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Host immune suppression after small bowel/liver transplantation in rats

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Abstract Simultaneous liver grafting in the Lewis ($RT1^l$)-to-DA ($RT1^a$) rat strain combination protects small intestinal grafts from rejection. The present study examined host immune responses after combined small bowel/liver transplantation (SBL) in this model. Orthotopic liver transplantation and heterotopic small intestinal transplantation were performed simultaneously and compared with isolated small bowel allografts (SBA) and isolated small bowel isografts (SBI). All rats were sacrificed on postoperative day (POD) 7 or 14 for immunological and histological studies. The mean time to rejection of the SBA was 6.6 ± 0.3 days. In contrast, there was no clinical or histological evidence of intestinal rejection in SBL recipients during the 14 days of follow-up. The SBL recipients showed clinical and histological evidence of graft-versus-host disease (GVHD). Lmphocyte proliferation and IL-2 production in response to donor antigens were suppressed after SBL transplantation compared with the SBA or the SBI controls ($P < 0.05$). Cell-mediated cytotoxicity and lymphocytotoxic antibody production against donor cells were also significantly inhibited in the SBL recipients compared with the SBA control group ($P < 0.05$). We conclude that SBL transplantation in the Lewis-toDA rat strain combination: (1) suppresses host alloimmune responses, (2) prevents early intestinal rejection, and (3) favors the development of GVHD.

Key words Immune suppression, small bowel/liver transplantation, rat

Introduction

Clinical experience over the past two decades has demonstrated that liver allografts are better tolerated than other solid organ grafts [21, 25]. In addition to being less prone to rejection, liver grafts in some species have also been shown to induce donor-specific tolerance without the need for immunosuppressive treatment [16]. Donor-specific tolerance after liver grafting was first described in pigs by Calne et al. [3]. Later, Kamada and colleagues demonstrated that liver grafting protected kidney and heart allografts, but not skin grafts, from rejection in selected rat strain combinations [15]. A similar phenomenon may also occur in humans. Gonwa and colleagues have reported that kidney recipients with simultaneous liver allografts have 50% fewer rejection episodes than patients with isolated kidney allografts [9].

In marked contrast to the experience with clinical liver transplantation, isolated intestinal transplants have been plagued by frequent, severe graft rejection [26]. Small bowel/liver allografts (SBL), on the other hand, have had a low rate of rejection in our experience [11, 20]. The reduced frequency of intestinal rejection following SBL transplantation in humans has been attributed to the immunosuppressive effect of simultaneous liver grafting [11]. To test this hypothesis and to identify possible mechanisms, we developed a rat model of SBL transplantation [29]. In pilot experiments we found no evidence of intestinal rejection on postoperative days (POD)7 and 14 in DA ($RT1^a$) rats with Lewis ($RT1^l$) SBL allografts [28].

The present study extends these observations and examines host immune responsiveness in vitro after SBL transplantation.

Materials and methods

Animals

Inbred male Lewis ($RT1^l$) rats were purchased from Harlan-Sprague Dawley (Indianapolis, Ind., USA). DA ($RT1^a$) male rats were purchased from Trudeau (New York, N.Y., USA). The rats weighed 250–300 g. Animal use and care conformed to the guidelines established by the Canadian Council on Animal Care [4].

Surgical model

A detailed description of the surgical technique for SBL in rats has been reported elsewhere [29]. The intestinal and liver grafts were harvested from separate donors. A non-arterialized orthotopic liver transplantation was performed first. The intestinal transplantation was performed after completion of the liver grafting. The end of the donor aorta was sutured to the side of the recipient's aorta. The end of the donor portal vein was then anastomosed to the end of the recipient's left renal vein using the cuff technique. Both ends of the intestinal graft were exteriorized as stomas. The native gut was left intact. The rats received 12–15 ml of lactated Ringer's solution during the operation [4].

Experimental groups

Three types of transplantation were performed in DA rats: (1) combined Lewis small bowel/liver allografts (SBL), (2) isolated Lewis small bowel allografts (SBA), and (3) isolated DA small bowel isografts (SBI). Each group included 12 animals. Half of the rats in each group were sacrificed on POD 7; the remaining rats were followed until death or sacrificed on POD 14. Intestinal rejection was defined as the development of a palpable abdominal mass with enterostomal closure [18]. The solitary liver grafting in the Lewis-to-DA rat strain combination has been well documented by Zimmermann et al. Liver allografts are spontaneously tolerated in this strain combination with a mean survival of 197 days without immunosuppression [30] and were not included in this study.

Cell proliferation assay

Mixed lymphocyte reactions (MLR) were performed as follows. Recipient splenocytes were prepared at sacrifice in RPMI 1640 medium supplemented with 5% FCS, 100 U/ml penicillin and streptomycin, 2 mM glutamine and 2×10^{-5} M 2-ME (Sigma, St. Louis, Mo., USA) and used as the responder cells. The responder cells (2×10^5) were mixed with 4×10^5 irradiated Lewis splenocytes (2500 rad) and then placed into 96-well, round-bottom tissue cultures plates (Linbro Flow Laboratories, Mclean, Va., USA) in triplicate in a total culture volume of 200 µl/well. Plates were cultured at 37°C in a 5% CO_2 humidified incubator. On day 5, each well was pulsed with 1 uCi of tritiated thymidine (^{3}H-TdR, 40–60 Ci/mmol; Amersham, UK) for 16 h. Cells were harvested onto fiber glass paper and ^{3}H-TdR incorporation was counted with a Beckman scintillation counter [19]. The responder cells alone incorporated less than 1000 cpm.

Cell-mediated cytotoxicity assay

The assay used was described by Bumgardner et al. [2] with slight modifications. Target cells were prepared by culturing Lewis splenic cells (5×10^6 cells/ml) with concanavalin A (5µg/ml; Sigma) for 2 days and labeling these cells with sodium chromate (250–500 mCi/mg, ^{51}Cr, Amersham). ^{51}Cr-labeled target cells (4×10^4 cells/well) were placed into 96-well, round-bottom culture plates along with varying numbers of recipient splenocytes to achieve the desired effector/target cell ratio. Total culture volume was 200 µl in each well and all assays were performed in triplicate. The plates were centrifuged at 700 *g* for 10 min after 4 h of incubation. Supernatant (100 µl) was removed from each well and counted in a Beckman gamma counter. Total counts were determined by lysing the target cells with 0.1% Triton (Sigma). The supernatant from target cells, which were cultured with medium alone, was used to determine the spontaneous release. Background ^{51}Cr release was 10%–15%. Cytotoxicity was calculated using this formula:

$$\frac{\text{Experimental cpm} - \text{spntaneous cpm}}{\text{Total cpm} - \text{spntaneous cpm}} \times 100$$

Interleukin-2 (IL-2) assay

An IL-2-dependent cell line (CTLL-2) was used to assay IL-2 activity as previously described [8]. Culture supernatants obtained from the MLR at 3 days were serially diluted with complete RPMI 1640 medium in 96-well, falt-bottom culture plates (Linbro Flow Laboratories) in triplicate. CTLL-2 cells (1×10^4) were added into each well. The total culture volume was 200 µl per well. The cultures were incubated for 20 h at 37°C in a 5% CO_2 humidified incubator and then pulsed for 4 h with 1 uCi of ^{3}H-TdR per well. The cultures were harvested onto fiber glass paper and counted with a Beckman scintillation counter. Cultures containing complete RPMI 1640 medium were used as a negative control. Serial dilutions of a known concentration of IL-2 were used as a positive control (160 µ/ml; Cedarlane Laboratories, Ontario, Canada). To facilitate comparisons between experiments, the data were converted into units using the principle of probit analysis described by Gillis et al. [8].

Lymphocytotoxic antibody assay

The complement-dependent cytotoxicity assay was performed using a two-step incubation method. Lewis rat splenic cells (1×10^5 cells/well) were incubated for 30 min at room temperature with various dilutions of serum obtained from recipients at sacrifice. Serum from isograft transplants was used as a control. After the incubation, 50 µl Low-tox rabbit serum (Cedarlane) was added into each well as the source of complement and then incubated for another 60 min at 37°C. Cell lysis was calculated using the trypan blue exclusion method. Background cell lysis was 8% in the control group.

Pathology

Samples were obtained at necropsy from: thymus, lungs, liver, spleen, native and transplanted gut, mesenteric lmyph nodes, and skin. Tissue samples were fixed in 10% buffered formaldehyde, embedded in paraffin, cut at 3 µm, and stained with hematoxylin-eosin. The tissue sections were examined microscopically for evidence of rejection and GVHD.

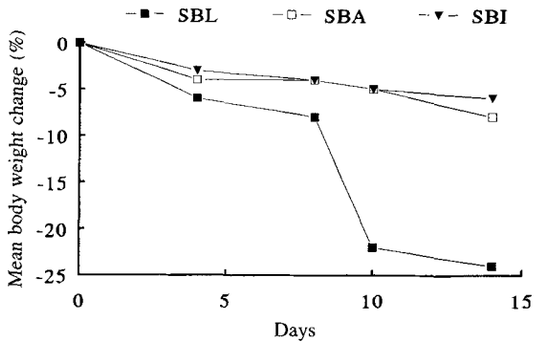

Fig. 1 Weight change after transplantation in rats with small bowel/liver allografts (SBL), isolated small bowel allografts (SBA), and isolated small bowel isografts (SBI)

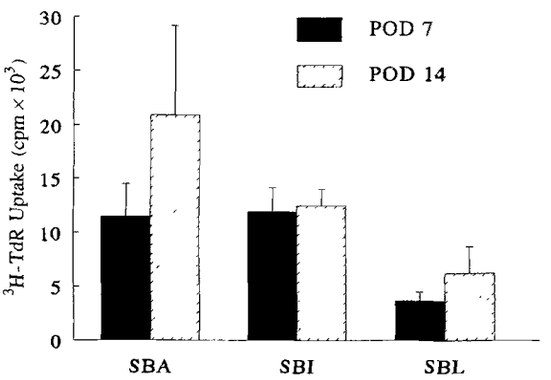

Fig. 2 Mixed lymphocyte reactions on the 7th and 14th postoperative days in rats with small bowel/liver allografts (SBL), isolated small bowel allografts (SBA), and isolated small bowel isografts (SBI). The responder cells alone incorporated less than 1000 cpm

Statistical analysis

The immunological data are expressed as the mean ± the standard deviation of the six animals in each group. The data were compared using an analysis of variance with a P value less than 0.05 considered significant.

Results

Simultaneous liver grafting protects intestinal allografts from rejection

The mean time to rejection of the SBA was 6.6 ± 0.3 days. Rejection of the heterotopic intestinal allografts was well tolerated; these animals remained active and their body weight change was comparable to that in the SBI control group (Fig. 1). Histological examination of the SBA showed heavy lymphocytic infiltation with blunted villi and decreased numbers of goblet cells on POD 7 and full-thickness necrosis of the allografts by POD 14. In contrast, none of the rats with SBL or SBI transplants had clinical or histological evidence of intestinal rejection during the 14 days of follow-up. Rats with SBL allografts showed clinical and histological evidence of GVHD, as manifested by diarrhea, cachexia, and progressive weight loss (Fig. 1). Two of the rats in this group died from interstitial pneumonitis on POD 8 and 10. Necropsy in the SBL recipients revealed fibrosis of the thymus and splenomegaly. Histological examination demonstrated: (1) thymocyte depletion with effacement of the corticomedullary junction and medullary attenuation, (2) loss of the normal follicular architecture in the spleen and the mesenteric lymph nodes, and (3) infiltration of the host's thymus, spleen, and lymph nodes with immunoblasts. Other typical histological features of GVHD were not observed in the skin, the lungs, or the native gut of the SBL recipients. None of these changes were present in the SBA or SBI recipients.

Lymphocyte proliferation and cytotoxicity to donor antigens

Results of the MLR are shown in Fig. 2. Lymphocytic proliferation was present but significantly inhibited in the SBL recipients on POD 7 and 14 compared with the SBI and the SBA controls ($P < 0.05$). The development of strong cell-mediated cytotoxicity against donor antigens on POD 7 and 14 correlated well with the clinical evidence of severe allograft rejection in the SBA recipients. Cell-mediated cytotoxicity was significantly suppressed in the SBL group on POD 7 and 14 compared with the SBA group ($P < 0.05$; Fig. 3).

IL-2 activity

IL-2 activity in the SBA group was markedly elevated on POD 7 compared with the SBI group. Splenocytes from the SBL recipients had significantly lower levels of IL-2 production than the SBA and SBI rats in response to the donor stimulator cells on both POD 7 and 14, as shown in Fig. 4 ($P < 0.05$).

Lymphocytotoxic antibody production

Anti-RT1^l antibodies were present on POD 7 and were maintained at a very high level on POD 14 in the SBA recipients. The titers of the lymphocytotoxic antibody in the peripheral circulation were significantly lower in the SBL group on POD 7 and 14 ($P < 0.05$; Fig. 5).

Discussion

This study provides further evidence that simultaneous liver grafting can protect the intestinal allograft from rejection. In the Lewis-to-DA rat strain combination, we

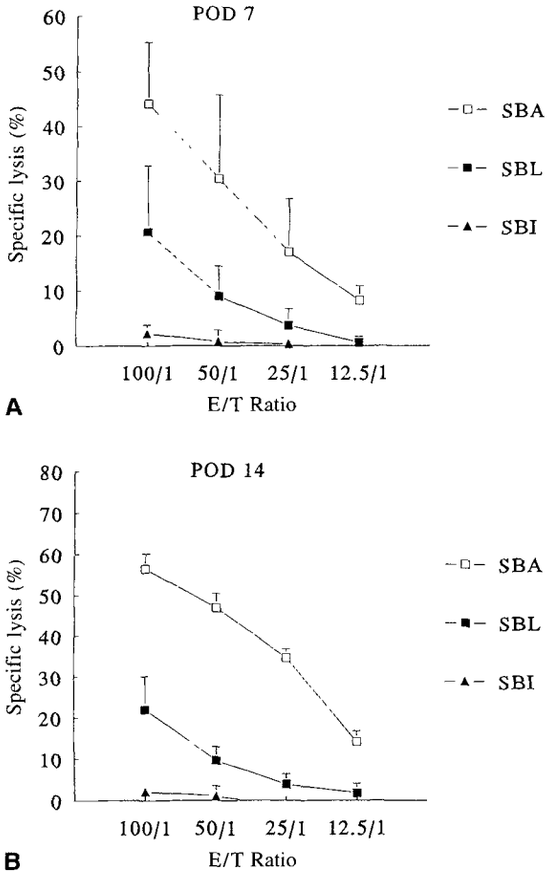

Fig. 3 Donor-specific, cell-mediated cytotoxicity on the 7th and 14th postoperative days in rats with small bowel/liver allografts (SBL), isolated small bowel allografts (SBA), and isolated small bowel isografts (SBI). Background ^{51}Cr release was 10%–15%

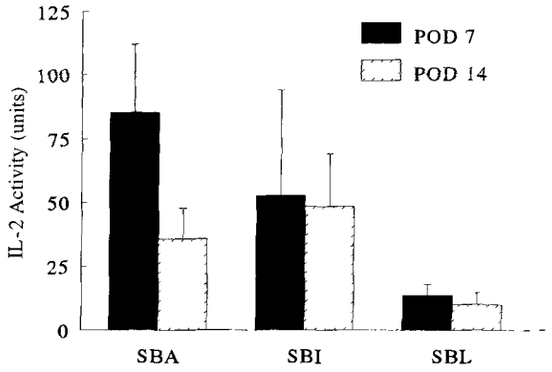

Fig. 4 IL-2 production by splenocytes harvested from rats with small bowel/liver allografts (SBL), isolated small bowel allografts (SBA), and isolated small bowel isografts (SBI) on the 7th and 14th postoperative days after stimulation for 3 days in a mixed lymphocyte culture with irradiated donor splenocytes

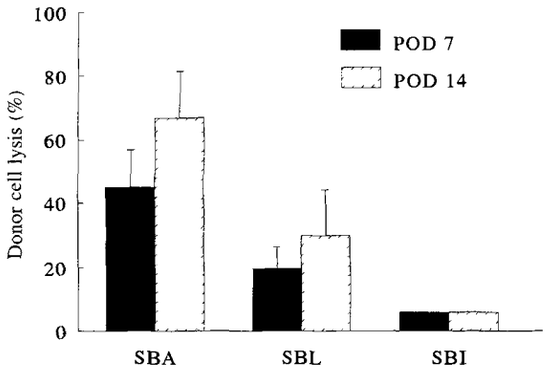

Fig. 5 Donor-specific lymphocytotoxic antibody production on the 7th and 14th postoperative days in rats with small bowel/liver allografts (SBL), isolated small bowel allografts (SBA), and isolated small bowel isografts (SBI). Background cell lysis was 8% in the SBI control group

found that SBL grafting diminished host alloimmune responses, prevented early intestinal rejection, and promoted the development of GVHD. The clinical applicability of the present study is unclear since the animals were sacrificed on POD 7 or 14 to obtain tissue samples. Further research is required to determine whether the immunological protection provided by simultaneous liver grafting in this model will persist over longer period of time.

Immune suppression with liver grafting has been attributed to many mechanisms including: the induction of T suppresssor cells [12], the selective depletion of cytotoxic T cell clones [13], the release of soluble MHC class I antigens from the liver graft, and the production of anti-MHC class II antibodies [5, 14, 17]. In the present study, we found that IL-2 production was decreased and cell-mediated cytotoxicity was inhibited after SBL. These data suggest that another potential mechanism for immune suppression after liver grafting is the preferential stimulation of antigen-specific Th_2 cells and/or the inhibition of Th_1 cells. Th_1 cells release IL-2 and IFN-γ and mediate delayed-type hypersensitivity reactions, macrophage activation, and cytotoxic T cell maturation. Th_2 cells produce IL-4 and IL-5, thereby stimulating antibody production [1]. Differential activation of Th_2 cells has been associated with tolerance to allogeneic heart grafts induced by cyclosporin [22]. Alloantigen delivered by portal venous system has also been shown to preferentially activate IL-4-producing cells rather than IL-2-producing cells [10].

Graft rejection is usually the predominant immune response after isolated intestinal allotransplantation. In the present study, however, the SBL recipients developed GVHD with no evidence of graft rejection. The immune suppression produced by liver grafting may have favored the development of GVHD mediated by the larger number of donor immune cells transplanted with the SBL grafts. Migration of the graft-derived lymphocytes into the hosts' lymphoid tissues during GVHD may have contributed to

immune suppression by damaging the thymus and producing a prostaglandin-mediated impairment of B-cell function [27]. Other changes in immune function that occur with GVHD include an increase in natural killer cell activity [6, 7] and the induction of T suppressor cells [24].

SBL transplantation is uniquely suited for the treatment of patients who have developed liver failure while on total parental nutrition. Although the experimental data are encouraging, it is still unclear whether simultaneous liver grafting will diminish the risk of intestinal rejection in humans. In contrast to the low graft rejection rates in patients who were treated with cyclosporin after SBL at our center [11, 20], the Pittsburgh group has reported high rejection rates in patients treated with FK 506 after SBL [23]. Sepsis has been a frequent problem after SBL at both centers. The present data suggest that this complication may be due to the profound immunosuppression produced by the combined effects of anti-rejection therapy, liver grafting, and GVHD after SBL.

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