Successful Orthotopic Total Bowel Allotransplantation in the Rat Utilizing Low-Dose Cyclosporine Therapy

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THE EFFICACY of cyclosporine (CsA) in orthotopic bowel transplantation (OBT) was evaluated in rats. The short-course low-dose CsA regimen (5 mg/kg for 14 days) provided the highest rate of indefinite survival (>100 days, >80%). Donor-specific hyporesponsiveness was demonstrated in in vitro experiments utilizing skin grafts.

MATERIALS AND METHODS

Lewis (Le; RT1[l]), Brown Norway (BN; RT1[n]), ACI (ACI; RT1[a]), and Buffalo rats (BUF; RT1[b]) obtained commercially and F_1 hybrid rats between Le and BN (F_1; RT1[l,n]) bred in our laboratory were used. The rat model of OBT with portal venous drainage developed in our laboratory[1,2] was utilized. CsA for intravenous use (Sandoz) was given subcutaneously. The recipients were always Le. Early postoperative mortality was approximately 20%. Those animals were eliminated from further studies.

The animals were divided into five groups: group I (N = 10), Le-to-Le, isogeneic transplantation; group II (N = 7) BN-to-Le, allogeneic transplantation without immunosuppression; group III (N = 10), BN-to-Le, allogeneic transplantation with CsA, 15 mg/kg for 14 days; group IV (N = 17), BN-to-Le, allogeneic transplantation with low-dose CsA, 5 mg/kg for 14 days; and group V (N = 5), F_1-to-Le, semiallogeneic transplantation with low-dose CsA, 5 mg/kg for 14 days.

Four long-term survivors (>100 days) in group IV treated with low-dose CsA were challenged by donor-specific skin grafts (BN skin) three times, by third-party skin grafts (ACI skin) twice, and by another third-party skin graft (BUF skin) once. Fifteen Le rats served as controls. The skin grafting followed Billingham's method as modified by Grogan and associates.[3]

RESULTS

Nine animals in group I (90%), four in group III (40%), 14 in group IV (82.4%), and four in group V (80%) survived 100 days or longer and were healthy until they were electively killed. The deaths were mainly attributable to nonimmunological sequelae—i.e., infection, intestinal obstruction, or nutritional problems—but one animal in group III and one in group IV might have died of rejection or graft-versus-host reaction (GVHR), as mentioned below. Without CsA, all animals in group II died of rejection within ten days after OBT (Table 1).

Table 1. Experimental Groups and Survival

Group	No.	Graft	CsA[a]	Survival (Days)
I	10	Le	None	19, >100 × 9 (90%)
II	7	BN	None	6, 6, 7, 7, 7, 7, 7, 6.7 ± 0.5 (mean ± SD)
III	10	BN	15	7, 8, 8, 8, 11, 16 >100 × 4 (40%)
IV	17	BN	5	32, 30, 62, >100 × 14 (82.4%)
V	5	F_1	5	54, >100 × 4 (80%)

[a]mg/kg/d for 14 days.

Interestingly, all long-term survivors in groups III and IV, which received fully allogeneic grafts (BN grafts), lost weight dramatically between the 30th and 60th postoperative days (POD) after recovering from the initial early postoperative body weight loss and then began to regain body weight. The animals in groups I and V, which received isografts or F_1 semiallogeneic grafts, did not exhibit such changes. This weight loss usually was accompanied by severe diarrhea, voracious appetite for both food and drink, and proliferative dermatitis with hair loss. These clinical findings may be attributable to GVHR, mild rejection, or both. In this period, the two animals mentioned above died.

The long-term survivors in group IV rejected the third-party skin grafts in the same fashion as did normal Le rats and also exhibited the second-set phenomenon. Although those animals that retained the donor-specific skin grafts challenged three times longer than did normal Le ($P < .05$), they rejected the second BN grafts earlier than the first BN skin grafts and the third BN skin grafts earlier than the second BN skin grafts ($P < .05$, Table 2). Despite the skin graft challenges, the bowel grafts functioned well, and the recipients survived longer than 300 days until they were electively sacrificed.

DISCUSSION

OBT remains one of the most difficult forms of organ transplantation even with CsA treatment. Although CsA provided some sporadic long-term survivors in the dog model, the rate was low and many recipients died early after OBT.[4] Few studies of the efficacy of CsA in OBT with portal venous reconstruction have been reported, probably because of the technical difficulties of the procedure. We developed a very stable OBT model in rats, and the efficacy of CsA in OBT was tested in this model.

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Table 2. Survival (Days) of Skin Grafts in Long-Term Survivors Bearing Bowel Grafts

	BN[a]			ACI		BUF
Skin Graft	1st	2nd	3rd	1st	2nd	1st
Long-term survivors	31	23	12	11	<8	8
	46	30	12	10	<8	8
	60	30	18	8	<8	8
	73	32	22	8	<8	10
Mean ± SD (n = 4)	52.5 ± 18.1[b]>	28.8 ± 3.9[b]>	16.0 ± 4.9[b]	9.3 ± 1.5	<8	8.5 ± 1.0
Normal Le	9 × 7	<8 × 10		8 × 2		
	10 × 2			10 × 1		
	11 × 1			11 × 2		
Mean ± SD	9.4 ± 0.7[c]	<8		9.6 ± 1.5		
	(N = 10)	(N = 10)		(N = 5)		

[a]BN = donor-specific skin graft; ACI = first third-party skin graft; BUF = second third-party skin graft.
[b]versus [c] Significantly different, $P < .05$; > = significantly longer, $P < .05$.

This study showed that temporary low doses of CsA (5 mg/kg for 14 days) rather than high doses (15 mg/kg for 14 days) were sufficient for OBT in rats across major histocompatibility antigen. This finding is similar to that in orthotopic kidney transplantation.[5]

Only the recipients with fully allogeneic grafts, which may cause GVHR, lost weight temporarily but markedly after the cessation of CsA. This event was apparently attributable to immunological factors, since the isotransplanted rats did not exhibit this loss of body weight. GVHR may be the mechanism involved, because the recipients with F_1 grafts, which do not have the potential to induce GVHR, did not lose weight. Otherwise, a mild rejection might have occurred, because alloantigenicity is stronger in BN grafts than in F_1 grafts. Perhaps both rejection and GVHR occurred simultaneously. The precise mechanism merits further study. At any rate, the recipients of OBT actively acquired tolerance after the cessation of CsA. This phenomenon may be similar to tolerance in heart transplantation in rats after the unstable phase, in which the grafted heart was easily rejected by challenging the donor-specific skin graft after the cessation of CsA,[6] or spontaneous tolerance in liver transplantation in rats after the demonstration of signs of rejection.[7]

The long-term survivors showed donor-specific hyporesponsiveness in in vitro experiments utilizing skin grafts. Interestingly, those animals showed the second-set or the third-set-like phenomenon against donor-specific skin grafts, although the second and the third BN skin grafts in the long-term survivors bearing BN orthotopic bowel transplants survived longer than did those on the normal Le rats. On the other hand, the bowel grafts were not rejected and functioned well. These results suggest that the host might have responded to skin-specific antigens in addition to the histocompatibility antigens on the bowel graft, that the suppressor mechanism works locally, or that the alloantigenicity of the bowel graft itself was altered. The precise reason is unclear.

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