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## Lymphocyte Traffic and Graft-Versus-Host Disease After Fully Allogeneic Small Bowel Transplantation

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GRAFT-versus-host disease (GVHD), in which immunocompetent cells in the graft react against the recipient, has been one of the concerns after small bowel transplantation, because of the large amount of lymphocytes in gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes. In rodent experiments, GVHD has been extensively studied using convenient unidirectional transplantation from parent strains to F1 hybrids.<sup>1</sup> However, in fully allogeneic transplantation and large animal experiments, rejection and not GVHD has been the dominant phenomenon. We found that small bowel transplantation from Lewis to Brown Norway rats induced fatal GVHD when transient immunosuppression with FK 506 was employed, similar to the study shown with cyclosporine (CyA).<sup>2</sup>

We had previously concentrated on recipient lymphoid trafficking after small bowel transplantation and have shown that most of the lymphoid tissues in small bowel allograft are replaced by the recipient hemato lymphoid cells.<sup>3</sup> In the current study, we focused on the donor lymphoid tissues implanted with the graft.

The fate of the donor lymphoid cells in the recipient and its relationship to the development of GVHD was examined.

### MATERIALS AND METHODS

#### Animals

Inbred male Lewis rats (*RT-1<sup>l</sup>*, LEW) and Brown Norway rats (*RT-1<sup>n</sup>*, BN) weighing 200 to 300 g were obtained from Harlan Sprague Dawley (Indianapolis, Ind), and were maintained in conventional animal facilities under standard conditions.

#### Orthotopic Small Intestine Transplantation With Portal Drainage

The entire donor small intestine from the ligament of Treitz to the ileocecal valve was isolated on a vascular pedicle consisting of the superior mesenteric artery on a segment of aorta and portal vein. After the graft was perfused with 10 mL of cold lactated Ringer's solution, the lumen was washed with cold neomycin sulfate solution. End-to-side vascular anastomoses were performed between the graft aorta and recipient infrarenal aorta and graft portal vein and recipient portal vein. The entire recipient small intestine was resected. Intestinal continuity was restored by proximal and distal intestinal anastomoses.

## FK 506

Intramuscular (IM) FK 506, dissolved in carrier solvent (HCO-60 and D-mannitol) (Fujisawa Pharmaceutical Co, Osaka, Japan) was diluted in normal saline and injected in the thigh for 14 days starting on the day of surgery.

## Histopathology of GVHD

Tissues were obtained from the animals showing GVHD. Mesenteric lymph nodes and Peyer's patches from jejunum and ileum were obtained from the grafts. Samples of recipient tissues, including the liver, spleen, mesenteric lymph nodes, duodenum, thymus, lymph node, and skin (ear and tongue) were also taken. Tissues were stained with H&E, and L-21-6 (mouse anti-LEW class II monoclonal antibody, kindly provided by Dr Yagihashi, University of Pittsburgh, Department of Surgery) as described previously.<sup>3</sup>

## Flow Cytometry

Animals were sacrificed for the flow cytometric analysis 7 days after transplantation and when they developed GVHD (LEW to BN) to determine the distribution of donor and recipient lymphocytes. Lymphocytes were obtained from graft mesenteric lymph nodes by gently mincing the tissues in RPMI (Gibco, Grand Island, NY), followed by nylon mesh filtering. Lymphocytes from Peyer's patches were obtained by isolating the patches, followed by vigorous mechanical agitation and filtration similar to above. Biotinylated rat monoclonal antibody 163, which is specific for the *RT1.A<sup>l</sup>* antigen on LEW, or 42, which is specific for the *RT1.A<sup>n</sup>* antigen on BN<sup>3</sup> (kindly provided by Dr H. W. Kunz, University of Pittsburgh, Department of Pathology) were added to lymphocyte suspension for 45 minutes at 4°C. After washing, FITC-conjugated streptavidin (Pharmingen, San Diego, Calif) was added for another 10 minutes at 4°C. The samples were analyzed using a FACScan flow cytometer, and the percentage of cells stained positive with each monoclonal antibody was determined.

## RESULTS

### Animal Survivals

When BN grafts were transplanted into LEW recipients, untreated animals died of rejection with a median survival of 10.5 days (Table 1). FK 506 treatment (0.64 mg/kg for 14 days) was effective in prolonging the animal survival to more than 100 days, without any signs of GVHD. However, this short-term treatment has been shown to result in chronic-type graft rejection.<sup>3</sup>

By contrast, reversing the direction of transplantation (LEW to BN) induced fatal GVHD when the same dose and duration of FK 506 was administered. Without any treatment, BN recipients rejected LEW grafts within 13 days. After FK 506 treatment, the BN recipients developed erythema between 20 and 25 days after transplantation (6 to 11 days after cessation of FK 506); thereafter, the recipients expressed skin erosion, hyperkeratosis, body weight loss, and eventual death, with median survival of 29.0 days.

### Flow Cytometry

Seven days after transplantation, 70% to 80% of the lymphocytes in graft mesenteric lymph nodes were recipient phenotype in both combinations. In BN recipients showing clinical GVHD 30 to 35 days after transplantation, most of the lymphocytes in the grafts were also recipient origin (Table 2).

### Histopathology

Histologic examination of the skin of BN recipients with clinical GVHD revealed apoptosis, basal layer lymphocyte infiltration, and vacuolization, similar to the findings seen in GVHD

after bone marrow transplantation. Phenotypic analysis of the dermal infiltrates revealed many donor class II-positive (L-21-6+) cells. Donor cells (L-21-6+) were also detected in the spleen, mesenteric lymph nodes, and thymus in GVHD animals. Donor class II-positive cells in the recipient thymus were rare early in the course of GVHD, and were largely populated in medulla. The number of donor cells in the recipient thymus increased as GVHD worsened, but remained a relatively small population.

## DISCUSSION

The rapid movement of recipient lymphocytes into the graft lymphoid tissue and the migration of the donor lymphocytes, which has been considered to cause GVHD, into the recipient tissues occurred soon after the transplantation under FK 506. This two-way traffic between the graft and recipient was not significantly different in animals who developed GVHD, compared to those who did not, when examined on day 7 after grafting. When animals actually developed GVHD, most of the cells in the small bowel allografts were of recipient origin. Donor-phenotype cells were found in the spleen, mesenteric lymph nodes, and skin of the recipients with GVHD. Donor-type cells were found in the thymus only when the GVHD was advanced and the thymus was atrophic. Whether extended FK 506 treatment can offer sanctuary to donor hematopoietic cells for prolonged periods of time, resulting in delayed emergence of GVHD, is currently under investigation.

In the past, the traffic of mature T cells from the thymus to the periphery has been considered unidirectional. However, re-entry of mature activated T cells into the thymus has been shown to occur.<sup>5</sup> This may explain why donor MHC class II-positive cells were seen in the recipients only after the animals developed GVHD in this study. The significance of matured donor-type cells in the recipient thymus after small bowel transplantation is unknown. However, it has been shown that mature allogeneic T cells enter the recipient thymus after bone marrow transplantation and may be responsible for the pathogenesis of chronic GVHD.<sup>6</sup>

This study has shown that the balance between rejection and GVHD, after small bowel transplantation, is affected by donor and recipient strain combinations and the dosage and duration of immunosuppressive therapy. Therefore, fully allogeneic transplantation will be more realistic to study the rare occurrence of GVHD after small bowel transplantation.

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**Table 1**

## Animal Survival After Small Bowel Transplantation

Donor	Recipient	FK 506	Survival (d)	Median (d)
BN	LEW	None	9, 9, 10, 11, 12, 14	10.5
		0.64 mg/kg × 14	>100 × 9	>100
LEW	BN	None	11, 12, 13	12.0
		0.64 mg/kg × 14	17, * 27, * 28, * 29, * 30, * 36, * 36*	29.0*

\* Animals died with GVHD.

**Table 2**

Percentages of Donor or Recipient Phenotype Lymphocytes in Graft Mesenteric Lymph Nodes

Donor-Recipient Combination	Days after Transplantation	Donor-Type	Recipient-Type
BN-LEW	7	27.7 ± 3.5	69.4 ± 1.6
LEW-BN	7	18.9 ± 2.8	80.4 ± 7.0
	30-35 (with GVHD)	3.8 ± 2.9	96.2 ± 2.8

Results are expressed as mean ± SD (percent of positive cells).

Two to three animals/group were examined.