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Comparison of potentiality to induce graft-*versus*-host reaction with small bowel, pancreas/spleen, and liver transplantation in the rat

E. KOBAYASHI*†, N. KAMADA†, S. ENOSAWA‡, N. TOYAMA*†, L. DELRIVIERE†, S. GOTO†§, Y. IL KIM§ & M. MIYATA* **Department of Surgery, Jichi Medical School, Omiya Medical Centre, Omiya, Japan, †Department of Surgery, University of Queensland, Queensland, Australia, ‡Department of Experimental Surgery, National Children's Medical Research Centre, Tokyo, and §First Department of Surgery, Oita Medical College, Oita, Japan*

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SUMMARY

Although small bowel transplantation (SBT), or pancreas-spleen transplantation (PST) often lead to lethal graft-*versus*-host reaction (GVHR) in experimental animals, fatal GVHR is rare after clinical liver transplantation. This study describes a modified model of SBT and PST in the rat using cuff techniques applied to the renal artery and vein of the recipient. The ability of LEW ($RT1^l$) or BN ($RT1^n$) lymphocytes accompanying intestinal, splenic, or hepatic grafts to induce lethal GVHR in (LEW × BN) F_1 hybrid recipients was compared. SBT and PST experiments showed that lethal GVHR always occurred in LEW-into-F_1 combination, but was much less frequent in BN-into-F_1 SBT. In mixed lymphocyte reaction (MLR), LEW mesenteric or splenic T cells showed significantly higher proliferative responses against BN stimulators than did BN mesenteric or splenic T cells against LEW. Adoptive cell transfer experiments using mesenteric or splenic cells also showed that LEW cells were higher responders than BN. In contrast with SBT and PST results, a lethal GVHR was not induced after liver or pancreas grafting alone in either parent-to-F_1 combination. In MLR, hepatic T cells from either parent failed to elicit a proliferative response against allostimulators. These results indicate that the occurrence of lethal GVHR is dependent upon the reactivity of parental lymphocytes against allo-antigenicity of F_1 hybrids and also upon the lymphoid tissue transplanted. The lack of alloreactivity of hepatic T cells accounts for the absence of lethal GVHR after liver grafting.

Keywords graft-*versus*-host reaction small bowel transplantation liver transplantation pancreas/spleen transplantation

INTRODUCTION

Graft-*versus*-host reaction (GVHR) after organ transplantation is thought to result from the simultaneous transfer of donor lymphocytes into the recipient. Small bowel transplantation (SBT) and pancreas-spleen transplantation (PST) in experimental animals often lead to lethal GVHR [1–10]. It has been demonstrated using the rat model in parent-to-F_1 hybrid combinations that lymphocytes of the intestinal or splenic graft attack host tissues [3–6,9,10]. Although liver grafts transfer large numbers of lymphocytes into the recipient [11], GVHR has been seen only occasionally after clinical liver transplantation [12–14]. Hepatic lymphocytes may have properties different from those of other lymphoid organs [15].

In this study we describe a highly successful method of SBT and PST in the rat, using cuff techniques. Using this model we studied the ability of mesenteric, splenic and hepatic lymphocytes accompanying parent small bowel, spleen and liver grafts to induce lethal GVHR in F_1 hybrids. The ability of injected BN or LEW mesenteric and splenic lymphocytes to induce GVHR in F_1 recipients was also investigated. *In vitro* mixed lymphocyte reaction (MLR) was used to assess the alloproliferative response to BN and LEW mesenteric, splenic and hepatic cells.

Correspondence: Eiji Kobayashi, MD, Department of Surgery, University of Queensland and QIMR, The Bancroft Centre, 300 Herston Rd., Herston, Qld. 4029, Australia.

MATERIALS AND METHODS

Rats

Male BN (MHC haplotype $RT1^n$) and LEW ($RT1^l$) inbred rats weighing 230–270 g were used as donors, and (LEW × BN)F_1 hybrid rats weighing 250–290 g, as recipients. All rats were purchased from Seiwa Experimental Animals Co. Ltd. (Japan).

Surgical techniques

All operations were carried out under ether anaesthesia.

Heterotopic SBT. The intestinal graft was prepared using a modification of the method of Monchik & Russell [3]. In brief,

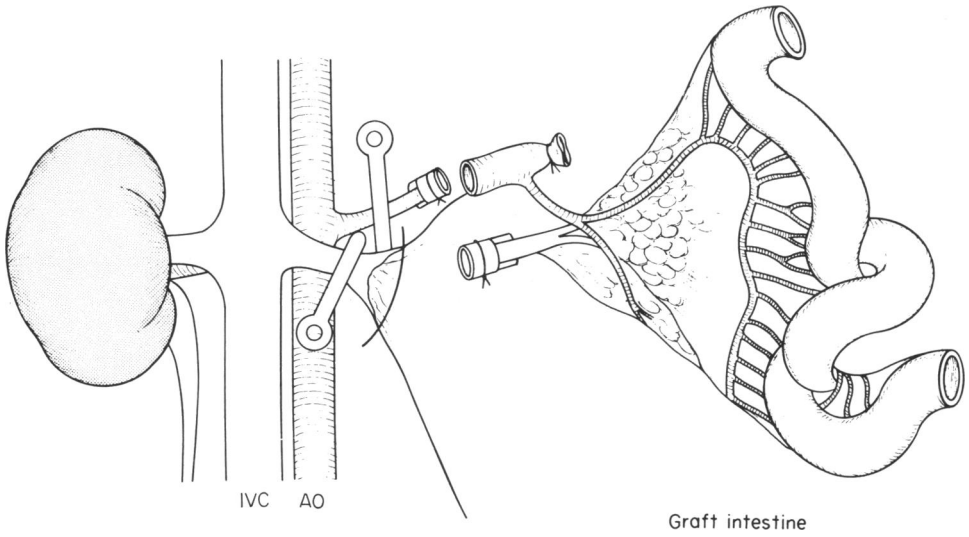

Fig. 1. Attachment of the intestine graft using the cuff technique. The venous anastomosis was performed first, between the cuffed donor portal vein (PV) (16 G cuff) and the recipient renal vein kept taut by traction. The arterial anastomosis was then carried out by inserting the recipient renal artery, cuffed with 22 G tube, into the donor aortic cuff containing the superior mesenteric artery. AO, Aorta; IVC, infrahepatic vena cava.

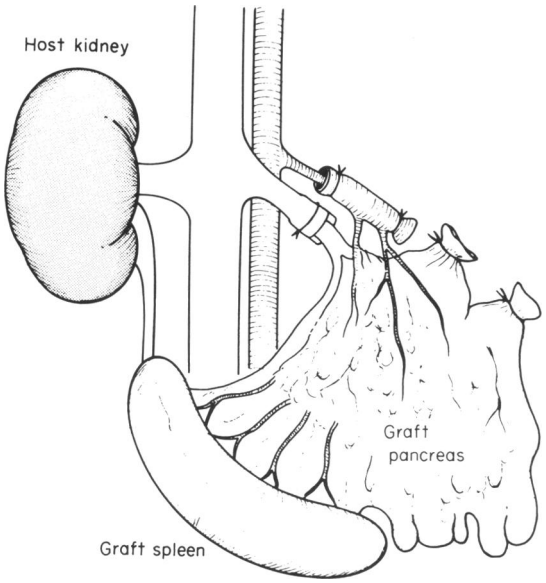

Fig. 2. Schema of the technique of pancreas-spleen transplantation using cuff method. The anastomoses were carried out between the donor cuffed portal vein (PV) (16 G cuff) and aortic patch and the recipient renal vein and cuffed artery (22 G cuff), respectively.

the aorta, at the superior mesenteric artery, and portal vein (PV) were freed of their surrounding tissues. After systemic heparinization, approximately 10 cm of the terminal ileum including mesenteric lymphoid tissue was prepared with separation of vascular pedicles. In a cold saline bath, the PV of the graft was cuffed with polyethylene tubing (16 G, Cat. code SROT1851C, Terumo Co. Ltd., Tokyo, Japan). The recipient operation was carried out with a modification of the method of Kamada for rat renal transplantation [16]. The venous anastomosis was performed first, between the cuffed donor PV and the recipient renal vein, keeping tension by traction. The arterialization was then done by inserting the recipient renal artery, cuffed with 20 G or 22 G polyethylene tubing (Terumo), into the donor aortic cuff containing the superior mesenteric artery (Fig. 1). Both ends of the intestinal graft were exteriorized as stomas.

Heterotopic PST and pancreas transplantation. The harvesting of the pancreas-spleen graft was performed using a modification of the pancreatic-duct ligation method [10]. Briefly, the aorta, at the celiac origin, and the PV were skeletonized. After systemic heparinization, the duct at the pancreatic head was ligated and the pancreas-spleen graft, including vascular pedicles, was removed and then prepared in a cold bath. After the PV of the graft was cuffed with 16 G tubing, intra-abdominal PST was performed using the same method as for SBT (Fig. 2). Pancreas transplantation (PT) alone was carried out by splenectomy after revascularization of the *en bloc* pancreas-spleen graft.

Orthotopic liver transplantation (OLT). The technique used has been described fully elsewhere [17].

Assessment of GVHR

SBT, PST, PT, and OLT recipients were examined daily for evidence of cachexia, cutaneous erythema, diarrhoea and reduced activity. Surviving animals were killed at 60 days and tissues submitted for histology.

Tissue samples were fixed in 10% formalin in water, dehydrated and embedded in paraffin. Sections were cut with a microtome, deparaffinated in alcohol and xylene, and stained with haematoxylin and eosin (H&E). Animals which died before 60 days were autopsied and relevant tissue was similarly assessed.

Induction of GVHR with mesenteric or splenic lymphocytes

Mesenteric lymph nodes or spleen from BN or LEW rats were minced and passed through a 60 mesh wire sieve as previously described [18]. Cells were washed once with RPMI 1640 medium (300 ***g***, 10 min), counted and inoculated into F_1 recipients via the tail vein.

Table 1. Survival time of small bowel, pancreas-spleen, pancreas, or liver grafts in parent-to-F_1 or F_1-to-F_1 combinations

Transplanted organ	Combination	*n*	Survival in days*	Mean survival time ± s.d.
Small bowel	LEW-into-F_1†	5	14, 15, 16 (2), 17,	15·6 ± 1·1
	BN-into-F_1	5	17‡, >60(4)	>60
	F_1-into-F_1	5	>60(5)	>60
Pancreas-spleen	LEW-into-F_1	5	14(2), 15, 16(2)	15·0 ± 1·0
	BN-into-F_1	5	15, 17, 18(2), 19	17·4 ± 1·5
	F_1-into-F_1	5	>60(5)	>60
Pancreas	LEW-into-F_1	4	>60(4)	>60
	BN-into-F_1	4	>60(4)	>60
Liver	LEW-into-F_1§	5	>60(5)	>60
	BN-into-F_1§	5	>60 (5)	>60

* Number of recipients in parentheses.

† All stomas on F_1 recipients implanted with parent intestine grafts were not closed during observation.

‡ Only one F_1 recipient implanted with BN intestine graft showed typical symptoms of graft-*versus*-host reaction (GVHR). Autopsy showed haemorrhagic pneumonia and splenomegaly.

§ Histological examination showed that some lymphocytes were detected in the grafted liver at 7 and 14 days after orthotopic liver transplantation (OLT), but no obvious changes from syngeneic OLT at 60 days.

Mixed lymphocyte reaction

Splenic, mesenteric and hepatic lymphocytes were prepared by passing minced tissue through a 60 mesh wire sieve, followed by a Ficoll–Conray density gradient. T cells were purified by passage through a nylon fibre column (Wako Pure Chemicals Ltd., Japan). Mitomycin C-treated 2×10^5 stimulator spleen cells were cultured with 1×10^5 responder T cells in a 96-well microtitre plate for 3 days. Twenty hours before harvesting, 0·5 μCi of ^{3}H-thymidine (NET02720, NEN) was added to each well. The incorporated radioactivity was counted in a scintillation counter. Since the number of passenger lymphocytes in the pancreas was negligible, it was not examined.

RESULTS

Survival of F_1 hybrids after SBT, PST, PT or OLT

Table 1 shows the survival of (LEW × BN)F_1 rats receiving intestinal, pancreas-spleen, pancreas or liver grafts from either parental strain. All the F_1 recipients of LEW intestinal grafts had died by 17 days with typical GVHR symptoms, showing weight loss, erythema and diarrhoea. Histological examination showed the presence of large numbers of infiltrating immunoblasts in the skin, liver, spleen, salivary glands and ileum. In contrast, with BN as SBT donors, only one of five recipients died with typical GVHR. The remaining four survived in good health to the study end point of 60 days. They showed no consistent histological changes at that time. F_1 recipients of LEW or BN pancreas-spleen grafts also died early at 15·0 ± 1·0 or 17·4 ± 1·5 days respectively after grafting. However, PT alone in either parent-into-F_1 combination did not show any symptoms or histological evidence of GVHR at 60 days after grafting. F_1 rats receiving either LEW or BN liver grafts also survived to 60 days with no signs of GVHR in either group. The histology showed infiltration of some lymphocytes into the grafted liver at 14 days, but no obvious microscopic changes at 60 days.

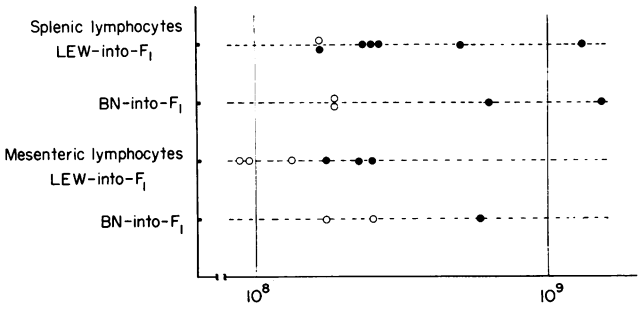

Fig. 3. Induction of lethal graft-*versus*-host reaction by mesenteric or splenic lymphocytes. Variable numbers of BN or LEW splenic or mesenteric lymphocytes were injected intravenously into the tail vein of F_1 recipients. ○, Individual surviving F_1 rats; ●, F_1 recipients dying of GVHR. Abscissa axis shows the number of lymphocytes injected in log units. F_1 rats receiving more than $2{\cdot}2 \times 10^8$ LEW splenic lymphocytes died at 21·0 ± 1·2 days (mean ± s.d. in five rats) with typical symptoms of GVHR, while more than $8{\cdot}0 \times 10^8$ BN splenic lymphocytes were needed to induce a lethal GVHR; $2{\cdot}4$–$4{\cdot}0 \times 10^8$ LEW mesenteric lymphocytes also killed F_1 hosts, but the same dose of BN mesenteric lymphocytes did not.

Induction of GVHR by mesenteric or splenic lymphocytes

Mesenteric or splenic lymphocytes from LEW or BN donors were injected into F_1 recipients to induce GVHR. The results obtained are shown in Fig. 3. Greater than $2{\cdot}2 \times 10^{10}$ LEW splenic lymphocytes induced a lethal GVHR in all recipients, while BN splenic lymphocytes were much less effective, the threshold for induction of GVHR being $8{\cdot}0 \times 10^8$ cells. Transfer

Table 2. Mixed lymphocyte reaction between BN and LEW

Stimulator*	Responder*	^{3}H-TdR uptake (ct/min)†
BN	LEW	
	Splenic T cells	17 110 ± 9500
	Mesenteric T cells	22 307 ± 5137
	Hepatic T cells	627 ± 276
LEW	BN	
	Splenic T cells	3091 ± 365
	Mesenteric T cells	8711 ± 3079
	Hepatic T cells	302 ± 32

* Stimulator spleen cells were treated with mitomycin C and responder cells were purified by passage through a nylon wool column. Experimental details are described in Materials and Methods.

† Two assays were performed and the results obtained are shown in mean ± range.

of mesenteric lymphocytes also showed that LEW cells were higher responders than BN against the F_1 hosts.

MLR of splenic, mesenteric or hepatic lymphocytes

In order to investigate the discrepancies in GVHR induction using different donors and organs, the responses of splenic, mesenteric and hepatic lymphocytes in MLR (BN against LEW and *vice versa*) were examined (Table 2). LEW splenic and mesenteric T cells gave high proliferative responses against BN stimulators, whereas LEW hepatic T cells showed no proliferation. In the reverse combination, the response of BN lymphocytes was weaker in all cases; BN mesenteric T cells were the best responders, while hepatic T cells completely failed to proliferate.

DISCUSSION

Although the conventional techniques of SBT and PST in the rat have been reported and widely used [3,9], the methods require expert microsurgical skills and long operation times, whereas the method described in this series is relatively simple, with a consequently high success rate. This method can also be applied to small bowel—or pancreas—liver combined transplantation in the rat.

GVHR after organ transplantation is thought to result from reactivity of donor lymphocytes in the transplanted organ against host antigen [4–6]. However, characteristics of lymphocytes vary depending on the type of transplanted organ. In particular, hepatic lymphocytes are postulated to be self-reactive and forbidden clones under certain non-physiological conditions [15].

Our results showed that a lethal GVHR always occurred after SBT and PST in LEW-into-F_1 combination, but was much less frequent in BN-into-F_1 SBT. However, even in the latter combination, a lethal GVHR was always induced when a longer length of intestinal graft was implanted (manuscript in preparation). The MLR results correlated well with GVHR induction; LEW splenic and mesenteric T cells caused significantly greater proliferative responses against BN stimulators than did BN mesenteric T cells against LEW. Furthermore, GVHR was induced by adoptive transfer of $2{\cdot}2 \times 10^8$–$4{\cdot}0 \times 10^8$ splenic lymphocytes in the LEW-to-F_1 but not in the BN-to-F_1 combination at the same dose. The BN strain has been shown to produce poor *in vitro* proliferative responses to mitogens and allogeneic cells [19] and only high doses of BN splenic lymphocytes ($8{\cdot}0 \times 10^8$ or greater) induced a lethal GVHR. The transfer experiments of mesenteric lymphocytes showed the same results. PST always led to a lethal GVHR, while PT alone in either parent-into-F_1 combination did not. These results indicate that the occurrence of a lethal GVHR depends on the quantity of donor T cells and on the type of the lymphoid tissue of origin.

In contrast to the findings in SBT and PST, lethal GVHR was not induced after liver grafting in either parent-to-F_1 combination. F_1 recipients survived to the study end point despite lymphocyte proliferation within the grafted liver. In fact, hepatic T cells gave no proliferative response against allostimulators in MLR, although they responded to mitogen stimulation. This indicates a significant difference in immune response potential of T cells from the liver compared with those from other lymphoid organs, and may account for the absence of GVHR after OLT. A few clinical cases of generalized, lethal GVHR after liver transplantation have been reported [7–9]; some of these may have been due to blood transfusion during operation. We have previously reported that GVHR of F_1 recipients surgically treated was more strongly evoked when recipients were injected with parent lymphocytes at the time of operation than when F_1 rats received the same dose of parent cells without operation [18].

In the parent-to-F_1 combinations tested, SBT and PST induced a lethal GVHR, whereas PT and OLT did not. Hepatic T cells lacked the ability to mount an alloproliferative response *in vitro*; in this they differed from splenic and mesenteric T cells. The occurrence of a lethal GVHR in F_1 hybrids was determined by the dose and origin of the parental cells transferred. The immune response potential of hepatic T cells deserves further study.

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