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Donor cell infiltration of recipient tissue as an indicator of small bowel allograft rejection in the rat

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Abstract. This study assessed whether screening of host tissues for graft cells could be used as an effective monitor of rejection following small bowel transplantation. Allogeneic rat small bowel transplantation was performed with or without cyclosporin (CyA) immunosuppression and cellular infiltration of host tissues assessed by immunohistological staining. Without immunosuppression, grafts were completely rejected within 1 week. CyA treatment for 7 days preserved the graft for 28 days although there was histological evidence of mild rejection in some of the animals studied. Continuous CyA treatment preserved the graft for up to 56 days. The peripheral lymph nodes and spleens of untreated animals were transiently infiltrated by low numbers of donor cells that disappeared by day 6. There was a marked donor cell infiltration of the lymph nodes and spleens of 7-day, CyA-treated animals that was maintained during the administration of immunosuppressive therapy but that declined thereafter. Continuous CyA treatment sustained donor cell infiltration up to day 56. These findings suggest the presence of donor cells in recipient lymph nodes and spleen to be indicative of effective control of rejection and their disappearance to be predictive of developing rejection responses. Examination of recipient peripheral tissues for donor cells may provide an improved technique for monitoring clinical small bowel transplantation.

Key words: Small bowel transplantation – Rejection, small bowel transplantation – Cell infiltration, small bowel transplantation

Introduction

Small intestinal failure, associated with massive resection or inadequate function, may be severe enough to require permanent total parenteral nutrition. This treatment can be associated with serious complications and for some patients the outlook is bleak. Small bowel transplantation may offer such patients their only hope of survival. Despite rapid progress in many areas of transplantation and the introduction of cyclosporin (CyA), there has been limited clinical success with small bowel transplantation [4, 6, 18]. The major barriers to successful small bowel transplantation are the immunological responses directed towards both host and donor tissues that result in rejection and graft-versus-host disease (GVHD) and life-threatening sepsis.

Early and accurate diagnosis of rejection is essential as the capacity of the intestine to recover adequate function after treatment of a rejection crisis is unknown. Rejection leads to a loss of mucosal barrier function, translocation of bacteria into the host and sepsis [10, 16]. In the limited clinical experience to date, multiple deep mucosal biopsies are required on a daily basis [3, 5, 7]. However, repeated biopsy may be difficult when a functional orthotopic small bowel allograft is relatively inaccessible. In addition, the presence of host cells in graft tissue does not necessarily infer rejection because the gut and its associated lymphoid tissues are components of the "common mucosal immune network" through which leukocytes pass as part of their normal migratory behaviour [2]. Definitive diagnosis is also difficult as ischaemic injury with villous tip loss, followed by reactive inflammation within the crypts, may mimic immunological damage [13].

In light of the major difficulties with interpretation of graft biopsy material, alternative monitoring procedures need to be assessed. Ongoing acute rejection and GVHD cannot co-exist within one individual, and donor cells will only persist in host tissues in the absence of active rejection. Therefore, identification of donor cells in more accessible recipient tissues might provide an improved indicator of impending rejection.

Materials and methods

Small bowel transplantation

Transplantation was performed between PVG (RT1c) donors and DA (RT1a) recipient male rats weighing 200–250 g. The small intestine was transplanted as a heterotopic accessory graft with two cuta-

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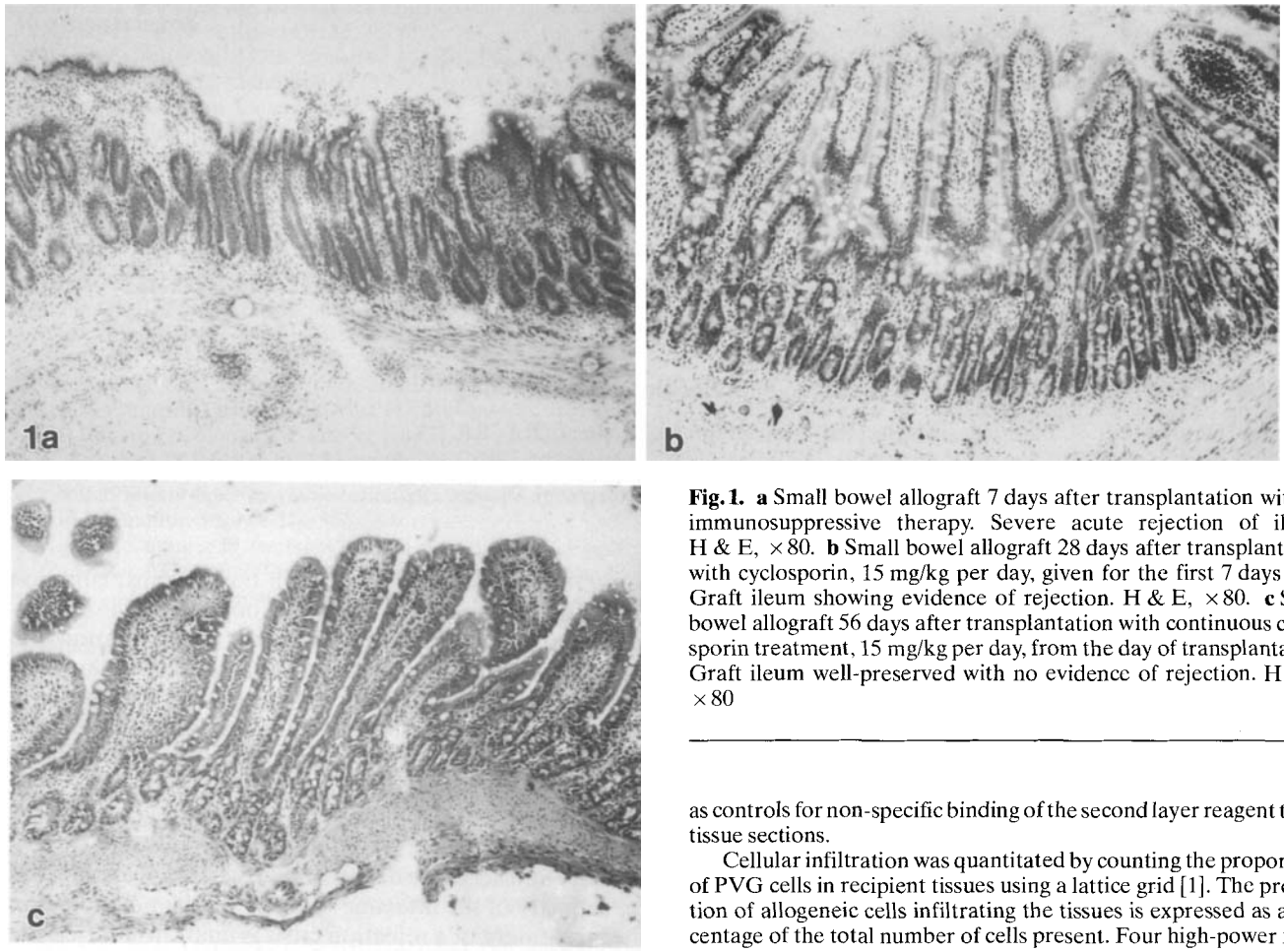

Fig. 1. **a** Small bowel allograft 7 days after transplantation with no immunosuppressive therapy. Severe acute rejection of ileum. H & E, ×80. **b** Small bowel allograft 28 days after transplantation with cyclosporin, 15 mg/kg per day, given for the first 7 days only. Graft ileum showing evidence of rejection. H & E, ×80. **c** Small bowel allograft 56 days after transplantation with continuous cyclosporin treatment, 15 mg/kg per day, from the day of transplantation. Graft ileum well-preserved with no evidence of rejection. H & E, ×80

neous stomata, using standard microvascular techniques [15]. The portal vein and superior mesenteric artery with a cuff of aorta were anastomosed end-to-side to the recipient inferior vena cava and infra-renal aorta, respectively. Recipients survived well (less than 10% operative mortality) as their native intestine was left intact to continue to provide nutrition. All rats received 1 mg gentamicin post-operatively. Animals were placed into one of three experimental groups:

Group 1: No immunosuppression. Rats were sacrificed daily (four rats/day) from day 1 to day 7 post-transplantation.

Group 2: CyA dissolved in olive oil (15 mg/kg per day) for 7 days by gavage. Rats were sacrificed daily from day 1 to day 7 post-transplantation and additional rats sacrificed on days 14, 21 and 28 (four rats/day).

Group 3: CyA (15 mg/kg per day) continuously. Rats were sacrificed on days 28 and 56 (six rats/day).

Immunohistochemical analysis of recipient tissues

Recipient peripheral lymph nodes and spleen were harvested. Tissue was cut into 5-mm blocks, frozen in liquid N_2 and stored at −70°C. Eight-micron cryostat sections were stained following an indirect immunoperoxidase staining procedure [14], using horseradish peroxidase-conjugated rabbit antiserum to mouse immunoglobulins (Dako, UK) as the second antibody, diaminobenzidine (0.1%) as the chromogen and haematoxylin to counterstain nuclei. The primary antibody used (1:100 dilution) was OX27, which recognises PVG MHC class I determinants and does not react with DA MHC class I antigens [9, 14]. Sections incubated without primary antibody acted as controls for non-specific binding of the second layer reagent to the tissue sections.

Cellular infiltration was quantitated by counting the proportions of PVG cells in recipient tissues using a lattice grid [1]. The proportion of allogeneic cells infiltrating the tissues is expressed as a percentage of the total number of cells present. Four high-power fields per section, each comprising 100 cells, were counted.

Results

Graft survival

The grafts of animals receiving no immunosuppression were completely rejected within 1 week. In accordance with previous studies [8, 12, 17], haematoxylin and eosin staining demonstrated progressive changes in allograft architecture, with initial sloughing of the villous tips progressing to mucosal destruction (Fig. 1). Cyclosporin treatment for 7 days preserved histological morphology for at least 28 days and continuous CyA administration for up to 56 days (Fig. 1).

Donor cell infiltration of host lymphoid tissues

Untreated animals. Donor cell infiltration of host peripheral lymph nodes and spleen began soon after transplantation. In all recipients the periarteriolar lymphatic sheathes (PALS) of the spleen became populated with graft leukocytes. Donor cells were seen in the host lymph nodes and spleen within 24 h of transplantation, accumulated over the first 4–5 days and then disappeared (Fig. 2), presumably through local destruction and failure of repopulation from the rejecting graft. Loss of donor cells in host tissues preceded histological evidence of acute graft rejection.

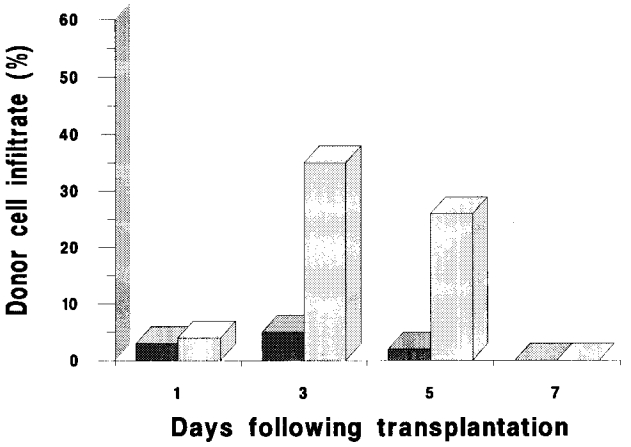

Fig. 2. Donor cell infiltration of host peripheral lymph node (■) and spleen (□) following fully allogeneic small bowel transplantation without cyclosporin A immunosuppression. Tissues were obtained at the times indicated and infiltration assessed using indirect immunohistochemistry. Data are presented as medians of four rats per time point

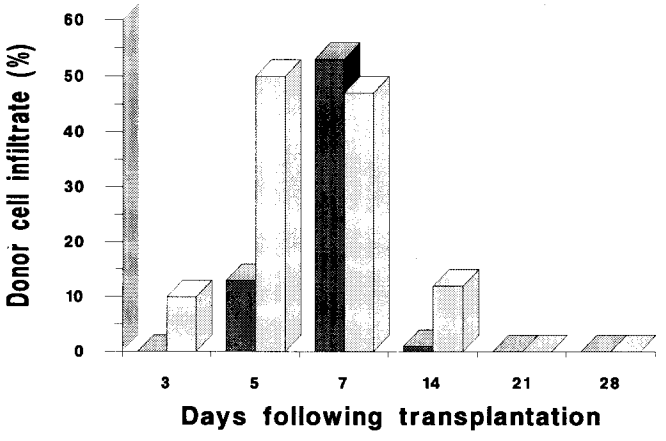

Fig. 3. Donor cell infiltration of host peripheral lymph node (■) and spleen (□) following fully allogeneic small bowel transplantation. Animals received cyclosporin immunosuppression for 7 days following transplantation. Tissues were obtained at the times indicated and infiltration assessed using indirect immunohistochemistry. Data are presented as medians of four rats per time point

CyA treated animals. In 7-day CyA-treated rats, the presence of donor cells in the peripheral lymph node and in the PALS of the host spleen was maintained during the administration of immunosuppressive therapy (Fig. 3). The donor cell infiltrate peaked at 7 days and declined following the cessation of CyA such that no donor cells were seen at 28 days (the latest time-point investigated). Donor cells persisted in the peripheral lymph node and spleen of animals receiving continuous CyA treatment (Fig. 4).

Discussion

The fully allogeneic rat model has proved valuable in elucidating the immunological consequences of small bowel transplantation. The availability of strain-specific monoclonal antibodies had demonstrated major cellular traffic between the host and transplanted tissues.

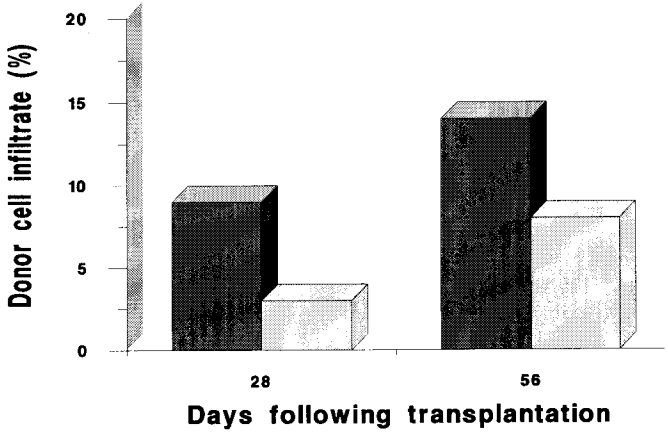

Fig. 4. Donor cell infiltration of host peripheral lymph node (■) and spleen (□) following fully allogeneic small bowel transplantation. Animals received continuous cyclosporin immunosuppression until the day of sacrifice. Tissues were obtained at the times indicated and infiltration assessed using indirect immunohistochemistry. Data are presented as medians of six rats per time point

Cell trafficking through the transplanted graft is a complex process that is driven not only by alloantigen recognition but also by mechanisms regulating the physiological migration of cells through mucosal tissues [2]. As a result, the demonstration of allogeneic cellular infiltrates in small bowel transplants is not necessarily diagnostic of rejection. Although histochemical staining of epithelial cells for intracytoplasmic enzymes has been advocated as an effective technique for reaching a prompt and definitive clinical diagnosis [17], ischaemic damage following transplantation may also reduce epithelial enzyme production.

The early loss of donor cells in untreated animals suggests a localised immune reaction. The finding that donor cells can be seen in recipient peripheral lymph node and spleen on day 56 in animals receiving continuous CyA treatment, whereas donor cells cannot be detected on day 21 if treatment ceases on day 7, indicates that donor cell disappearance may be associated with developing rejection and is prevented by effective immunosuppressive regimens. Although CyA treatment for 7 days preserved histological morphology for at least 28 days, previous studies using this model have shown pathological signs of rejection in the graft by day 40–56 after transplantation (personal observation). It is not clear when the grafts in groups 2 and 3 would have been rejected; however, if the donor cell disappearance from recipient spleen and lymph node in group 2 animals was not related to developing anti-graft reactivity, then a similar disappearance of donor cells would be expected in group 3 animals.

The presence of donor cells within the peripheral lymph nodes and spleen only when graft rejection is controlled provides a template for the examination of different treatment regimens in experimental small bowel transplantation and a potential technique for monitoring clinical small bowel allograft recipients. The presence of donor cells within the peripheral lymph nodes has previously been reported [11], and fine needle aspiration of

superficial nodes may provide a realistic means for monitoring small bowel transplantation in clinical practice since it would enable early detection of developing anti-graft reactivity in a situation where graft biopsy is difficult to interpret. Current work in our laboratory has detected donor cells in the peripheral blood following rat small bowel transplantation that disappear prior to histological evidence of rejection.

In summary, physiological trafficking of cells between mucosal sites makes the diagnosis of small bowel graft rejection by host cell infiltration of graft tissue difficult. The findings of this study suggest that the disappearance of donor cells from host tissues precedes allograft rejection and may be a useful means of assessing the efficacy of immunosuppressive regimens and monitoring small bowel transplant recipients.

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