Transplant Int (1988) 1: 135-139

TRANSPLANT
International

Small-bowel transplantation in the rat with a nonsuture cuff technique

Technical and immunological considerations

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Abstract. Small-bowel transplantation (SBT) using an nonsuture cuff technique was carried out on 137 rats. Preparation of the donor graft was carried out according to conventional procedures. Graft perfusion was done at a fixed pressure of 35 cm water. The left renal vessels of the recipient were dissected, the native kidney removed, and the graft was connected to the vessels by a nonsuture cuff technique. Of the animals, 92% survived for at least 5 days posttransplant. Three different combinations were investigated: (1) isografts; (2) semisyngeneic grafts from nontreated Lewis → (Lewis × DA) F1 hybrids; and (3) semisyngeneic grafts from rabbit antilymphocyte globulin (ALG)-pretreated Lewis → (Lewis × DA) F1. In group 1, 80% of the grafts were unaffected after 1 month; flow studies showed slight or no impairment of circulation in the graft. In group 2, the recipients developed clinical signs of graft-versus-host disease (GVHD) after 1 week, and at the end of the 2nd week the animals showed signs of severe illness, leading to death due to GVHD. There was also a higher percentage of complications in this group. In group 3, 65% of the animals died. However, 27% showed intact grafts and no signs of GVDH after 1 month, indicating that antibody pretreatment of the donor may successfully prevent GVHD SBT.

Key words: Small bowel transplantation - ALG - Graft versus host disease.

In patients suffering from short bowel syndrome (SBS), long-term total parenteral nutrition is the only available treatment at present [7]. However, this is associated with several complications, particularly among pediatric patients, and the survival time is limited [9]. An ultimate solution for SBS would be successful small-bowel transplantation (SBT). Only eight cases have been presented since the first attempts in the late 1960s, and none of these was successful [2, 11]. Graft-versus-host disease (GVHD) has probably substantially contributed to the poor results [2]. Considerable knowledge of GVHD has been obtained from bone-marrow transplantation models [13, 18]. However, complete organ grafts are vascularized, and the bowel contains a large quantity of educated immune cells. Studies aimed at the adequate control of GVHD in SBT should therefore include these organ-specific circumstances.

In 1971, Monchik and Russel [16] described a microsurgical technique for SBT in the rat model. Since then, their technical procedure has been used to gather immunological observations about the nature of GVHD after SBT [12]. In the present study, we used a simplified technique to provide satisfactory technical conditions for studying different aspects of SBT, such as the prevention of GVHD.

Materials and methods

Animals

Inbred Lewis and DA rats were obtained from Möllegard, Skensved, Denmark. Lewis and (Lewis × DA) F1 hybrids were bred in our own animal quarters. The animals weighed 200-250 g and received food and water ad libitum.

Donor pretreatment

One group of donor animals ($n=26$) received 1 ml rabbit anti-rat-lymphocyte globulin (ALG) IV 1 day before the operation.

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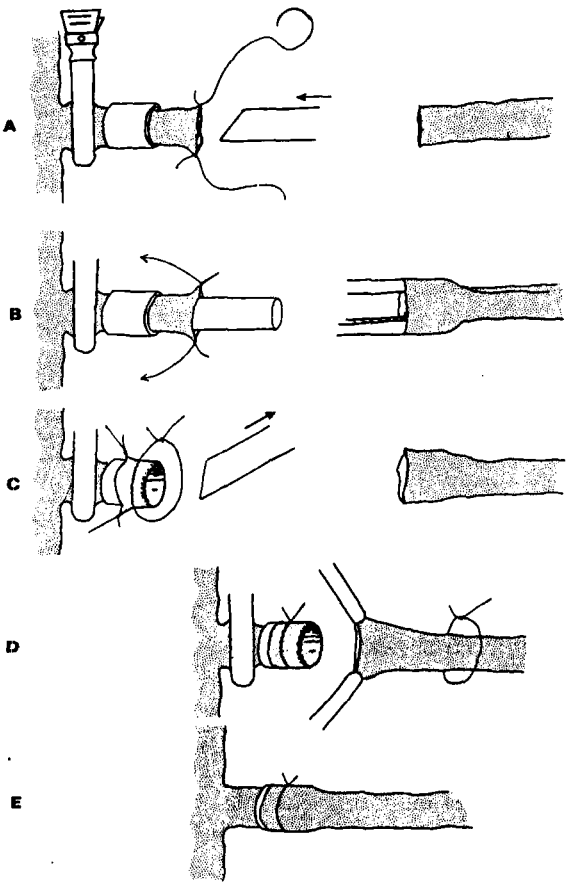

Fig. 1. Schematic illustration of the cuff technique as carried out in the vein. The vessel is clamped, a cuff placed around it *(A)*, a splint inserted *(B)*, and the vessel is turned over the cuff and secured *(C)*. The graft portal vein is carefully dilated *(B)*, pulled over the cuff *(D)*, and secured with a ligature *(E)*

The ALG was produced in our own laboratory by the SC inoculation of rabbits with a single dose of 1×10^9 rat thymocytes mixed with Freund's complete adjuvant. The rabbits were boostered IV with 1×10^9 rat thymocyte suspension 1 week prior to exsanguination. The serum was heat-inactivated at 56° C for 30 min and absorbed with an equal volume of washed erythrocytes. The Ig fraction was purified with ammonium sulfate precipitation and resuspended in phosphate-buffered saline. The titer of cytotoxic antibody to mouse splenocytes was adjusted to 1:1250–1:2500.

Donor operation

The preparation of the donor was carried out mainly as outlined by Monchik and Russel [16]. After preparation of the small bowel on a vascular pedicle, the aorta was clamped proximally and distally to the superior mesenteric artery; a silastic tube was then inserted in the aorta and perfusion was started at a fixed pressure of 35 cm H_2O with an iced histidine- and mannitol-containing buffer (J. Jacobsson, J. Wahlberg, L. Frödin, G. Tufveson, unpublished work). The perfusion required 3–5 ml perfusate and was completed within 1–2 min; meanwhile, the intestinal lumen was irrigated with 20 ml perfusate.

Recipient operation

The preparation of the recipient was done simultaneously to graft harvesting. The left kidney was exposed via a midline incision. The renal artery and vein were freed from surrounding tissue, clamps were placed as proximally as possible, and the kidney was removed after the division of the ureter and vessels. For anastomosis of the vessels, a cuff technique was used mainly as outlined by Olausson et al. [17] (Fig. 1). A plastic cuff with an inner diameter (ID) of 0.75 mm was placed around the artery, a splint was inserted into the lumen, and the vessel was turned inside out over the cuff and fixed with a ligature. The vein was prepared according to the same principle using a cuff with an ID of 1.4 mm. The vessels were carefully dilated and pulled over the cuffs. The anastomoses were secured with a 6/0 silk ligature. The oral end of the graft was closed blindly while an end-to-side anastomosis of the aboral end to the proximal colon was made.

Animal harvesting

The animals were monitored daily after surgery; their weight and general condition were recorded. If signs of severe morbidity were obvious, the animal was killed the same day. Healthy animals were kept for a minimal period of 30 days. The appearance of the organs (spleen, liver, graft, native small bowel, and lymph glands) was assessed, and samples were taken for histological analysis. Animals found dead are included in the survival curves, although no samples were taken.

Histology

Samples of skin, graft, small bowel, spleen, and liver were fixed in 4% formaldehyde, processed, and stained with hematoxylin and eosin for routine light microscopy.

Measurements of intestinal blood flow

Intestinal blood flow (IBF) was measured by an autoradiographic method previously described by Sakurada et al. [19], with modifications by Abdul-Rahman et al. [1]. The animals were anesthetized with IP Ekviticin (5 ml/kg) during blood-flow measurement. Catheters were inserted in one jugular vein and carotid artery. A relaparotomy was carried out and the transplanted and native intestine were identified, whereafter the abdomen was closed and the animal was allowed a steady-state period of 30 min. Thereafter, 40 μCi ^{14}C-iodoantipyrine (IAP) (New England Nuclear, Boston, Mass.) in 1 ml saline was infused IV at a constant rate for 45 s. Repeated arterial blood samples were obtained before and at short intervals during the infusion. The exact time of each blood sample was recorded and used toghether with the isotope concentration of each sample to describe the temporal profile of ^{14}C-IAP concentration. An abrupt cessation of blood flow as well as access to the intestine was obtained with a guillotine procedure using a double-edged chisel. Pieces of the graft and of the native intestine (2 cm long) at approximately the same level were excised, pinned to a cork plate, and frozen within 60 s in isopentane. Autoradiographs were obtained from 20-μm-thick longitudinal sections cut at −20° C and from standards of known activity (Amersham International Ltd., Amersham UK) using Kodak SB 5 X-ray film. The film was exposed for 1 week. Local ^{14}C activities in tissue were determined from the optical

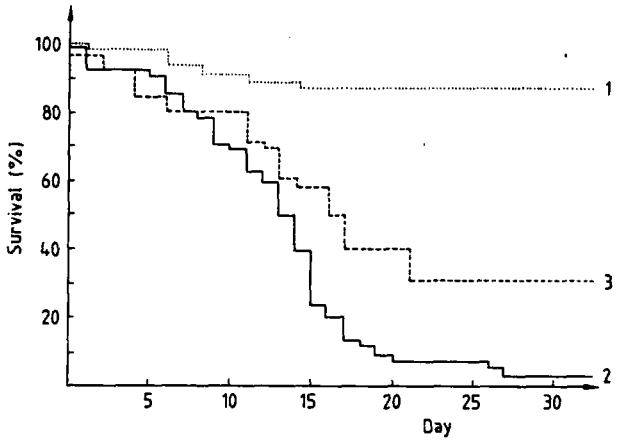

Fig. 2. Survival in three different groups: *1* isogenically grafted animals ($n=55$); *2* semisyngeneic grafts from Lewis → Lewis × DA ($n=57$); *3* semisyngeneic grafts from ALG-pretreated Lewis → Lewis × DA ($n=26$). No exclusions were made due to technical failures, nor were animals sacrificed prior to signs of severe illness

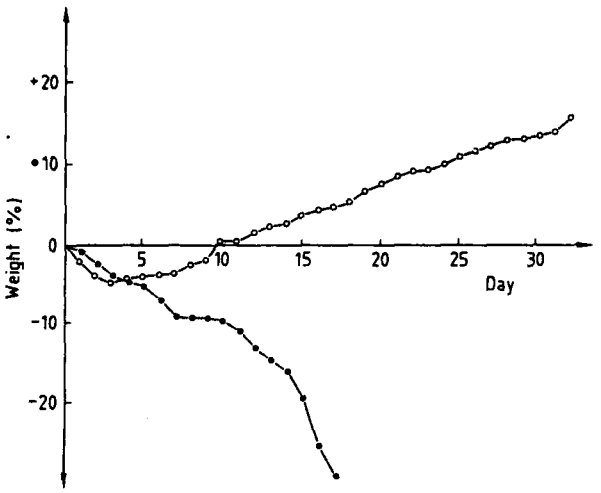

Fig. 3. Weight curve for two groups. O—O Isografts [Lewis × DA → Lewis × DA ($n=17$)]; ●—● semisyngeneic grafts [Lewis → Lewis × DA ($n=19$)]

densities of the intestinal tissue, using a densitometer with an aperture of 0.1 mm (Microdensitometer 3 CS, Joyce Loebel, Gateshead 11, UK). Six determinations of ^{14}C activities were made at each millimeter along the intestine. The mean of these six values was recorded and the IBF was calculated using the equation given by Sakurada et al. [19]. A tissue-blood partition coefficient of 1.0 for ^{14}C-IAP was used [8].

Results

A total of 137 animals underwent surgery as follows: (1) isografts, Lew → Lew or (Lew × DA) F1 → (Lew × DA) F1 ($n=55$); (2) Lew → (Lew × DA) F1 ($n=57$); and (3) Lew (ALG-pretreated) → (Lew × DA) F1 ($n=26$). Intraoperative thrombosis or hemorrhage from the anastomosis were not noted in any case. The mean graft ischemic time in the study was 10 ± 1.4 min. The overall 5-day animal survival was 92% ($n=130$). Figure 2 summarizes the total survival results.

Isografts

In the group receiving isografts, 54 of 55 animals survived for more than 5 days. Five animals in this group developed signs of abdominal abscess or died for unknown reasons during the 1st month. Five of the rats that survived during the observation time had a palpable mass in the abdomen but recovered, gained weight, and did not shown any sign of morbidity at the time of sacrifice. The grafts in these animals were either encapsulated or had been resorbed. The remaining 44 animals in this group (80%) gained weight (Fig. 3) and showed no clinical or histological signs of morbidity. Histopathology showed that the investigated organs (spleen, liver, skin, graft, and native bowel) were all normal, the exception being a slight thickening of the basal membrane in the graft. No villous atrophy or inflammation in the transplanted bowel could be seen.

The values of IBF measured in syngeneically transplanted animals 40 days posttransplantation are shown in Table 1. The distribution was slightly variable along the graft as well as along the native small bowel. The degree of heterogeneity, measured as the range between the lowest and highest values in each sample, did not differ between transplanted and host intestine (Fig. 4). In general, the graft exhibited blood flow values slightly lower than those in the corresponding host bowel (Table 1).

Semisyngeneic grafts

In the second group [Lewis → (Lewis × DA) F1], there was a high mortality during the first 10 days ($n=13$ or 23%). The most common cause of death was peritonitis or abscess, sometimes in connection with ileus. In two cases pneumonia was the cause of death. However, 58% did well for the first 5 or 6 days, when redness of the ears and paws could be noted. After a few more days, weight loss began to be obvious (Fig. 3), and at day 14 the animals showed signs of fully developed GVHD.

At this stage the animals were sacrificed. The most prominent sign at autopsy was gross enlargement of the spleen and an enlarged mesenteric lymph node in the graft. The native small bowel was

Table 1. Intestinal blood flow (ml/min per 100 g tissue) in the graft and corresponding part of the host ileum. Median of 10-25 isolated determinations and range (in parentheses) are shown

Animal no.	Intestinal blood flow		
	Native bowel	Graft	Graft/native bowel (%)
1	144 (77-214)	61 (31- 80)	42
2	139 (95-267)	70 (46-106)	50
3	113 (43-157)	37 (19- 74)	33
4	84 (52-177)	79 (40-135)	94
5	158 (84-269)	118 (68-140)	75
6	116 (73-183)	113 (62-154)	97
7	127 (66-192)	180 (112-449)	142
8	58 (35- 73)	41 (24- 56)	71
9	121 (73-183)	113 (62-153)	93

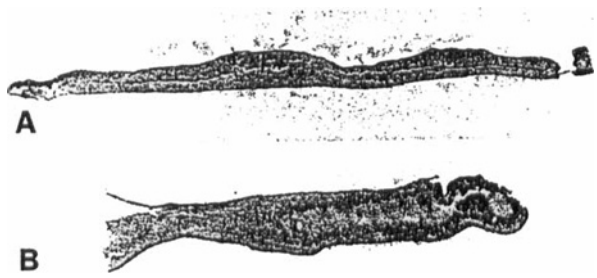

Fig. 4 A, B. X-ray images for the measurement of blood flow in the graft (A) and native bowel (B) 40 days post-transplantation. The degree of positive staining is correlated to the tissue blood flow (for technique, see Material and methods)

also affected, especially in the terminal part, showing a thin and inflammated bowel wall and prominent Peyer's patches. The graft appeared macroscopically intact. Seven animals (12%) with skin and mucous manifestations were found to have an abscess or encapsulated graft. All but four animals (7%) were dead after day 19. All of these had a previous history of abdominal tumors and at laparotomy the graft was necrotic or had become an encapsulated fibrous structure.

The animals with clinical signs of GVHD also demonstrated pronounced histopathological changes compatible with GVHD; however, the graft showed no vascular necrosis and only minor changes, with slight edema and an increase in mononuclear cells in the serosa.

Donor pretreatment

The ALG-pretreated group showed a somewhat different picture (Fig. 2); 35% ($n=9$) of the animals survived for more than 30 days. Among these were two that had encapsulated or resorbed grafts, whereas seven had viable grafts and no signs of GVHD. In the remainder of the animals, signs of GVHD appeared after 7-10 days and the disease had fully developed by days 16-21.

Discussion

In the present study we demonstrated good technical results for SBT between syngeneic rats (80% graft survival), which could be attributed to several factors, e.g., short ischemic time (nonsuture technique), gentle perfusion (hydrostatic pressure, 35 cm water), and good flow characteristics of the perfusion fluid (short perfusion time). The nonsuture technique is also rapid, reducing the second warm ischemic time.

Microscopical evaluation of the isografts showed an essentially intact mucosal morphology, thus implying an adequate nutritive blood flow. The blood flow to the graft was somewhat lower than that of the corresponding native bowel. This may be explained by the following. First, the graft was not placed in continuity, i.e., was not functioning as a part of the resorbing intestinal area. Second, the graft was denervated and thus lacked the central autonomic control. A third and less probable explanation is that a relative stricture of the arterial anastomosis could not be excluded. However, in six of eight cases, the total IBF was within the same range as that previously observed in normal rat ileum using a microspheric technique [10]. The segmental variability in blood flow in the graft was on the same order as that in the native bowel, indicating that intact microcirculation existed in the whole graft. The only potential drawback of this technique is that it requires a left-side nephrectomy, but this is well accepted even in clinical transplantation practice.

A major obstacle for clinical SBT is GVHD [2, 11], caused mainly by the large number of immune cells transferred with the graft [5]. In the present communication we report the development of signs of fatal GVHD. Our technical success rate appears to be somewhat lower (60%) than in the syngeneically transplanted animals. This observation is not well understood, but at least two explanations are possible. First, the local immune reaction due to GVHD makes the tissues less resistant to infections. Second, GVHD is also fatal in the absence of classic clinical signs.

Cyclosporin treatment of the recipient [4, 12], or irradiation of the donor or the graft ex vivo [14, 16] may prevent fatal GVHD in rats. However,

there are some doubts as to the possibility of using cyclosporin alone to maintain graft function in dogs after SBT [3, 6]. Therefore, measures designed to reduce immune cell transfer may facilitate SBT by reducing the immunological problem to that of conventional graft rejection. In the present communication we report that the administration of rabbit ALG to the donor prevents the development of fatal GVHD in 30% of the animals. The effect is seemingly an all-or-nothing effect, since the kinetics of mortality in treated and nontreated animals are similar during the first 14 days posttransplantation. The effect resembles that of antithymocyte globuline (ATG) on organ graft rejection, where a single dose of ATG induces permanent graft survival in 20%–70% of the animals in some strain combinations [15]. Understanding of the biological mechanisms underlying the difficulties in SBT should be facilitated by the procedure described. In particular, studies aimed at a further definition of the nature of GVHD occurring after SBT and its prevention will be simplified.

Acknowledgements. This project was supported by grants from Olli and Elof Ericsson's Foundation for Scientific Research, Her Royal Highness the Crown Princess Louise's Fund for Scientific Research, the Fortia Fund, and the Swedish Medical Research Council. For technical assistance we thank Eva Sandström, Ester Roos-Engstrand, and Ulla Karlsson.

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