Experimental Small-Bowel Transplantation: Alternative Strategies for Graft Prolongation

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● **Alternative combined immunosuppressive therapy was tested in canine orthotopic bowel transplantation. Despite sporadic long-term survival, cyclosporine is still questionably effective. Triple-drug therapy (cyclosporine, azathioprine, and prednisone) combined with antilymphocyte serum or with a short segment graft was effective in reducing the early postoperative mortality due to acute rejection but did not alter the long-term survival rate. There was no apparent relationship between the serum cyclosporine levels and survival. The long-term survivors (longer than 100 days) maintained relatively low serum trough levels of cyclosporine. These results suggest that orthotopic bowel transplantation in the dog, and probably in the human as well, requires improved immunosuppressive regimens.**

INDEX WORDS: Intestinal transplantation.

EARLIER STUDIES in our laboratory had indicated a nine-fold increase in graft survival with ectopic small-bowel transplants in the dog with cyclosporine (CsA) immunosuppression compared with untreated controls. Such extensive graft prolongation had not been possible with azathioprine (AZA) immunosuppression.[1-3] Although a similar extended mean survival time could be duplicated with orthotopic transplantation in the dog, early mortality was high in our experience and that of others, and long-term survival was achieved only sporadically.[4-6] Infections, nutritional disorders, and complicated immunologic problems are inherent in orthotopic small-bowel transplantation. Possible immunologic mechanisms for the discrepancy in survival rates between ectopic and orthotopic grafts include: (1) absence of graft-versus-host reaction (GVHR) in the ectopic graft because of its limited length and size; (2) decreased antigen load provided by the small size of the ectopic graft compared with the total orthotopic graft; and (3) better CsA absorption by the native gut in the ectopic model. Strategies were devised to remedy these perceived deficiencies in the orthotopic model in an effort to obtain better and more consistent graft prolongation. These measures included: (1) use of the short segment graft in the orthotopic position; (2) varied CsA administration regimens including early intravenous (IV) induction before reverting to oral CsA, intraperitoneal administration, and high-dosage oral CsA; and (3) multiple immunosuppressive drug combinations. Experimental groups that incorporated combinations of these alternative strategies and evaluated their usefulness in orthotopic graft prolongation were devised. The results form the basis of this report.

MATERIALS AND METHODS

Experimental Models and Treatment

Adult mongrel dogs (approximately 20 kg body weight) were divided into five experimental groups (Table 1). Group A consisted of untreated controls (n = 6). These animals received a total orthotopic graft but no immunosuppression. Group B consisted of dogs that received total orthotopic graft and IV CsA and oral prednisone (PS) (n = 12). CsA dosage was initially 16 mg/kg/d and tapered to 8 mg/kg/d. Oral PS dosage was initially 10 mg/d and was gradually reduced to 5 mg/d. Group C included dogs that received short segment (90 cm) orthotopic graft and oral CsA and PS (n = 5). CsA dosage was 20 mg/kg twice daily (total, 40 mg/kg/d) and PS at 5 mg/d. Group D included dogs that received triple-drug therapy with total orthotopic graft and CsA, AZA, PS, and antilymphocyte serum (ALS) (n = 4). These animals were given oral PS (10 to 5 mg/d), AZA (25 to 50 mg/d), and oral CsA (20 mg/kg twice daily: total, 40 mg/kg/d), after a short course of IV CsA (10 mg/kg/d for 3 to 7 days). In addition, they received IV ALS (5 mL for 2 days preoperatively and 5 ml for 3 to 7 days postoperatively); the ALS was produced in rabbits in our laboratory. Group E consisted of dogs that received triple-drug therapy group with short segment graft (n = 5). These animals were treated with oral PS (10 mg/d), oral AZA (50 to 25 mg/d), and intraperitoneal (IP) CsA (15 to 20 mg/kg/d). The IP route was chosen to permit better absorption of CsA and greater ease in administration.

All recipients treated with oral CsA were given CsA 16 hours before operation. IV CsA was begun on the day of surgery. IP CsA, filtered through a sterilization filter (0.20 μm), was instilled prior to closure of the abdomen and subsequently instilled by needle puncture. CsA for oral use was used for both the oral route and the IP route, and CsA for intravenous use was used for the IV route. The animals treated with PS received intramuscular methylprednisone for 3 days after operation (500 mg on the operative day, 250 mg on the first and second postoperative days), and then oral PS (5 to 10 mg).

Animals that died within 7 days after surgery were eliminated as technical failures from all groups. The incidence of technical failure was approximately 30% in each group.

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Table 1. Experimental Groups

Group	No.	Type of Graft	Immunosuppression
A	6	Total orthotopic	None
B	12	Total orthotopic	CsA* + PS†
C	5	Segmental orthotopic	CsA‡ + PS†
D	4	Total orthotopic	CsA§ + PS† + AZA‖ + ALS#
E	5	Segmental orthotopic	CsA** + PS† + AZA‖

*IV, 16 mg/kg/d at start, tapered to 8 mg/kg/d.

†Orally, 5 to 10 mg/d, after methylprednisolone intramuscularly for 2 days.

‡Orally, 20 mg/kg for 2 days.

§Orally, 20 mg/kg/d, after short course of IV CsA.

‖Orally, 2 to 3 mg/kg/d.

#IV, 5 mL/d for 2 days preoperatively, 5 mL/d for 3 to 7 days, postoperatively.

**Intraperitoneally, 10 to 20 mg/kg/d.

Preoperative Preparation

All experimental animals received neomycin (2 g) daily for 3 days preoperatively. The animals were fasted, except for medication, for 24 hours prior to surgery. Eight animals were given Sustacal (Mead Johnson Nutritional, Evansville, IN) (350 mL) simultaneously with CsA 16 hours before surgery.

Operative Procedure

A modification of our technique for total orthotopic small-bowel transplantation, previously described,[4,7] was used. The small intestine from the inferior border of the pancreas to the terminal ileum was resected on its vascular pedicle, leaving only an inch of ileum proximal to the ileocecal junction, and flushed with heparinized lactated Ringer's solution (10 U/mL) at room temperature and then at 4°C for preservation. For total orthotopic small-bowel transplantation, the preserved graft in its entirety was used except for the ends, which were resected prior to performing anastomoses. For the short segment graft, we used a 110 cm-long section of jejunum, excising as much mesentery as was safely possible to exclude, and attached the mesenteric lymph nodes to it. The grafts were preserved in cold (4°C) lactated Ringer's solution. The vascular connection was orthotopic, the donor's superior mesenteric artery and vein being anastomosed to the respective vessels in the recipient. Intestinal continuity was restored end-to-end. A Thiry-Vella loop, 6 inches in length, was constructed from the proximal end of the graft for monitoring purposes.

Postoperative Care

All operated animals received 2,000 mL of 5% glucose-lactated Ringer's solution IV daily for 5 days after surgery. They were also given penicillin G (450,000 U) and kanamycin (0.5 g) intramuscularly twice a day for 5 days postoperatively. Water was allowed on the third postoperative day and a normal diet on the fifth postoperative day.

Experimental Protocol

Blood samples were drawn for complete blood cell count, serum chemistries, and serum trough levels of CsA in the morning of postoperative days 2, 5, and 10 and every 10 days thereafter. The trough levels of CsA were measured before surgery on eight dogs that had been given Sustacal simultaneously with CsA on the day before surgery and the levels were compared with those of eight other dogs selected randomly. CsA levels were measured by radioimmunoassay (Sandoz Ltd, Basle, Switzerland). The dogs were weighed daily during the first postoperative week and every 10 days thereafter. Mucosal biopsies from the Thiry-Vella loop were taken on days 5 and 10 postoperatively and every 10 days thereafter.

RESULTS

Graft Survival

Survival data for the various subgroups are presented in Fig 1 and Table 2. The control animals, that received no immunosuppression (group A), survived 9.2 ± 0.9 days (mean ± SE). The animals treated with IV CsA and oral PS (group B) survived significantly longer than did the control animals (52.2 ± 14.0 days; $P < .05$). There was modest prolongation compared with controls when short segment grafts were treated with a combination of oral CsA and PS (group C; 22.2 ± 9.0 days). Of the triple-drug therapy groups, total length allografts pretreated with ALG (group D) survived 54.0 ± 5.5 days; the group with short segment bowel grafts (group E) survived 65.4 ± 14.8 days. Animals in the latter two groups survived significantly longer than did the control animals ($P < .05$) but not longer than did those in group B (those that received IV CsA and PS). However, it is noteworthy that no animal in the triple-drug therapy groups (groups D and E) died within 30 days after surgery, while 6 of 12 animals in group B died within this period (Table 2), a difference that is statistically significant ($P < .05$). On the other hand, the percentage of animals surviving longer than 60 days was 33.3% both in the triple-drug therapy groups (groups D and E) and in group B. The incidence of long survival (more than 100 days) was 25% in group B and 11.1% in groups D and E; this difference was not significant.

Two animals in group E (those that received short segment graft treated with triple drug therapy) died from peritonitis, accompanied by severe hepatic dys-

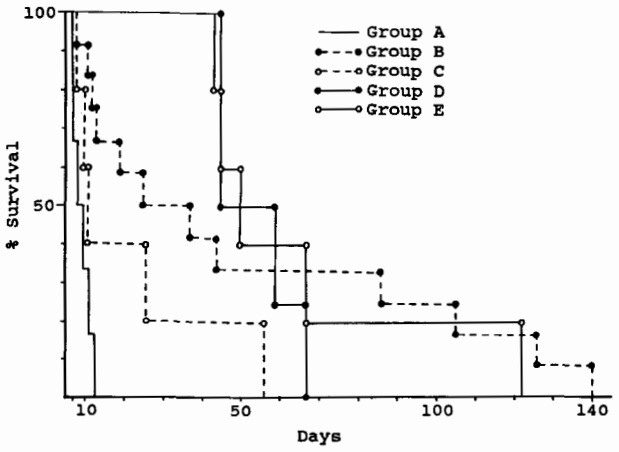

Fig 1. Cumulative survival rates of experimental groups. No animals died before day 40 postoperatively in groups D and E.

Table 2. Survival of Experimental Groups

Group	Survival (days)	(Mean ± SE)		Survival Distribution (days)					
				< 30		30-60		> 60	
A	7, 7, 8, 10, 11, 12	(9.2 ± 0.9)		6		0		0	
B	8, 11, 12, 13, 19, 25, 37, 44, 86, 105, 126, 140	(52.2 ± 14.0*)		6†		2		4	
C	8, 10, 11, 26, 56	(22.2 ± 9.0)		4		1		0	
D	45, 45, 59, 67	(54.0 ± 5.5*)		0		3		1	
			(60.3 ± 8.3)		(0)		(6)		(3)‡
E	43, 45, 50, 67, 122	(65.4 ± 14.8*)		0		3		2	

*Significantly different *v* group A, $P < .05$.

† *v* ‡: significant, $P < .05$.

function (glutamic oxalacetic transaminase [GOT] > 1,000 IU). One other animal in this group died of pneumonia. These complications were probably related to the IP injection of oil-based CsA and prolonged high levels of CsA combined with prednisone mentioned below. One healthy dog in this group was electively sacrificed on day 122 postoperatively.

CsA Trough Levels

Serum trough levels of CsA in groups B, D, and E are shown in Fig 2. These levels remained very low in the animals in group C (not shown in Fig 2) and group D that were given oral CsA. In group B, i.e., the IV CsA group, CsA trough levels were maintained in the therapeutic range. Serum CsA levels in group E (those that received IP CsA) were extraordinarily high, including three animals that maintained serum levels of approximately 1,000 ng/mL. As mentioned above, two of these animals had severe peritonitis and the other had severe pneumonia. However, the longest-term survivor in this group maintained very low trough levels of CsA.

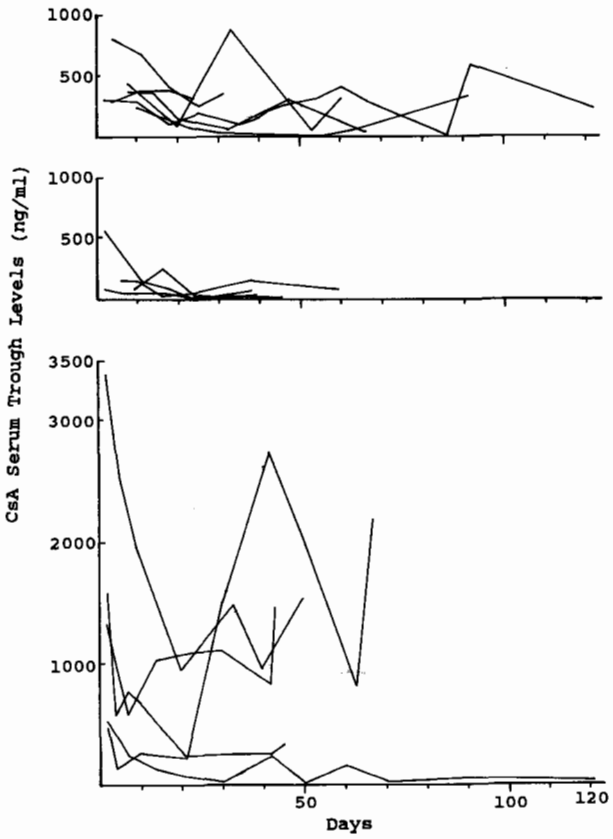

Fig 2. Serum CsA trough levels. Serum trough levels were extremely low in group E, while those in group D were extremely high.

All animals that were treated and survived longer than 10 days were assigned to one of three subgroups according to survival time: the long-term survivors group (longer than 100 days; n = 4), the moderate-term survivors group (30 to 100 days; n = 12), and the short-term survivors group (10 to 30 days; n = 8). The serum trough levels of CsA of these groups were compared (Table 3). On day 10 postoperatively, the mean values were 289 ± 58, 498 ± 150, and 600 ± 228 ng/mL (mean ± SE), respectively. On day 30 postoperatively, those of the former two groups were 222 ± 166 and 477 ± 175 ng/mL, respectively. These differences are not significant, nor was there any relationship between CsA serum levels and duration of graft survival. This is especially noteworthy in group E, in which all animals had prolonged graft survival despite the fact that CsA trough levels were apparently lower than the therapeutic range.

Body Weight Changes

Percent body weight changes of the animals in groups B, D, and E are presented in Fig 3A. No appreciable differences were noted between these three groups.

The long-term survivors (longer than 100 days) of all groups initially lost weight in the first 20 days after transplantation but began to recover and subsequently maintained their body weight at a stable level until just prior to death. The moderate-term survivors (30 to 100 days) of all groups lost weight continuously (Fig 3B). This difference was significant on day 40 postoperatively ($P < .05$).

Table 3. Serum CsA Trough Levels

	Day 10 Postoperatively (ng/mL)	Day 30 Postoperatively (ng/mL)
Long-term survivors (n = 4; over 100 days)	289 ± 58	222 ± 166
Moderate-term survivors (n = 12; 30 to 100 days)	498 ± 150	477 ± 175
Short-term survivors (n = 8; 10 to 30 days)	600 ± 228	—

NOTE. Mean ± SE.

CsA Absorption and Sustacal

The serum CsA levels at 16 hours after oral CsA administration were 51.3 ± 12.5 ng/mL (mean ± SE) without Sustacal (n = 8) and 671 ± 74 ng/mL with Sustacal (n = 8). This difference is significant ($P < .01$). These results suggest that Sustacal alters CsA absorption from the gut to yield higher serum levels.

DISCUSSION

Enthusiasm generated by the advent of cyclosporine with regard to small-bowel transplantation has since waned. Although CsA has proved to be a potent immunosuppressive agent following transplantation of several other organs, small-bowel transplantation remains an unsolved clinical and experimental problem. While sporadic long-term survival in large animals has been obtained by several groups, the rate of consistent graft prolongation has been disappointing and early mortality high.[4-6] A major cause of mortality is rejection, apparently resistant to the CsA regimen used (with or without prednisone). The multiple drug therapy used in the present study was designed to investigate whether such a combination could reduce the mortality resulting from recalcitrant rejection.

It has been suggested that better graft survival in the pig can be achieved with consistently higher CsA levels.[8,9] Such a relationship was not noted in our study. Long-term survivors (over 100 days) grouped together from the various protocols in this study had relatively low trough levels of CsA (222 ± 166 ng/mL; mean ± SE on day 30 postoperatively). In group E (those that received IP CsA), the serum CsA levels were very high, but the incidence of infection was also extremely high. The graft prolongation achieved by high CsA levels may be compromised by a higher than usual infection rate. A potential strategy not investigated in this experiment would be that of multiple drug therapy with relatively low CsA dosage, augmented by antirejection "pulse" therapy during certain rejection episodes. Recently, we showed that short-course, low dosages of CsA are effective in orthotopic bowel transplantation in rats.[10] Currently, however, suitable monitoring techniques to detect impending rejection are not well developed in this organ system.

The small-bowel allograft differs from most other solid organ transplants in its high lymphoid content. It is estimated that one fourth of the intestinal mucosa is composed of lymphoid cells.[11] In addition, the lymphoid cells in Peyer's patches and in the numerous mesenteric lymph nodes provide a vehicle for GVHR in this organ transplant. Lillehei et al suspected a GVHR to be the responsible mechanism in a number of their laboratory deaths after small-bowel transplantation without overt signs of rejection.[12] This presumption has been disputed, and certainly other explanations, such as septicemia, could have accounted for these nonrejection deaths. Some authors have even suggested that GVHR does not occur in two-way systems such as in the outbred dog.[5,6] Recently, we showed that GVHR may occur in the dog.[13] Theoreti-

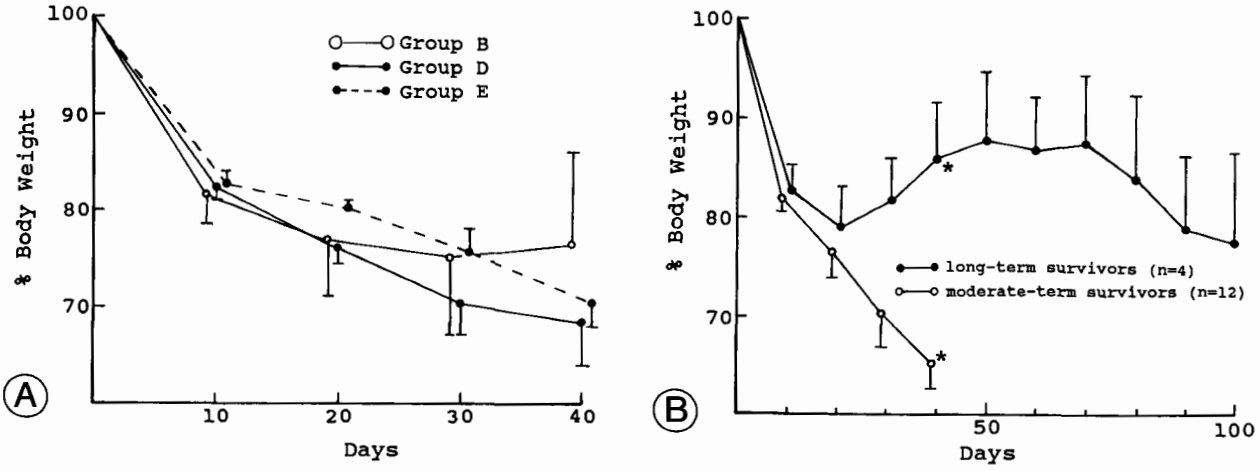

Fig 3. (A) Body weight changes. No differences are discernible between the groups. (B) Body weight changes of long-term and moderate-term survivors. *, significant, $P < .05$.

cally, T-cell depletion of the small-bowel graft should reduce the incidence of GVHR as it does in bone marrow transplantation.[14,15] It was shown that such a strategy in the rat can provide for graft prolongation in a one-way GVHR rat model. In this system, pretreatment of the donor by radiation[16] or by ALS[17] resulted in prolongation of the recipient's survival. Pretreatment of the graft by radiation or antithymocyte globulin was also tested in a dog model.[18] The ALS induction used in group D was undertaken to deplete the lymphoid population in the graft. Use of a short segment orthotopic graft is based on a similar rationale. In group C, the survival of short segment orthotopic grafts was disappointing and undoubtedly attributable to poor CsA absorption (serum trough levels < 100 ng). However, no differences in survival were noted between groups D and E, both of which used multiple drug therapies, one with orthotopic long segment grafts and the other with orthotopic short segment grafts. There was no early mortality (under 30 days) in either of these groups. Although this represents a considerable improvement over group B (using orthotopic long segment grafts with CsA and PS alone), in which half of the animals died within 30 days, additional improvement to achieve long-term graft survival is clearly necessary.

Even in autografts surviving long term without rejection, nutritional function of the transplanted gut has been shown to be impaired.[19] In this context, the functional adequacy of a short segment orthotopic graft remains of concern. In this study, body weight changes of experimental animals were different in long-term survivors (over 100 days) and those that survived between 30 to 100 days. The former maintained a stable weight while the latter continuously and precipitously lost weight. One of the long-term survivors had a short segment graft and maintained body weight well, suggesting that freedom from rejection is probably more important than is the length of the graft itself in maintenance of body weight and functional adequacy of the graft.

Sustacal, a partially digested food supplement, was shown to enhance CsA absorption through the transplanted gut. The basis of this finding remains unclear. The effect of food on the absorption of oral CsA remains a controversial issue.[20,21]

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