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Lymphocyte Trafficking Using In Situ Hybridization and Physioanatomy of the Intestinal Immune System After Human Small Bowel Transplantation

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THE gut-associated lymphoid tissue (GALT) plays a major role in gastrointestinal immunity, but is thought to represent an immunologic obstacle to successful small bowel transplantation.¹ We have shown that the GALT and mesenteric lymph nodes are preferential sites of allograft rejection in animals, most likely because of the high content of passenger leukocytes.² However, with intense immune suppression, much of the GALT in allografts is replaced by recipient lymphoid cells in the absence of rejection.³ This study focused on the immune anatomy of the GALT in human small bowel allografts.

MATERIALS AND METHODS

Between May 2, 1990 and August 21, 1991, eight patients underwent small bowel plus liver or small bowel transplantation alone (one patient) at the University of Pittsburgh for short gut syndrome and associated liver disease. There were three adults (ages 21 to 31 years) and six children (ages 1 to 4 years) composed of four males and five females. Baseline immunosuppression consisted of FK 506 and steroids.

Routine H&E sections of serial allograft small bowel biopsies were assessed for rejection. Cell repopulation studies were conducted using a fluorescent DNA probe for the Y-chromosome (Oncor, Inc. Gaithersburg, Md) in two cases where the donor and recipient were sex mismatched (female to male, $n = 1$; male to female, $n = 1$). IgA localization in the allograft bowels was studied using monoclonal anti-IgA and antisecretory component (SC) antibodies in five patients. T- and B-cell phenotyping on paraffin-embedded sections was achieved using L60 (T-cell) and L26 (B-cell) antibodies. Primary antibodies were obtained from Dako, Inc. Carpinteria, Calif. Reagents for avidin-biotin immunoperoxidase staining were purchased from Vector Labs, Inc, Burlingame, Calif.

RESULTS

Immune cells (lymphocytes, plasma cells, eosinophils, etc) were always present in routine H&E sections of allograft biopsies in the lamina propria (LP) and submucosa. In the absence of infectious organisms and when the infiltrate was associated with gland or crypt infiltration, damage, and repair, rejection was diagnosed. However, the presence of plasma cells and eosinophils in the LP and lymphoid nodules (often with germinal centers) unassociated with tissue damage or architectural distortion, was considered as part of the GALT.

Hybridization studies for the Y-chromosome revealed that recipient hemato-lymphoid cells (negative in female recipients of male donors; positive in male recipients of

female donors) infiltrated the graft within the first week after transplantation. The majority of cells in the lamina propria were replaced by recipient cells by 70 to 80 days, whereas the epithelial and stromal elements remained of donor origin (opposite of infiltrative cells) throughout the follow-up periods of 485 and 321 days. The presence of recipient cells in the allografted tissues was independent of the histologic and clinical diagnosis of rejection.

Furthermore, in lymphoid nodules from nonrejecting grafts, T cells were located in interfollicular areas whereas B cells were present in follicles. Intraepithelial lymphocytes were also phenotypically T cells, as expected. IgA-containing plasma cells in the lamina propria were observed in all patients, as was secretory IgA on the epithelial surface. The above findings are all consistent with an anatomically intact mucosal immune system, despite the different genetic origin of the immune cells versus the epithelium, stroma, and blood vessels.

DISCUSSION

The normal presence of immune cells in the small intestine provides an intense immunologic stimulus after transplantation and complicates assessment of routine allograft biopsies for rejection. Previous animal and human studies using major histocompatibility complex type-specific antibodies have shown that complex trafficking of donor and recipient lymphoid cells occurs after small bowel transplantation.^{3,4} In humans and animals, donor cells can be found in the peripheral circulation and tissues,⁴ where they can elicit a GVH reaction. In contrast, recipient lymphoid cells can repopulate the allograft GALT without causing rejection.^{3,5} We have confirmed and extended these findings using a totally separate methodology (in situ hybridization). Furthermore, in the GALT, the recipient cells can recapitulate the appropriate immune architecture. These findings suggest that: (a) the influx of recipient lymphoid cells into the graft is, in part, a result of normal lymphoid trafficking; and (b) although small bowel allografts are

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chimeric after transplantation, the mucosal immune system is likely to be at least partially intact.

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