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LONG-TERM SURVIVAL OF ORTHOTOPIC BOWEL ALLOGRAFTS IN THE RAT TREATED WITH SHORT-TERM LOW-DOSE CYCLOSPORINE[1]

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Orthotopic bowel transplantation continues to be a "difficult" procedure, both experimentally and clinically, despite the advent of cyclosporine. Even with high-dosage CsA administration, long-term survival has been sporadic and short-term mortality high. In the present study, a low-dosage CsA regimen (5 mg/kg/day for 2 weeks postoperatively) produced prolonged survival in BN-Lewis donor/recipient combinations utilizing an orthotopic bowel transplantation model with portal venous anastomosis. The transplanted bowel continued to survive despite the lack of maintenance dosage after initial induction. The recipient animals evinced donor-specific hyporesponsiveness as donor skin grafts were rejected in a slow, indolent fashion. Third-party skin grafts acutely rejected in normal fashion. It was remarkable that the bowel grafts continued to survive in good condition despite the rejection of donor-specific and nonspecific challenging skin grafts placed on the animals. The precise mechanism of this donor-specific hyporesponsiveness is not known.

The advent of cyclosporine has prompted renewed interest in transplantation of the bowel. Several groups, including our own, have reported on the efficacy of CsA utilizing both ectopic and orthotopic models in the dog (*1–4*) as well as the rat (*5–9*). Although CSA provides significant prolongation of survival as compared to azathioprine, early mortality has been high, and long-term survival sporadic and unpredictable in the dog. Because of these discouraging experimental results, clinical trials have been limited, and small bowel allotransplantation remains an unsolved problem.

Kirkman et al. (*5*) have reported the results of CsA therapy utilizing an ectopic auxiliary rat model. In 1-way reaction (F_1 hybrid-parent), rejection was completely controlled with a CsA dosage of 15 mg/kg/day for 7 days. The same treatment, however, failed to prevent graft-versus-host reaction in a parent-F_1 combination. It is generally recognized that survival of orthotopic grafts is inferior to that of the ectopic model, probably because of differences in antigen load and the incidence of graft-versus-host reactions and many nonimmunologic complications (infection, nutritional problems, etc.). Because of the highly demanding technical nature of the orthotopic model in the rat, only a few studies have been reported. Lee and Schraut were able to show successful prolongation of orthotopic small bowel allografts in the rat utilizing CsA (*6, 7*). However, a high-dosage regimen (15 mg/kg/day on alternate days for 28 days) was necessary. Such a regimen in the dog has been disappointing. Because of the foregoing experiences, it has generally been accepted that the small bowel is a "difficult" organ to transplant, requiring very high doses of CsA for successful allotransplantation.

In the process of evaluating an orthotopic total bowel allotransplantation model in the rat, we discovered that prolonged survival can be obtained in an MHC incompatible combination (BN-Lewis) utilizing low-dose CsA (5 mg/kg/day) administered for as little as 2 weeks after transplantation. These results and a discussion of preliminary attempts to elucidate the responsible mechanisms are reported below.

MATERIALS AND METHODS

Animals. Lewis rats (Le; $RT1^l$) were always recipients. ACI rats (ACI; $RT1^a$), Brown Norway rats (BN; $RT1^n$), F_1 hybrid rats between ACI and Le [F_1(ACI×Le); $RT1^{a,l}$] and F_1 hybrid rats between BN and Le [F_1(BN×Le); $RT1^{l,n}$] served as donors. Le, ACI, and BN rats were obtained commercially, and both F_1 hybrid rats were bred in our laboratory. All animals weighed 250–300 g.

Operative procedure. The operative procedure has been described in detail elsewhere (*10*). In brief, the procedure is as follows.

Donor procedure: The total bowel segment from the jejunum to the distal colon is isolated after ligating and dissecting the inferior pancreaticoduodenal vessels (Fig. 1). The vascular bed is flushed with cold (4°C) Euro-Collins' solution in situ through the aorta. After resecting the graft with the vascular pedicle (consisting of the superior mesenteric artery and vein), the lumen of the graft is gently irrigated with cold (4°C) lactated Ringer's solution followed by cold (4°C) Euro-Collins' solution. The graft is preserved in cold (4°C) lactated Ringer's solution.

Recipient procedure: Before celiotomy, an infusion line is placed in the right femoral vein for continuous infusion during operation and thereafter. The total bowel segment from the 3rd portion of the duodenum (5 mm distal to the influx of the bile duct) to the distal colon is resected, preserving the root of the superior mesenteric artery and vein, and avoiding interruption of the splenic venous flow. The remaining colon with the rectum is placed in the pelvic space. End-to-end anastomoses of the superior mesenteric artery and vein between the graft and recipient vessels are achieved under microscopic magnification utilizing 10-0 nylon suture with a 100-μm needle. Intestinal continuity is restored with a retrocolic duodenojejunostomy and a skin level colostomy on the left flank (Fig. 2).

Postoperative care: The venous infusion was continued for as long as possible, until the line came off spontaneously by the movement of the rat. Antibiotics (10 mg cefazolin sodium and 1 mg kanamycin) were given subcutaneously twice a day for 5 days. Ten ml of 5% glucose-lactated Ringer's solution was given subcutaneously twice a day for 4 days. Water was permitted on the 1st postoperative day, and normal rat chow on the 3rd or 4th postoperative day.

The average operative time was about 150 min, and the early postoperative mortality (within 4 days) approximately 20% in this study. The rats that died within 4 days were excluded from further consideration. Thus, the number of each group mentioned below indicates the number of rats that survived longer than 5 days.

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Experimental groups. Experimental protocols for 7 groups are given in Table 1.

Group 1: Isografts LE-LE (n=9); no immunosuppression.

Group 2: Allografts ACI-LE (n=7); no immunosuppression.

Group 3: Allografts BN-LE (n=5); no immunosuppression.

Group 4: One-way (rejection only) allografts F_1 (ACI×Le)-Le (n=8); no immunosuppression.

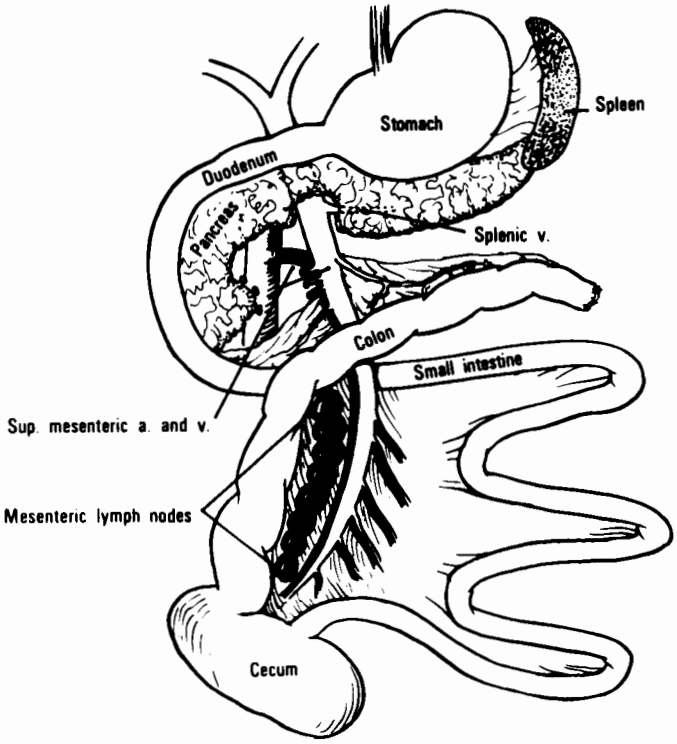

FIGURE 1. Schematic anatomy of orthotopic total bowel transplantation in rats.

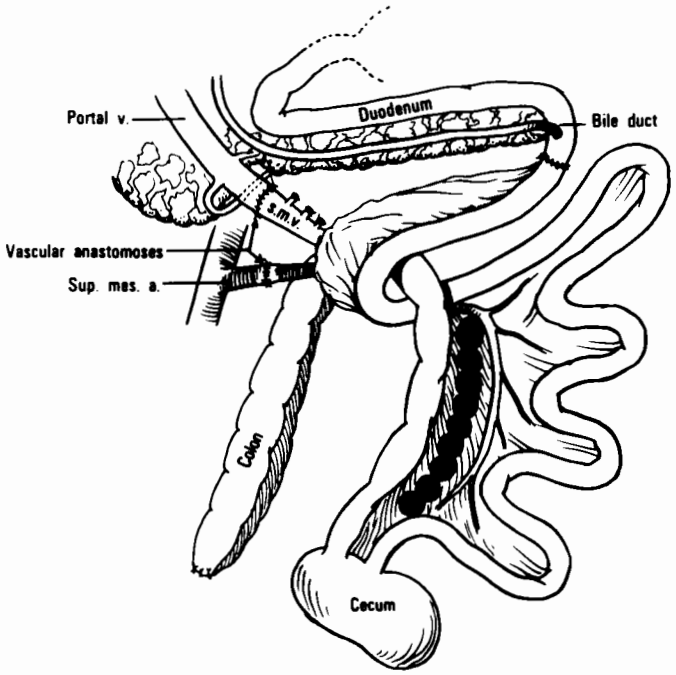

FIGURE 2. Schema of reconstruction.

Group 5: Allografts BN-Le with *high dosage of CsA* (n=10); the recipients received CsA (15 mg/kg/day for 14 days), beginning on the day of operation and continued for 13 consecutive days.

Group 6: Allografts BN-Le with *low dosage of CsA* (n=9); the recipients received CsA (5 mg/kg/day for 14 days) on the same schedule as in group 5.

Group 7: One-way (rejection only) allografts F_1(BN×Le)-Le (n=5); the recipients received low-dose CsA (5 mg/kg/day for 14 days) on the same schedule as in group 5.

CsA was administered subcutaneously. CsA for intravenous use (Sandoz) was used after being diluted 1:5 with saline.

Body weight of the recipients was measured every day for 100 days after transplantation or until death. The mucosa of the colostomy was inspected, and the abdomen palpated daily.

Serum trough levels of CsA were examined in all recipients treated with CsA on any day between the 7th and 10th postoperative days. The blood sample was obtained by cutting the tip of the tail. CsA was measured by RIA (Sandoz).

With few exceptions, recipients were sacrificed before death.

Donor-specific skin grafts in long-term survivors following orthotopic bowel transplantation. Five long-term survivors (>100 days) following orthotopic bowel transplantation in group 6 (low-dose CsA) received simultaneously a donor-specific skin graft (BN skin) and a 3rd-party skin graft (ACI skin) by standard technique (*11*, *12*) 110 days after bowel transplantation. The skin grafts were inspected daily after removing the bandage 7 days after grafting. Body weight of these animals was measured daily for 40 days following skin grafting. As control groups, 10 normal Le rats received BN skin grafts and 5 normal Le received ACI skin grafts.

Statistical analysis. All results are expressed as mean ±SD. Data were analyzed using the unpaired Student's *t* test (2-tailed).

RESULTS

Survival following orthotopic bowel transplantation. Tables 2 and 3 show survival times with and without CsA treatment. Figure 3 shows the cumulative survival rate.

Isogeneic transplantation: Eight of 9 rats in group 1 have survived longer than 100 days, and 7 rats longer than 200 days. The remaining animal died of emaciation, probably due to nonimmunologic graft failure, on the 19th postoperative day.

Nontreated allotransplantation: Among the 2-way (rejection and GVHR) allotransplanted groups, the mean survival time of group 2, with ACI graft, was 7.0±0.6 days and that of group

TABLE 1. Experimental protocols

Group	Donor	No.	CsA (mg/kg×14 days)
1	Le (isogeneic)	8	None
2	ACI (allogeneic)	7	None
3	BN (allogeneic)	5	None
4	F_1(ACI×Le) (semiallogeneic)	8	None
5	BN (allogeneic)	10	15
6	BN (allogeneic)	9	5
7	F_1(BN×Le) (semiallogeneic)	5	5

TABLE 2. Survival following orthotopic bowel transplantation without CsA treatment

Group	Donor	No.	Survival (days; mean ±SD)[a]
1	Le (isogeneic)	8	7 rats (87.5%) >200
2	ACI (allogeneic)	7	A: 7.0±0.6
3	BN (allogeneic)	5	A: 6.8±0.4
4	F_1(ACI×Le) (semiallogeneic)	8	B: 9.8±1.5

[a] Significant, $P<0.01$, A vs. B.

TABLE 3. Survival following orthotopic bowel transplantation with CsA treatment

Group	Donor	CsA (mg/kg×14 days)	No.	Survival (days)
1	Le (isogeneic)	None	8	7 rats (87.5%) >200
5	BN (allogeneic)	15	10	4 rats (40.0%) >100
6	BN (allogeneic)	5	9	8 rats (88.9%) >100
7	F_1(BN×Le) (semiallogeneic)	5	5	4 rats (80.0%) >100

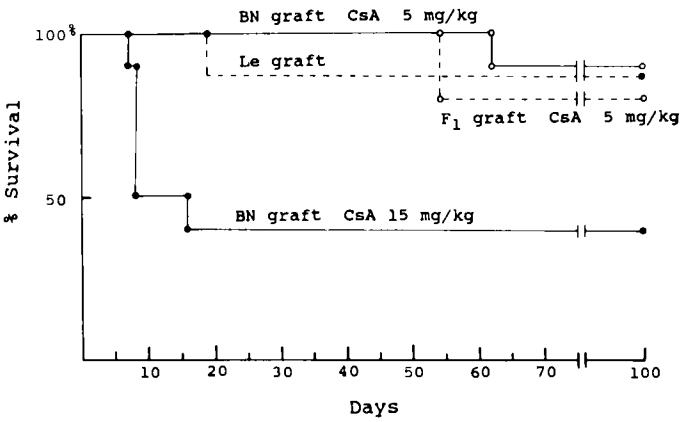

FIGURE 3. Cumulative survival following orthotopic bowel transplantation.

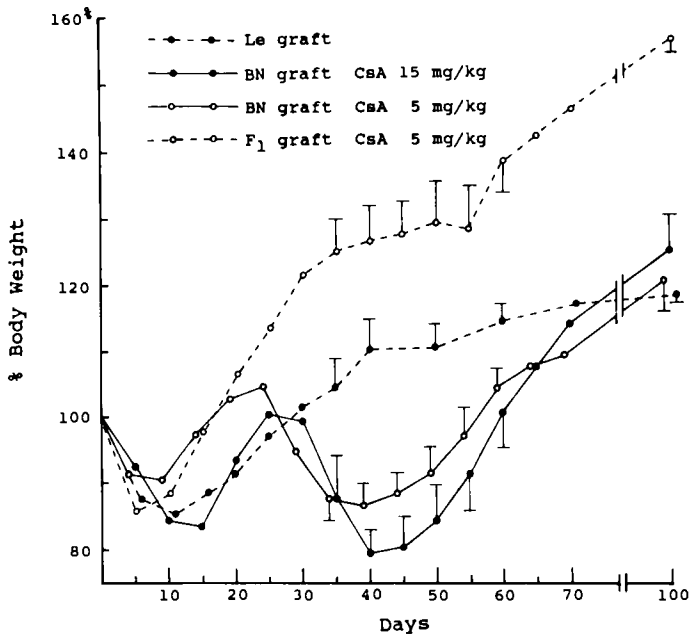

FIGURE 4. Body weight changes following orthotopic bowel transplantation. Note the different patterns of body weight changes. Two BN graft groups lost weight significantly between the 35th and 50th POD compared with isograft group and F_1 graft group ($P<0.05$).

3, with BN graft, 6.8±0.4 days. As expected, no difference was discernible between these 2 groups. The recipients in group 4, 1-way allotransplantation (rejection only) with F_1(ACI×Le) grafts, survived significantly longer than did 2-way allotransplantation groups 2 and 3 (9.8±1.5 days; $P<0.01$).

CsA-treated allotransplantation: Four of 10 rats (40%) in group 5 (treated with high dosage of CsA), 8 of 9 rats (88.9%) in group 6 (treated with low dosage of CsA), and 4 of 5 rats (80%) in group 7, with 1-way (rejection only) allografts (treated with low dosage of CsA), have survived longer than 100 days, and they are in good condition at present. In group 5 (high-dose CsA), at least 4 rats died from infectious complications: panperitonitis of unknown origin, or severe subcutaneous infection spreading from the colostomy. Cause of death in the other 2 rats in group 5 was not established. One rat in group 6 died on the 62nd postoperative day from stricture of the colostomy, a result of ulcer formation around the colostomy during the peculiar weight loss mentioned below. One rat in group 7 had right hemiplegia following operation and died on the 54th postoperative day with hematuria in the distended bladder.

The mean trough level of CsA in group 5 was significantly higher than those in groups 6 and 7 (3701±1316 vs. 440±213 and 532±167 ng/ml, respectively; $P<0.01$).

Clinical course and body weight changes.

Nontreated allotransplantation: Generally, the rats drank and ate well after resuming oral intake. Invariably upon resumption of eating, they had loose (but not watery) stools. The body weight decreased steadily. They abruptly stopped drinking and eating and remained motionless with a humped-back posture, concomitant with the appearance of a dried mucosa of the colostomy. The rats were sacrificed 1 day after these findings were noted.

Isogeneic transplantation and CsA-treated allotransplantation (Fig. 4): All rats had a satisfactory appetite. After losing 10–20% of preoperative weight in 10 days postoperatively, these animals had attained preoperative weight by the 30th postoperative day. Although the isografted rats in group 1 and the 1-way (rejection only) allografted rats also gained body weight gradually after that time, the 2-way allografted rats treated with CsA in groups 5 and 6, without exception, suddenly lost weight again, accompanied by diarrhea and consequent extensive dermatitis around the colostomy. The pH of this diarrheal fluid was around 8. Some rats developed a cutaneous ulcer around the colostomy. Weight loss was maximal on the 40th postoperative day and then began to recover gradually and spontaneously. Significant differences in body weight were discernible between groups 1 and 7 and groups 5 and 6 during this period (Fig. 4). The 1-way (rejection only) allografted rats gained body weight significantly as compared with the other 3 groups.

Skin graft survival in long-term survivors following bowel transplantation (Table 4). The control BN skin grafts and ACI grafts on normal Le rats were rejected acutely within 11 days. All ACI skin grafts on the long-term survivors (BN-Lewis, group 6) following bowel transplantation were also rejected acutely within 11 days (8, 8, 9, 10, and 11 days; 9.2±1.3). Five BN skin grafts on the long-term survivors were rejected chronically (31, 32, 46, 65, and 73 days; 49.4±19.1). This is significantly longer than 3rd-party ACI skin graft survival following bowel transplantation (BN-Lewis) and control skin grafts (BN or ACI) on normal Le rats ($P<0.01$).

TABLE 4. Skin graft survival

Skin graft recipients	Skin graft survival (days)[a]			
	BN	(Mean ±SD)	ACI	(Mean ±SD)
Long-term survivors	31,32,46,65,73	A: 49.4±19.1	8,8,9,10,11	9.2±1.3 (B)
Normal Le	9,9,9,9,9,9,9,10,10,11	B: 9.4±0.7	8,8,10,11,11	9.6±1.5

[a] Significant, $P<0.01$, A vs. B.

DISCUSSION

Significant results of this study were threefold: (1) even a low dosage of CsA (5 mg/kg/day) given for a short period of time (2 weeks) provided long-term survival following orthotopic small bowel transplantation; (2) untreated 1-way allografted rats (rejection only) survived longer than untreated 2-way allografted rats; and (3) long-term survivors treated with CsA following bowel transplantation acquired donor-specific hyporesponsiveness; neither chronic rejection of donor-specific skin graft nor acute rejection of 3rd-party skin graft affected the continued survival of the chronically accepted bowel graft.

Several groups have investigated the efficacy of CsA in small bowel transplantation in the rat and in larger animals. In the dog (*1–4*) and the pig (*13*), even though prolongation of survival can be demonstrated with CsA administration, indefinite survival is sporadic and is accompanied by a generally high mortality rate. In the rat, several groups have investigated the efficacy of CsA utilizing either the ectopic model of Monchik (*5, 8, 9, 14*) or an orthotopic model (*6, 7*). In general, the ectopic model is associated with improved mortality figures and survival time in both the rat and the dog. Schraut and Lee, using the orthotopic model of Kort (*15*) in the rat, demonstrated indefinite small graft survival with a high-dose regimen of CsA (15 mg/kg/day) on alternate days for 30 days) (*7*).

In our laboratory, an orthotopic model somewhat different from that of Kort, utilizing transplantation of both small and large bowel, is employed. The model is attended by a low technical failure rate and is reproducible. This preparation has been utilized for the studies reported herein. A very high mortality (60%) was noted with high-dosage CsA, predominately due to infectious complications. In an effort to reduce the magnitude of this problem, a small dosage of CsA was employed and was found to provide indefinite survival of small bowel grafts. This was surprising, inasmuch as we and others have generally considered the small bowel to be a "difficult" organ to immunosuppress requiring higher than usual doses of immunosuppressive agents used with other organ transplants. It was also surprising that continued good graft function persisted even though CsA was stopped after 2 weeks of administration at relatively low dosage levels. This model is analogous to the kidney allograft model (*16*). Low-dose CsA administration also appears to be efficacious in the clinical setting. Very recently, improved results of orthotopic bowel transplantation with systemic venous drainage were reported utilizing low-dose CsA (5 mg/kg) for 30 days (*17*) or high-dose CsA (15 mg/kg) for 7 days (*18*). These results lend support to our finding that the immunosuppression of orthotopic bowel transplantation in the rat model is not "difficult," as had been assumed.

Low-dose CsA in this study achieved donor-specific hyporesponsiveness in vitro following orthotopic bowel transplantation. Long-term survivors of bowel grafts still possess the ability to reject donor-specific skin grafts, albeit in a chronic slow fashion. Third-party skin grafts were rejected acutely as in nonbowel transplanted controls. It was remarkable that the transplanted bowel continued to survive in good condition despite rejection of donor-specific and 3rd-party skin grafts. These observations are quite similar to those noted in the heart transplantation model in the rat (*19*). The precise nature of this specific hyporesponsiveness remains to be elucidated.

An interesting aspect of the study was the peculiar weight loss exhibited by the various groups. All groups evinced a 10–20% initial weight loss following transplantation, but began to recover around 2 weeks postoperatively. The observed weight changes are different among the different experimental groups thereafter. Isografts and 1-way allografts (no GVHR or rejection only) continued to demonstrate weight gain from this point. Two-way allograft models (both low-dose and high-dose CsA regimen) evinced an additional period of weight loss starting around 30 days posttransplantation. The 2nd episode of weight loss proved in some instances to be quite as marked as the immediate postoperative weight loss, amounting to 20% of preoperative weight. There was spontaneous recovery thereafter, starting around 40 to 50 days posttransplantation, and the animals eventually exceeded their preoperative weight by approximately 20% at 100 days. The 2nd episode of weight loss could be postponed but not abolished by a short course of CsA (5 mg/kg/day) administered for 7 days starting on the 21st postoperative day (unpublished data from our laboratory). The underlying bases of these body changes are unknown but are probably related either to immune reaction, GVHR or rejection, or both.

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CONTROLLED CYCLOSPORINE CONVERSION AT THREE MONTHS AFTER RENAL TRANSPLANTATION

LONG-TERM RESULTS

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The long-term results of conversion of cyclosporine to azathioprine and those of continuous CsA therapy were evaluated in a prospective study of 66 renal transplant patients who had been randomly assigned to each treatment group at 3 months following transplantation. The start point of the study was thus at 3 months posttransplant; no differences in the three-year patient and graft survival were found; these consisted of 97% and 94% in the converted group and 100% and 94% in the nonconverted group, respectively. The incidence of one or more antirejection treatments did not differ between the two groups at 3–12 months (16% vs. 17%) or after 12 months (12% vs. 9%). The incidence of hypertension at different intervals ranged from 79% to 100% in the group on continuous CsA therapy versus 50 to 58% in the converted patients. The degree of proteinuria in the 2 groups was not different at at 12 months. At 24 and 36 months the proteinuria (g/24 hr) was higher in the converted group (0.51±0.18 and 0.53±0.13; mean ± SEM) versus the CsA group (0.15±0.04 and 0.21±0.09). At 3 years, the mean creatinine clearance for the patients converted to Aza was higher than that found for the continuously CsA-treated patients (67±8 and 59±6 ml/min; mean ± SEM). This study shows that early CsA conversion to Aza gives a slightly better 3-year graft function, although not significantly different, compared with continuous CsA therapy without differences in patient or graft survival.

Cyclosporine has contributed substantially to improvements of the results of organ transplantation (*1*) but drug toxicity, especially in the kidney, and high costs may limit its continuous use. To date, irreversible renal failure from CsA has been reported in both cardiac (*2*) and renal transplant patients (*3, 4*). Studies of patients who received CsA for the treatment of uveitis (*5*) have suggested that histological CsA nephrotoxicity may be related to prolonged episodes of clinical CsA nephrotoxicity and it has been suggested that chronic CsA nephrotoxicity may be avoided by conversion of CsA to Aza several weeks or months after transplantation (*6–10*). On the other hand, several mostly uncontrolled studies have suggested that conversion of CsA to Aza in renal transplant patients is associated with an increased risk of rejection. Only one controlled clinical

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