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Intestinal allograft survival in the rat following pretreatment with donor-specific UV-B-irradiated leukocytes and peritransplant immunosuppression with cyclosporine

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Abstract. Previous studies from our laboratory showed that pretreatment with ultraviolet-B-irradiated donor leukocytes (UV-B DL) combined with brief peritransplant cyclosporine (CyA) resulted in indefinite survival of Wistar/Furth rat cardiac allografts in Lewis recipients. This study was designed to examine the effect of pretransplant UV-B DL with or without peritransplant CyA on orthotopic intestinal allografts in the same rat strain combination. The results showed that while low-dose CyA treatment alone (10 mg/kg i.m. on days 0, +1, and +2) had no effect on intestinal allograft rejection, 20 mg/kg (on days 0, +1, and +2) CyA significantly ($P \leq 0.001$) prolonged graft survival, with 33% of the hosts surviving indefinitely. The highest dose of CyA (30 mg on days 0, +1, and +2) abrogated rejection, but most transplant recipients succumbed to infection and functional ileus due to a toxic side effect of CyA. Pretreatment with UV-B DL on days −14 and −7 alone did not prolong intestinal allograft survival. Combination of a subtherapeutic CyA dose (20 or 10 mg/kg) given on days 0, +1, and +2 with pretransplant UV-B DL on days −14 and −7 did not alter the survival of intestinal allografts compared to treatment with CyA alone. This suggests that pretreatment with UV-B DL with or without peritransplant administration of CyA has no effect on intestinal allograft survival, in contrast to the effect of such combined treatment on cardiac allograft survival, where indefinite graft survival is observed. This difference in the effect of pretransplant UV-B DL on intestinal and cardiac allograft survival is most likely due to organ-specific immunogenicity, particularly to the relative density of class I and II histocompatibility antigens present in heart or intestine.

Key words: Intestinal transplantation, in the rat – Pretreatment, in intestinal transplantation – Irradiated leukocytes, in intestinal transplantation

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Use of the potent immunosuppressive agent FK 506 or of cyclosporine (CyA) has led to improvement in the survival of patients with small bowel transplantation [4]. In addition, numerous reports have demonstrated the superior effect of FK 506 or of CyA compared to conventional immunosuppression in experimental models of intestinal transplantation [5, 15]. Unfortunately, in the clinical situation graft failure after CyA treatment, even in combination with other immunosuppressive agents, remains a major limitation to successful intestinal transplantation [9]. Furthermore, the use of massive immunosuppression has led to significant morbidity in recipients of small bowel allografts from systemic infections [29]. For these reasons, the search for techniques that would reduce or avoid long-term use of immunosuppressive therapy has continued. Induction of donor-specific unresponsiveness via recipient pretreatment with donor antigens in the form of whole cells has been most promising in this regard [10]. The beneficial effect of pretransplant donor-specific transfusions (DST) in human renal allografts and in experimental animal organ transplantation has been documented in several reports [28].

The disadvantage of DST remains the risk of presensitization of the transplant recipient against the prospective donor [6]. To overcome this limitation of DST, we have focused on the use of ultraviolet-B (UV-B) irradiation of donor leukocytes (DL) prior to transfusion, which reduces or eliminates the immunogenicity of pretransplant DST and results in specific unresponsiveness to pancreatic islets and cardiac allografts [13, 17, 20] in the low-responder Lewis-to-ACI rat strain combination, while in the high-responder Wistor/Furth-to-Lewis combination pretreatment of recipients with UV-B DL required additional brief peritransplant treatment with CyA to induce 100% indefinite graft survival (> 120 days) [18]. These results encouraged us to examine whether donor-specific leukocyte transfusions modified by UV-B irradiation with or without peritransplant CyA can induce and/or maintain donor-specific unresponsiveness to rat intestinal allografts.

Table 1. Wistar/Furth intestinal allograft survival in Lewis rats pretreated with ultraviolet-B-irradiated donor leukocytes (UV-B DL), peritransplant cyclosporine (CyA), or a combination of UV-B DL and CyA

Group	*n*	Treatment	Recipient survival (days)	Median (days)
I	6	None	5, 7, 7, 10, 11, 12	8.5
IIa	11	CyA 30 mg/kg[a]	2 × 5[b], 6, 2 × 7[b], 2 × 8[b], 4 × > 120	8.0
IIb	6	CyA 20 mg/kg[a]	30[c], 51, 62, 69, 2 × > 120	65.5[e]
IIc	6	CyA 10 mg/kg[a]	5, 6, 6, 7, 25, 27	6.5
III	4	UV-B DL[d]	5, 7, 7, 7	7.0
IVa	7	CyA 20 mg/kg[a] + UV-B DL[d]	27, 43, 49, 51, 66, 87, > 120	51.0[e]
IVb	4	CyA 10 mg/kg[a] + UV-B DL[d]	6, 6, 7, 23	6.5

[a] On days 0, + 1, + 2
[b] Infection of the host and functional ileus of the transplant
[c] Acute graft-versus-host disease
[d] 10^8 Wistar/Furth leukocytes days − 7 and − 14
[e] U test: group IIb vs I $P < 0.001$; group IVa vs IIb not significant; group IVa vs I $P < 0.001$

Materials and methods

Animals

Lewis (RT-1[l]) and Wistar/Furth (W/F; RT-1[u]) male rats, each weighing approximately 250–300 g, were purchased from Harlan Sprague-Dawley, Inc., Indianapolis, Ind., USA. Lewis rats served as recipients of UV-B DL and intestinal grafts obtained from W/F rats.

Preparation and UV-B irradiation of splenic lymphocytes

Donor leukocytes were obtained from the spleen of donor W/F rats. The spleens were isolated, minced, and pressed gently through a 60-gauge mesh stainless steel screen. Lymphocytes were isolated and UV-B-irradiated as we have previously described [17]. The UV-B-irradiated cells were centrifuged and suspended in phosphate buffer solution at a concentration of 10^8 cells/ml prior to injection of 10^8 cells via the dorsal penile vein of Lewis recipients. The viability of cells determined by trypan-blue exclusion was consistently greater than 95 %.

Intestinal transplantation

Donors were fasted for 18–24 h with access only to water containing tetracycline. Anesthesia was induced with 4 % chloral hydrate given by intraperitoneal injection at a dosage of 1 mg/100 g body weight. As described previously [24], the intestine was removed from the donor with a vascular pedicle based upon the portal vein and an aortic cuff including the entry to the superior mesenteric artery. The donor organ was flushed with 3–5 ml cold (4 °C) heparinized (10 U/ml) lactated Ringer's solution and the intestinal lumen was rinsed with 20 ml cold 0.9 % sodium chloride solution containing 5 µg/ml gentamicin to remove remnants of intestinal contents. Then the graft was stored in cold normal saline solution. In the recipient, end-to-side anastomoses were performed between the abdominal aorta and the aortic cuff of the graft and the inferior vena cava and the portal vein, using a running suture. The ischemic time was about 30 min. After resection of the host's intestine from the duodenum to the last 1–2 cm of the terminal ileum, the donor graft was anastomosed orthotopically to the recipient intestine end-to-end using single inverted one-layer sutures.

Postoperatively the rats were fed with a 5 % glucose solution for 48 h, thereafter they were given normal diet and water ad libitum. Tetracycline was added to the water for the 1st postoperative week. The recipients were weighed daily and were evaluated for clinical evidence of graft-versus-host disease (GVHD). Animals surviving less than 4 days (technical failures) were excluded from data analysis. The technical success rate was approximately 90 %.

Immunosuppression

Cyclosporine was supplied dissolved in olive oil (Sandoz Pharmaceutical Co., East Hanover, N.J., USA) and further diluted with olive oil (Sigma Chemical Co., St. Louis, Mo., USA) to a concentration of 10 mg/ml. The drug was administered using deep intramuscular hind-limb injections.

Experimental design

Host survival as well as clinical or histologic evidence of graft rejection were assessed in Lewis recipients of W/F intestinal allografts divided into the following groups. Group I recipients (controls) were not treated. Animals in groups IIa, b, and c received different CyA doses: 30, 20, and 10 mg/kg i. m. respectively, on days 0, + 1, and + 2 in relation to transplantation. We then studied the effect of UV-B DL transfusions given on days − 14 and − 7 in group III recipients. Group IV a and b animals received transfusions of UV-B DL on days − 14 and − 7 combined with peritransplant CyA [20 (IVa) and 10 (IVb) mg/kg on days 0, + 1, and + 2]. In addition, we compared the postoperative weight changes of allograft recipients to those of syngeneically transplanted animals (Lewis-to-Lewis).

Histology

Post-mortem examinations were performed and tissue samples collected for routine histology following killing or death of recipients. Relevant morphologic evaluation of the intestine was done only in representative animals in each group. At the time of killing, the small intestine was placed in formalin and paraffin-embedded. Sections were cut at 3 µm and stained with hematoxylin-eosin and periodic acid-Schiff Statistical analysis

For statistical analysis of the results the Mann-Whitney U-test was used.

Results

Effect of peritransplant cyclosporine treatment on intestinal allograft survival

Control animals (Table 1, group I) died from acute rejection at 8.7 ± 2.5 days. Cyclosporine administered at the highest dose of 30 mg/kg i.m. on days 0, + 1, + 2 (group IIa) prevented allograft rejection and induced indefinite survival (> 120 days) in 4/11 recipients. However, the majority of the recipients died within 8 days. The post-

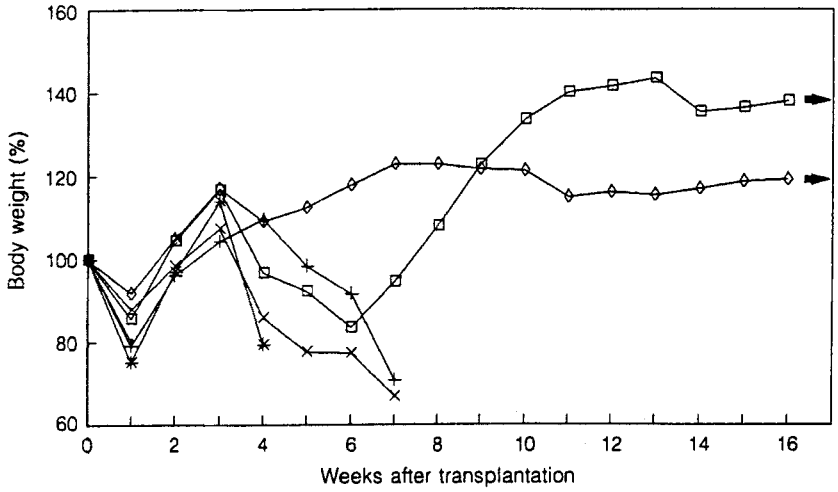

Fig. 1. Postoperative weight changes following fully allogeneic intestinal transplantation into untreated recipient and rats treated with 3 × 20 mg/kg cyclosporine alone (group II b) or after additional pretreatment using UV-B-irradiated donor-specific leukocytes (group IV a). The body weights are expressed in percentages. The number and abbreviation after the group indicates the survival time in days and summarizes the obduction finding of the animal represented in the weight curve. • Control-7d host-versus-graft reaction (HVGR); + group II b-51d HVGR; * group II b-30d graft-versus-host disease (GVHD); □ group II b, long-term survivor (LTS) > 120d; × group IV a-49d HVGR; ◇ group IV a, LTS > 120d

mortem examinations revealed no clinical or histologic evidence of graft rejection, and death of the animals was due to severe lung and abdominal infections; functional ileus of the transplant was also present as evidenced by gross dilatation of the duodenum proximal to the intestinal anastomosis, although no mechanical obstruction was found.

Acute rejection of the intestine was also prevented with 20 mg/kg i. m. cyclosporine given on days 0, + 1, + 2 (group II b); host survival was significantly prolonged ($P < 0.001$) with 33% of the recipients surviving indefinitely without major toxic side effects. The recipients recovered their preoperative weight in the 2nd week after transplantation (Fig. 1) and long-term survivors gained 120–140% of their initial weight. Late weight loss, starting approximately 2 weeks before death, indicated graft failure, since it correlated with the histologic finding of cellular graft rejection of the intestine (Fig. 2). Histologic features of acute cellular rejection seen in the intestine included shortening and blunting of the villi by edema with diffuse mononuclear leukocyte infiltration of the lamina propria and the intestinal epithelium. There was prominent edema and inflammation of the submucosa and muscularis propria. One animal in this group showed the typical signs of acute GVHD, i.e., diffuse skin erythema, hyperkeratosis of the foot pads, dermatitis, diarrhea, and weight loss, and died on day 30.

The lowest CyA dose of 10 mg/kg given on days 0, + 1, and + 2 (group II c) delayed acute rejection of intestinal allografts for 3 weeks in 2/6 recipients.

Effect of UV-B-irradiated DL transfusions alone and in combination with peritransplant CyA on intestinal allograft survival

Pretreatment with UV-B-irradiated DL on days − 14 and − 7 (Table 1, group III) had no effect on recipient or graft survival compared to those in unmodified control animals (group I).

When we examined the effect of pretransplant UV-B DL transfusions in combination with peritransplant

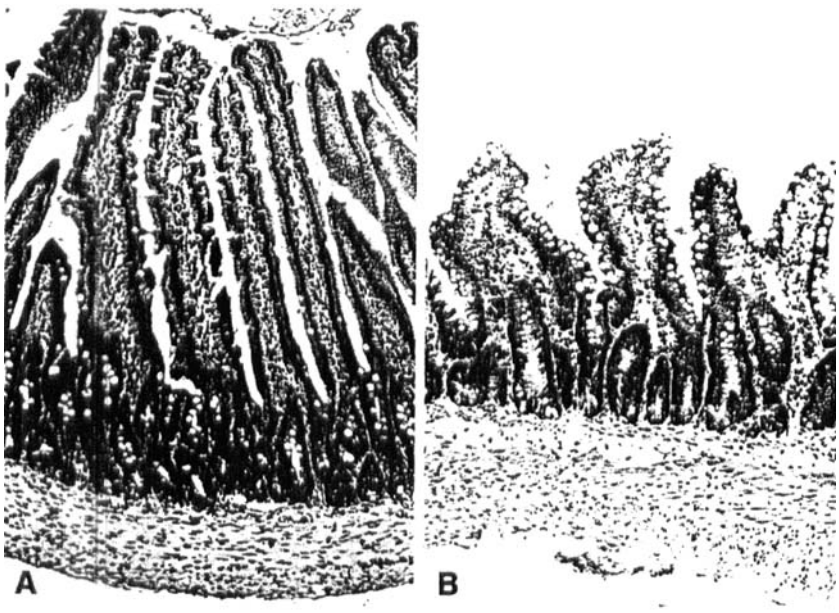

Fig. 2 A, B. Histologic examination of graft.
a Section of normal small intestine of the rat showing normal villus/crypt ratio, elongated villi, and absence of inflammatory cell infiltrate.
b Section of small intestine from a group III rat shows changes due to acute cellular rejection. The villi are shortened, blunted, and edematous with a diffuse mononuclear cellular infiltrate within the lamina propria and intestinal epithelium. There is also marked edema and inflammation of the submucosa and muscularis propria

CyA treatment using a dose of 20 or 10 mg/kg ×3 (groups IVa and IVb) – since the mortality of recipients treated with 30 mg/kg ×3 CyA was too high – the host survival following transplantation of intestinal allografts was not altered compared to recipient survival using CyA alone (group IIb and IIc). The weight changes and histology of intestinal allografts were similar in groups IVa and IIb and in groups IVb and IIc, respectively.

Discussion

This study confirms previous finding [7, 14] of significant prolongation of intestinal allografts in the rat following treatment with CyA. Our results agree with earlier reports which show that the fate of intestinal transplant recipients is dependent on the CyA dose and that a high dose of CyA is associated with 20–50% mortality due to infection of the host [12, 14]. Furthermore, we confirmed that intestinal allografts develop early functional ileus following administration of a high CyA dose [27].

We and others have focused on the beneficial effect of UV light in reducing sensitization after blood and platelet transfusions [3, 30]. Recent reports from our group have shown that pretransplant UV-B DL transfusions have a superior effect in prolonging cardiac allograft survival compared to transfusion of unmodified DL in the W/F-to-Lewis combination [18, 19]. This effect can be explained by a reduction of the immunogenicity of pretransplant DST [17, 20, 21] due to impairment of proper antigen presentation. Pretreatment with UV-B DL alone on days −14 and −7 or peritransplant CyA alone resulted in a significant prolongation of W/F cardiac allograft survival in Lewis recipients; moreover a combination of both treatments led to indefinite cardiac allograft survival in all recipients. In contrast to the findings in the cardiac allograft recipients, pretransplant transfusion of UV-B DL was ineffective in prolonging intestinal allograft survival. It is surprising that pretreatment with UV-B DL combined with peritransplant CyA had no synergistic effect on intestinal allografts as it does on allografts of other organs. The explanation for the observed disparity in cardiac and intestinal allograft survival is most likely organ-specific immunogenicity, which may be dependent on the content of "passenger leukocytes" and/or the presence of endothelial cells, both quantitatively and qualitatively, on the relative frequency of MHC class I and II antigens in the graft, and on the spontaneity with which MHC antigens can be induced in the graft.

Little is known about the effect of DST on the survival of intestinal allografts. In contrast to our findings in the W/F-to-Lewis rat model, Martinelli et al. [16] reported prolongation of host and intestinal allograft survival in the ACI-to-Lewis rat strain combination following DST pretreatment combined with peritransplant CyA in a model of orthotopic segmental small bowel transplantation; DST alone was not effective in prolonging allograft survival. Similarly, Bollinger et al. [1] reported prolonged survival of heterotopic intestinal transplants in the same rat strain combination following intravenous pretreatment of the recipient with mitomycin-C-treated DL alone. GVHD was not reported in either of the above studies. The effect of donor-specific blood transfusions on GVHR and HVGR was also investigated in the WAG-to-BN combination [26]. Interestingly, DST increased the severity of GVHD in the WAG-to-BN combination, whereas it ameliorated GVHD in the reciprocal combination of BN-to-WAG [2]. No effect on graft rejection was seen in either combination, even when peritransplant CyA was added to DST pretreatment in the BN-to-WAG combination. The differences in the results of these studies may be explained by the different rat strain combinations used and the finding that CyA is more effective in prolonging survival of segmental than total intestinal grafts [12], although segmental transplants remain a highly immunogenic graft [11, 31].

Of interest is the most recent report of en-bloc transplantation of the liver, pancreas, duodenum, and entire small intestine for short bowel syndrome and treatment of upper abdominal malignancies in man [32]. The result of the abdominal organ cluster transplantation is superior to that of small bowel transplantation alone. The experimental rodent model of multiple organ transplantation confirms the beneficial effect of the cluster transplantation over that of single organ (small bowel) transplantation [23]. These reports suggest that massive antigen load release from the transplanted lymphoid tissue in the organs modulates the immune response of the host. In addition, the immunomodulatory role of the liver favors prolonged graft survival. This may explain why DST with too little antigen load fails to prolong intestinal graft survival. Numerous studies have demonstrated that rat renal [10], cardiac [17, 19–21], liver [1], and pancreatic islet [13] allografts are prolonged by DST, whereas whole pancreas, skin, or intestinal allografts are not significantly prolonged by recipient pretreatment with DST [22]. The results of this study support our earlier suggestion that there is a hierarchy of immunogenicity within allografts [33]. Based on this hypothesis, reduction of the immunogenicity by limiting the graft size or by graft manipulation prior to transplantation seems to be a necessary step in achieving intestinal allograft survival in combination with DST.

In conclusion, it appears that induction of immunologic unresponsiveness requires strict attention to (a) the antigen dosage [25], (b) the specificity of the cell type used for pretreatment, (c) the timing of immunization, (d) the route of antigen administration, (e) the responder status of the host, and (f) the rat combination used [8]. In addition, the prolongation that follows DST appears to be influenced by the specific composition of tissue transplanted, since intestinal allografts are rejected, while cardiac allografts in the same rat strain combination survive indefinitely when the recipients are subjected to the same pretreatment protocol using UV-B-irradiated DL and a brief course of peritransplant cyclosporine.

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