximately 5–8 ml of infusate was required. After infusion in situ, the graft was removed by transecting the superior mesenteric artery and vein as high as feasible. After resection, the graft was gently irrigated through the cut end with cold (4°C) lactated Ringer's solution containing 0.1% kanamycin followed by Euro-Collins solution at 4°C. An additional needle hole in the cecum was found to be useful for more thorough irrigation of graft lumen.

Recipient Procedure

An intravenous catheter (utilizing the inferior epigastric vein) was placed in the anesthetized animal and lactated Ringer's solution continuously infused at approximately 1 ml/hour. During declamping of the vascular anastomosis, the animal often required bolus infusion of IV fluid (2–3 ml) to counteract hypotension that occurred during this period. A long midline incision was used and the entire intestine delivered out of the abdomen. The distal colon was ligated, the blind end being left in the pelvic cavity. The superior mesenteric artery and portal vein were cleared in a manner similar to that in the donor. The duodenum was divided approximately 0.5 cm distal to the entry of the common bile duct. Along a line from this point to the portal vein, the intervening pancreatic tissue corresponding to the uncinate process of the human was simply ligated and excised. With division of the superior mesenteric artery and vein, recipient bowel could now be removed and discarded. It was necessary to divide the superior mesenteric vein below the entry point of the splenic vein. Retention of the splenic vein was found to be important in preventing postoperative pancreatitis.

The cooled transplant was then placed in the recipient's abdomen and properly oriented. The graft was continuously moistened with cooled lactated Ringer's solution for proper preservation until the anastomoses were complete. The vascular anastomoses were performed between the superior mesenteric artery of the donor and recipient and the superior mesenteric vein of the donor and recipient in end-to-end fashion. Nylon sutures (10-0) were placed in interrupted fashion under microscopic magnification. The duodenums of the donor and recipient were anastomosed with interrupted 6-0 silk sutures as a single layer. The hole in the cecum was closed with a pursestring suture of 6-0 silk. The

distal end of the transplant was brought out as a colostomy on the left flank. The abdominal incision was closed in routine manner in two layers with 10-0 nylon sutures.

Postoperative Care

Intravenous fluid administration was maintained as long as was feasible, usually for 4–6 hours after completion of the procedure. Animals were kept warm under a heat lamp and were given fluid ad libitum from the day of operation. Normal diet was resumed on the third postoperative day. As a supplement, all animals received 10 ml of 5% dextrose in Ringer's lactate subcutaneously daily for 3 days after surgery. Cefazolin sodium (20 mg) was given subcutaneously twice per day for up to 5 days after surgery.

Immunosuppression

The recipients received cyclosporine A (IV preparation, 1:4 dilution with saline; Sandoz) at a dosage of 5 mg/kg/day given subcutaneously for 14 days beginning the day of surgery. No further immunosuppression was necessary after the initial 2 week course.

RESULTS

Eighty-nine percent of the transplanted animals survive indefinitely with the above regimen. The experimental animals generally lose 10–20% of their body weight in the first 10 postoperative days. A gradual weight gain begins after this period, all animals attaining or exceeding preoperative weight by postoperative day 13. Around 30–40 days after surgery, there is another period of weight loss accompanied by diarrhea and extensive dermatitis around the colostomy. This is thought to represent a graft-vs.-host reaction. Spontaneous resolution occurs and animals begin to gain weight again. The survivors exhibit interesting immunological phenomena not commonly observed in other allograft situations between genetically disparate donor/recipient combinations. The recipients reject donor-specific skin grafts in an indolent fashion.[1] Successive second and third set skin grafts are rejected somewhat more rapidly, but still in a very slow fashion compared to third-party skin grafts. Although the donor-specific skin grafts are being rejected, the animals have continued to maintain their small bowel allograft in intact fashion indefinitely. The unusual immunological mechanisms operative in this allograft model are currently under investigation.

DISCUSSION

During development of the model, several details were found to be crucial in reducing mortality. These include continuous intravenous fluid administration during surgery and the early postoperative period, use of Euro-Collins rather than Ringer's lactate for graft infusion, additional graft lumen irrigation with antibiotic-loaded Euro-Collins solution, and meticulous vascular technique with end-to-end vascular anastomosis. In our early experience, without intravenous fluid support, a large number of animals died in shock during the postoperative period. The use of Euro-Collins rather than lactated Ringer's solution for graft infusion markedly improved viability of the allograft. With Ringer's lactate solution, graft edema and postoperative bloody diarrhea occurred. This problem ceased after Euro-Collins solution was utilized for luminal as well as vascular infusion into the graft.

The low-dose cyclosporine regimen is particularly important to obtain long-term survival. Whereas a chronic autograft model and an acute allograft model[2] have succeeded in our laboratory, a long-term allograft model was not easily achieved. Use of cyclosporine at high doses[1] leads to increased postoperative mortality and reduced long-term survival.

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