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Graft-Versus-Host Disease in Fully Allogeneic Small Bowel Transplantation: Incidence of the Disease and Strain Combinations

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WE PREVIOUSLY reported that fully allogeneic small bowel transplantation (SBT) from Lewis (LEW) to Brown Norway (BN) rats caused fatal graft-versus-host disease (GVHD) when the recipients were treated with a short course of FK 506.[1] Graft-versus-host disease has also been well described after parent to F_1 hybrid SBT,[2] in which immune reaction between donor and recipient is completely unbalanced. This study was carried out to determine if certain strains or combinations were more or less susceptible to GVHD, similar to the parent to F_1 hybrid combination.

MATERIALS AND METHODS

Animals

Inbred male LEW (*RT-1*^{l}), BN (*RT-1*^{n}), ACI (*RT-1*^{a}), and PVG (*RT-1*^{c}) rats, weighing 200 to 300 g, purchased from Harlan Sprague-Dawley (Indianapolis, Ind), were used as donors or recipients.

Mixed Lymphocyte Reaction

One-way mixed lymphocyte reaction (MLR) was performed using mesenteric lymph node cells as described before.[3] Lymphocytes from normal LEW, BN, ACI, and PVG rats were used as responders and/or stimulators to examine in vivo proliferation activity.

Operation

Orthotopic SBT with portocaval drainage was performed by removing the entire donor small intestine from the ligament of Treitz to the ileocecal valve and by anastomosing end-to-side between graft aorta and recipient infrarenal aorta and graft portal vein and recipient vena cava. The recipient intestine was removed and intestinal continuity was restored by proximal and distal intestinal end-to-end anastomoses. The orthotopic liver transplantation (OLT) was carried out according to Kamada's method.[4] Arterial reconstruction was omitted. Body weight, activity, skin color, and defecation were checked daily for the first 14 days, followed by twice or more per week until day 150.

Experimental Design

Six strain combinations, including ACI-LEW, ACI-BN, ACI-PVG, LEW-BN, LEW-PVG, and BN-PVG were examined. In each combination, OLT was performed in both directions, and animal survival was compared. Because of the tolerogenic nature of the liver graft, some liver grafts were expected to survive indefinitely without immunosuppression.[5] Any discrepant graft and animal survival between the grafting directions suggests an immunologic imbalance between the strain combination.

SBT with or without immunosuppression was also performed for each strain combination in both directions to investigate the development of GVHD. For immunosuppression, intramuscular FK 506 dissolved in HCO-60 and D-mannitol carrier solvent (gift from Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan) was used with a daily dose of 0.64 mg/kg for 14 days, starting on the day of operation.

Statistical Analysis

Survival days were analyzed for statistical significance by the generalized Wilcoxon test.

RESULTS

Mixed Lymphocyte Reaction

As expected, lymphocytes from all strains used as responders proliferated more in the presence of allogeneic than syngeneic stimulator cells. Proliferative counts in recipients who developed GVHD (eg, BN responder and LEW stimulator) were not significantly different from those of the reversed responder-stimulator direction cases (Table 1).

Animal Survival After OLT

In ACI-BN and ACI-LEW combinations, animal survival after OLT was significantly influenced by the direction of grafting. In both combinations, ACI liver was acutely rejected by BN or LEW recipients with a median survival of 10 to 11 days. However, ACI recipients of BN or LEW liver grafts survived for more than 90 days. The direction of the grafting had less effect on survival in the BN-LEW and BN-PVG combinations. The results of PVG-ACI and PVG-LEW combinations are still being evaluated, although the direction of grafting did not significantly affect survival in these combinations.

Animal Survival After SBT

In all 12 combinations, untreated recipients died of rejection (pathologically confirmed) with median survival between 5 and 14 days. Untreated BN recipients of PVG or LEW grafts and one PVG recipient with a BN graft showed a transient skin rash, which resolved when the grafts were rejected. Two-week treatment with FK 506 effectively prolonged survival for more than 100 days in

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0041-1345/93/$3.00/+0

Table 1. Animal Survival After Orthotopic Liver or Small Bowel Transplantation

		Median Animal Survival Days (n)		
			SBT	
Donor	Recipient	OLT No Treatment	No Treatment	FK Treatment
ACI	BN	11 (5)*	8.5 (4)	91 (5)
BN	ACI	>100 (4)	14 (4)	>150 (4)
ACI	LEW	10 (9)*	5 (4)	>111 (3)
LEW	ACI	>89.5 (4)	8.5 (4)	>150 (4)
LEW	BN	30.5 (6)*	12 (3)†	29 (7)‡
BN	LEW	>100 (6)	10.5 (6)	>150 (10)
PVG	BN	25 (3)*	12 (5)†	42 (7)‡
BN	PVG	>50 (4)	11 (3)	>150 (7)
PVG	LEW	30 (3)	13 (3)	>150 (4)
LEW	PVG	>50 (3)	14 (5)	>150 (4)
ACI	PVG	>50 (6)	8 (3)	>150 (3)
PVG	ACI	>50 (3)	11 (3)	>150 (4)

*Strain combinations which show significantly different animal survival rates between the directions of transplantation.
†Animals which died of fatal GVHD.
‡Animals showed temporary GVHD before graft rejection.
§One of three animals showed temporary GVHD.

most of the combinations, except when BN rats were used as recipients. ACI grafts transplanted into BN recipients developed chronic rejection with median survival of 91 days. On the other hand, when BN recipients received a LEW or PVG graft, the recipients developed a skin rash, hyperkeratosis, hair loss, and weight loss after cessation of FK 506, and eventually died with median survival of 29 and 42 days, respectively.

DISCUSSION

GVHD after small bowel transplantation has been well documented, when immune reaction between the host and the recipient was completely unbalanced as seen in unidirectional transplantation from parent to F1 hybrid strains.[2] In this study, moderate immunologic imbalance was identified in certain rat strain combinations, such as ACI-BN and ACI-LEW, in which the liver graft was acutely rejected, but only in one grafting direction. In the reverse order, the graft was accepted. When a small bowel graft was used in these relatively unbalanced combinations, GVHD was not observed either with or without FK 506 immunosuppression. In 12 different fully allogeneic small bowel transplantations examined here, occurrence of GVHD was seen only in BN recipient of LEW or PVG grafts under FK 506. Similar fatal GVHD, described by DeBruin et al, was also observed in a BN recipient of WAG (*RT-1*^{u}) graft under CyA.[6] These findings suggest that lethal GVHD seen after small bowel transplantation under immunosuppression is somewhat unique to the BN strain. It is worth noting that the BN strain is also susceptible to chronically induced autoimmunity when the rats are fed mercuric chloride or golden sodium.[7] It may be purely coincidental, but the monoclonal antibody, L-21-6, which recognizes the invariant chain of the class II MHC molecule in rats, reacts with all rat strains tested (LEW, ACI, PVG, F334, and W/F) except BN (unpublished observation). Since the invariant chain is thought to protect the class II molecule from endogenous antigen binding, the difference detected by this antibody may be of functional significance.

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