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The pattern of rejection after combined stomach, small bowel, and pancreas transplantation in the rat

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Abstract. This study was designed to investigate whether in combined stomach, small bowel, and pancreas transplantation allograft rejection occurs in the individual organs concomitantly and with the same intensity. Heterotopic enbloc transplantation of the stomach, small bowel, and pancreas was performed in a Lewis-to-Brown Norway rat combination. Group 1 animals received no immunosuppressive therapy while animals in group 2 were treated with cyclosproin (10 mg/kg body weight, orally) daily. Grafts were histologically evaluated on the 5th (subgroups 1a and 2a) and 10th (subgroups 1b and 2b) postoperative days. The degree of rejection was defined as moderate, intermediate, or severe according to predefined criteria. The results indicate that the small bowel is more susceptible to rejection than either the stomach or the pancreas. Mucosal biopsies of the stomach are unlikely to provide a reliable guide to rejection in the small bowel.

Key words: Stomach transplantation, rat – Small bowel transplantation, rat – Rat, combined stomach/small bowel/pancreas transplantation

Introduction

Clinical transplantation of multiple abdominal viscera including the liver, pancreas, small bowel, and even the stomach has recently proved to be feasible [7]. Yet, despite powerful immunosuppressants, rejection still represents the major problem in both clinical and experimental small bowel transplantation. The function of the allografted small bowel has been shown to be impaired only during severe rejection [6]. Therefore, functional tests, such as the maltose absorption assay [6], and serological markers, such as N-acetyl-hexosaminidase [2], have proved to be of little value in detecting acute small bowel allograft rejection, and diagnosis is still largely based on morphology.

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Due to the fact that both the submucosa and the muscular layer are infiltrated by inflammatory cells during rejection, full-thickness biopsies of the intestinal wall are required [3, 5]. However, such biopsies pose a certain risk of perforating the graft. Furthermore, small bowel biopsies are difficult to obtain after restoration of continuity. The inclusion of the stomach with this organ cluster might offer several advantages. The stomach graft might serve as another source of mucosal biopsies and make reconstitution of the alimentary tract easier and more physiological.

We were interested in finding out whether in combined stomach, small bowel, and pancreas transplantation rejection occurs in the individual organs concomitantly and with the same intensity. If so, a multivisceral graft could be monitored by gastroscopic biopsies only.

Materials and methods

Combined stomach, small bowel, and pancreas transplantation was performed in rats using microsurgical techniques. The entire stomach, small bowel, and pancreas were harvested from inbred male Lewis (LEW) rats weighing between 200 g and 270 g and were transplanted into male Brown-Norway (BN) rats (Zentralinstitut für Versuchstierzucht, Hannover, Germany) of equal size. After in situ flushing of the graft via the aorta with 5 cc of cold saline solution, graft vessels (portal vein, aortic conduit with celiac axis, and superior mesenteric artery) were anastomosed to the infrarenal vena cava and aorta. All grafts were placed in a heterotopic position. The oral end of the gastric graft was closed, and the distal end ot the intestinal graft anastomosed in a Roux-en-Y fashion to the distal lileum of the recipient. Postoperatively, all animals were given water and standard rat chow ad libitum.

Three groups were studied. Group 1 consisted of BN recipients of grafts from LEW donors; they received no immunosuppression ($n = 11$). Group 2 consisted of BN recipients of LEW grafts that were given cyclosporin (10 mg/kg body weight per day) orally ($n = 10$). Group 3 consisted of LEW recipients of grafts from LEW donors; they received no treatment and served as controls ($n = 6$).

Five animals in each group were sacrificed 5 days after transplantation (subgroups 1a, 2a, and 3a), with the remaining animals sacrificed on day 10 (subgroups 1b, 2b, and 3b).

Four to six circumferential cross sections were cut from corresponding areas of each graft. Sections were stained with hematoxylin and eosin. Slides were evaluated by one of the authors according to criteria previously established for the small bowel [5]. In brief, early rejection episodes, defined as phase I, showed an infiltration of the mucosa and submucosa by mono- and polymorphonuclear leukocytes and by lymphocytes scattered along the myenteric ganglia. An intermediate stage of rejection, defined as phase II, was associated with a more severe infiltrate and with villous flattening and sloughing of epithelial cells. The muscular layer was invaded by numerous lymphoctes and neutrophils. At the end of the rejection episode, complete destruction of the mucosa and the muscular layer occurred (phase III).

According to these criteria, phase I represents a stage of rejection with mild inflammation of the graft and no damage to the epithelial cells. In phase II the inflammatory response is more pronounced and some epithelial damage is seen. In phase III the whole organ is severely damaged. Essentially similar histological criteria were adopted for assessing rejection of the stomach.

In the pancreas, phase I rejection was diagnosed when an interstitial edema with scattered mononuclear leukocytes and no epithelial damage was noted. Phase II exhibited a more pronounced inflammatory response and necrosis of epithelial cells. When more than four acini were destroyed per high-power field, the severity of rejection was referred to as phase III.

Results

The pattern of rejection after combined multivisceral transplantation of the stomach, small bowel, and pancreas is shown in Figs. 1 and 2.

In group 1a (no immunosuppression; 5 days; $n = 5$), four animals showed a phase I rejection in the stomach, small bowel, and pancreas. In one case the stomach and small bowel exhibited a phase II rejection, whereas the pancreas showed phase I rejection.

In group 1b (no immunosuppression; 10 days; $n = 6$), all but one of the six rats presented an intermediate type of rejection of the stomach, whereas the small bowel and pancreas were rejected more vigorously (phase III) in four and two animals, respectively.

In three animals in group 2a (immunosuppression with cyclosporin; 5 days; $n = 5$), no rejection was detected in any of the grafted organs. In the remaining two rats, phase I rejection was seen in all grafts.

In group 2b (immunosuppression with cyclosporin; 10 days, $n = 5$), a phase II rejection was the most common observation. However, in one stomach and in one small bowel a phase I rejection was seen. Two animals presented a phase I rejection in their pancreas.

In groups 3a and 3b (controls), all isogeneic grafts exhibited normal histology.

Discussion

Although the histological appearance of the stomach is similar to that of the small bowel, there are some specific features that may lead to a different pattern of allograft rejection. Besides the organ-specific type of epithelial cell, the most important difference is the lack of mucosa-associated lymphatic tissue in the stomach. Studies of the small bowel have revealed these tissues to be one of the first targets of allogeneic leukocytes [1].

The overall histological changes that developed during the rejection of stomach allografts were similar to those previously described for small bowel allografts. There were, however, some distinct differences, particularly during the early phases of the rejection process. In the stomach, a prominent submucosal edema was observed, something that did not develop in the small bowel. Compared to those in the small bowel, the number of mast cells in the stomach was greater. On the other hand, an increase in intraepithelial lymphocytes, regularly seen during small bowel allograft rejection, was not observed in gastric allografts. Damaged gastric glands are characteristic of a phase II rejection of the stomach. In contrast, histology of the small bowel reveals a loss of goblet cells and a cuboidal appearance of the epithelium at that stage.

Early reports have already shown that small bowel mucosal biopsies only are insufficient for recognizing early phases of rejection [3, 5]. The results of our study indicate that this is also true for the stomach. The lack of an increase in intraepithelial lymphocytes, serving as a marker of rejection in the small bowel [4], renders the situation even more difficult.

We were interested in knowing whether rejection in multivisceral allografts occurs concomitantly and with the same intensity in the individual organs. Our experiments indicated that the individual organs do not always exhibit the same intensity of rejection. We therefore suggest that each organ be individually monitored. However, according to our findings, it seems very unlikely that rejection of

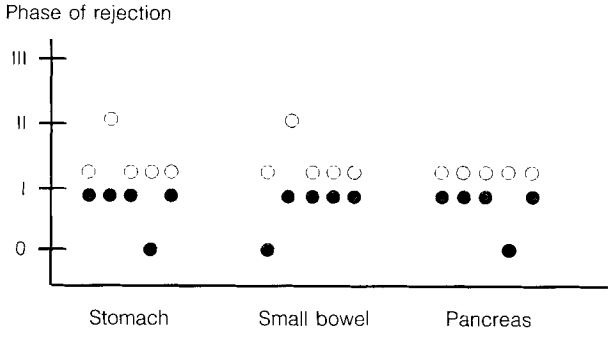

Fig. 1. Pattern of rejection after combined stomach, small bowel, and pancreas grafting after 5 days. ○ No immunosuppression; ● cyclosporin

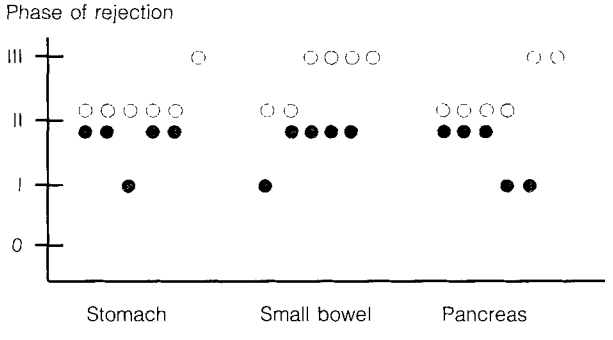

Fig. 2. Pattern of rejection after combined stomach, small bowel, and pancreas grafting after 10 days. ○ No immunosuppression; ● cyclosporin

the pancreas occurs without any signs of rejection of the stomach and/or small bowel.

One interesting finding was the low occurrence of a phase III rejection of the stomach in the untreated control animals. This might point to a decreased sensitivity of the stomach to a severe rejection. An explanation for this phenomenon might be the lack of mucosa-associated lymphatic tissue (MALT), which is a very immunogeneic part of the gut.

In conclusion, we suggest that inclusion of the stomach in a multivisceral organ cluster does not lead to an increased immunological risk. Monitoring of the graft by gastric biopsies alone is unlikely to be of great help.

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