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ORIGINAL ARTICLE

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Mucosal recipient-type mononuclear repopulation and low-grade chronic rejection occur simultaneously in indefinitely surviving recipients of small bowel allografts

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Abstract Lewis rat recipients of long-term, surviving, orthotopic Brown-Norway rat intestinal allografts, initially treated with cyclosporin A (CyA) or FK 506, were evaluated for their functional capacity and morphology over 1 year after the immunosuppressive therapy had been discontinued. Functional parameters such as nitrogen and fat balances, maltose absorption, blood chemistry, hematologic studies, and the weight gained by the allografted animals did not differ from those of syngeneically grafted or age-matched normal animals. Immunohistochemical studies showed that the lamina propria of the allografts was repopulated with recipient MHC class II + mononuclear cells and that a normal distribution of T helper, T suppressor/killer, and IgA + plasma cells had occurred. However, fibrous replacement of the mesenteric lymph nodes and Peyer's patches were detected in all, and an inflammatory obliterative arteriolopathy developed in the mesenteric vasculature of half of the allografted animals. No such findings were observed in recipients of syngeneic grafts. These results demonstrate that the limited use of potent immunosuppressive agents immediately after transplantation averts rejection and is followed by recipient-type mucosal lymphocytic repopulation. Simultaneously, a clinically not recognizable chronic rejection evolves. This suggests that the timely diagnosis of chronic rejection may not be possible with the use of standard tests of gut function and random mucosal biopsies alone.

Key words Small bowel transplantation, rat, chronic rejection · Repopulation, small bowel transplantation
Chronic rejection, small bowel transplantation/rat

Introduction

The introduction of cyclosporin A (CyA) and, more recently, of FK 506 has brought major advances in organ transplantation. Both agents have made experimental allogeneic small bowel transplantation in small animals possible [8, 10, 13], whereas heretofore such studies enjoyed only limited success. In contrast to the improving experimental results, survival of intestinal allografts with normal function in humans was accomplished in only three humans under CyA in over 15 published attempts until 1989 [14, 15, 17]. However, in the last 3 years, several investigators have reported on the successful use of CyA- and FK 506-based immunosuppression for clinical small bowel allotransplantation [3, 6, 7, 16] with functional graft survival up to 3 years in some cases as of now (E. Deltz, D. Grant, S. Todo personal communication). These encouraging results, showing that the initial post-transplant period could be controlled and that the patients could leave the hospital on regular enteral diets, led to the question of how long-surviving intestinal allografts would perform functionally and what the morpho-

logical and immunological status of these allografts would be.

The recent, intriguing observation by Murase et al., that using FK 506 immunsouppression to prevent intestinal allograft rejection caused the lymphoreticular elements of the graft to be replaced by those of the recipient [13], prompted us to determine if the replacement of the lymphoreticular elements of the intestine occurred in a manner that allowed for normal intestinal function and to further investigate the replacing mononuclear population. Thus, a standard model of orthotopic small bowel allotransplantation in the rat under both CyA and FK 506 immunosuppression was used to achieve long-term survival of intestinal allografts. Both the functional competence and the morphological integrity of the intestinal allograft and recipient animal were studied 1–1.5 years after transplantation, well after the immunosuppressive therapy utilized initially had been discontinued.

Materials and methods

Animals

Male Brown-Norway (BN, $RT1^n$) and Lewis (LEW, $RT1^1$) rats weighing 180–240g were purchased from Harlan Sprague-Dawley (Indianapolis, Ind., USA). The animals were maintained in the University of Pittsburgh animal facilities, which are AAALAC accredited and comply with the requirements for humane animal care as stipulated by the USDA and HHS. All animals were maintained on a 12-h light/dark cycle and were fed a commercially available rat chow and tap water ad libitum.

Experimental groups

LEW rats served as the recipients and received either syngeneic (LEW, group 1) or allogeneic (BN, groups 2 and 3) grafts. The animals in group 1 received no further therapy. In group 2, the recipients were treated postoperatively with CyA for 6 days and then every other day until the 28th postoperative day. The animals in group 3 received FK 506 for 5 days postoperatively. These animals were re-evaluated in terms of their graft structure and function 1–1.5 years following cessation of the immunosuppressive therapy. A fourth group, untreated LEW rats, was evaluated after 1 year of housing in the animal care facility and served as age-matched controls for the animals with syngeneic and allogeneic grafts. To assess possible effects of the immunosuppressive therapy on the small bowel grafts, one group of LEW rats, receiving syngeneic transplants, was treated with the 4-week course of CyA and another group of syngeneically transplanted animals was treated with the 5 day course of FK 506. Sections of these grafts were evaluated 3 months after the cessation of the immunosuppressive therapy.

Surgical procedure

One-step orthotopic small bowel transplantation was performed as described previously [9, 10]. Briefly, the graft was harvested by isolating the entire small bowel from Treitz's ligament to the ileocecal valve on a superior mesenteric artery (SMA)-portal vein (PV) pedicle. Revascularization consisted of end-to-side arterial and venous anastomoses between the SMA and PV of the graft and the infrarenal aorta and vena cava of the recipient. All grafts were immediately placed in gastrointestinal continuity, replacing an equal length of the recipient's own small bowel. Grafts not surviving beyond 5 days were considered technical failures and excluded from further analysis (the technical failure rate was approximately 15%).

Drugs

CyA (generously donated by Sandoz, E. Hanover, N.J., USA) was diluted in Intralipid (KabiVitrum, Alameda, Calif., USA) and injected intramuscularly at a dose of 15 mg/kg body weight per day from day 0 to 6 and every day until day 28. FK 506 (generously donated by Fujisawa Pharmaceutical, Osaka, Japan) was provided as a crystalline powder, suspended in saline, and injected intramuscularly at a dose of 2 mg/kg body weight per day.

Functional evaluation

All graft recipients were inspected and weighed daily for the first 3 postoperative months. Subsequently, they were inspected and weighed three times a week for the remainder of the study. One year after transplantation, hematologic and blood chemistry profiles were obtained using standard laboratory methods. Analysis of total serum bile acids was performed using a quantitative enzymatic kit (Sigma Chemical, St. Louis, Mo., USA).

Twenty-four-hour metabolic balances were performed and nitrogen and fat contents were analyzed as previously described [9]. A maltose absorption test was performed as previously described [1]. Briefly, 0,5 mg maltose per gram rat body weight was dissolved in 2.5 ml saline and injected into the duodenum of the recipient, just proximal to the site of graft anastomosis while occluding the recipient's proximal duodenum to avoid backflow. Serum glucose levels were determined at 15-min intervals for 1 h. This procedure ensured that only the capacity of the graft to absorb maltose was tested.

Morphologic evaluation

Four to five animals in each group were sacrificed and a necropsy was performed. The transplanted bowel, including the donor mesenteric lymph nodes, recipient bowel and mesenteric lmyph nodes, liver, spleen, pancreas, kidney, colon, heart, lung, brain, tongue, and skin were fixed in formalin and prepared [with hematoxylin and eosin (H&E) stain] for light microscopy. For immunohistochemical studies, specimens from the graft (small bowel and mesenteric lymph nodes) and from the recipient's spleen were embedded in optimal cold temperature compound (Tissue-Tek, Miles Laboratories, Elkhart, Ind., USA) and frozen rapidly. Cryostat sections were cut at 6 µm and stained with immunoperoxidase techniques as previously described [4], using the following monoclonal antibodies: O × 8 (T_c suppressor/cytotoxic cells), W3/25 (T_h helper cells and macrophages), O × 33 (B cells), and MARA-1 (IgA heavy chain). All of these antibodies were purchased from Bioproducts for Science (Indianapolis, Ind., USA). The mouse anti-rat IgG monoclonal antibody L-21-6, which recognizes LEW but not BN class II antigens [19], and the rat anti-rat IgG monoclonal antibody 42, directed against the BN class I antigen ($RT1^n$) [5], were used to determine the origin of the mononuclear cells in the lamina propria of the grafts.

Table 1 Experimental groups, therapy, and survival

Experimental group (n)	Transplant	Therapy	Survival (days)
1 ($n = 6$)	Isograft	None	477, 447, 446, 445, 441, 424[a]
2 ($n = 9$)	Allograft	CyA (28 days)	568, 562, 561, 560[b], 559, 558, 557, 557[c], 493
3 ($n = 8$)	Allograft	FK 506 (5 days)	499, 499, 492, 489, 484, 383, 226, 114[d]

[a] Technical failure (aspiration) during graft biopsy
[b] Died of bronchopneumonia with functioning graft
[c] Graft intussusception; animal died during reoperation
[d] Animal died of pulmonary edema with functioning graft

The evaluation of the immunohistology was performed in a semiquantitative fashion, using serial sections, First, standard histopathological grading was performed using the H&E-stained sections. Then, each experimental section was stained together with a section from a normal and a syngeneically grafted animal, using the different antibodies. Finally, the experimental immunoperoxidase sections were assessed in comparison with the syngeneic control sections and graded semiquantitatively (less, similar, or more positive staining).

Results

Clinical course

As depicted in Table 1, all animals not lost as a result of technical failures survived for more than 1 year. After initial recovery from the operation (approximately 3 days), all animals appeared healthy. Clinical signs of graft rejection or graft-versus-host-disease (GVHD), such as weight loss, diarrhea, hunched posture, and dermatitis, were not observed in any animal. The rate of body weight gain did not differ between the various experimental groups throughout the study (data not shown). One year after transplantation, blood was obtained by femoral vein puncture, and hematologic and serum chemistry profiles were determined. The results of red and white blood cell counts and the differential white blood cell counts did not differ between the various experimental groups and age-matched, untreated animals (data not shown). The only difference between the various groups with regard to the blood chemistry parameters assessed consisted of a significantly ($P \leq 0.01$ by unpaired Student's t-test) elevated total serum bile acid level in the transplanted animals compared to the control animals. This result was expected because the transplant procedure included a mesentericocaval shunt, which interrupts the enterohepatic circulation of bile acids. All other parameters of the chemistry profiles in the transplanted groups were within the normal range (data not shown).

Maltose absorption, nitrogen balance, and fat balance

Because it is a sensitive indicator of the mucosal brush border integrity, a maltose absorption test was carried out. The mean serum glucose levels achieved after maltose administration in the two groups of allografted animals did not differ from those observed in the animals with syngeneic grafts or in control (non-operated) animals and historical controls [1] (data not shown).

To further assess the function of the intestinal grafts, nitrogen and fat balances were performed. Again, no significant differences between the syngeneically or allogeneically grafted animals and the age-matched controls could be demonstrated (data not shown).

Macroscopic observations

At necropsy, the small bowel isografts in group 1 appeared normal. The shape and number of Peyer's patches per graft (35–40 cm; 17.3 ± 1.75 for syngeneic transplants, $n = 4$) were normal compared to age-matched, untreated animals (16.8 ± 1.5 for untreated LEW, $n = 4$). The graft mesenteries were translucent and the mesenteric fat content appeared normal in amount and distribution when compared with normal age-matched LEW rats. Moreover, normal mesenteric lymph nodes could be identified in all syngeneically grafted animals during macroscopic inspection. The recipients of syngeneic grafts treated with either CyA ($n = 4$) or FK 506 ($n = 4$) also showed a normal graft mesentery and normal mesenteric lymph nodes at necropsy.

A different picture was observed in all the allografted groups: the number of macroscopically identifiable Peyer's patches per graft (35–40 cm) was lower (11.3 ± 1.3 for the CyA group and 10 ± 1.4 for the FK 506 group, respectively); the Peyer's patches appeared scarred and shrunken; the mesentery of the intestinal allografts contained very little or no fat in any of the animals in groups 2 and 3, whereas the amount of colonic mesenteric fat appeared normal; the mesenteric lymph nodes of the allografts were condensed to small, fibrotic ridges in all animals, while the recipients' own mesenteric lymph nodes appeared normal.

Histology

Histological examination of the syngeneic grafts ($n = 5$, group 1) confirmed the macroscopic findings: mucosa, submucosa, architecture, and the degree of cellularity of the mesenteric lymph nodes and the Peyer's patches were normal (Fig. 1). No abnormalities of graft mesentery or its vessels were found. Moreover, the histological examination of the grafts from recipients of syngeneic transplants treated with either CyA ($n = 5$) or FK 506 ($n = 4$) showed

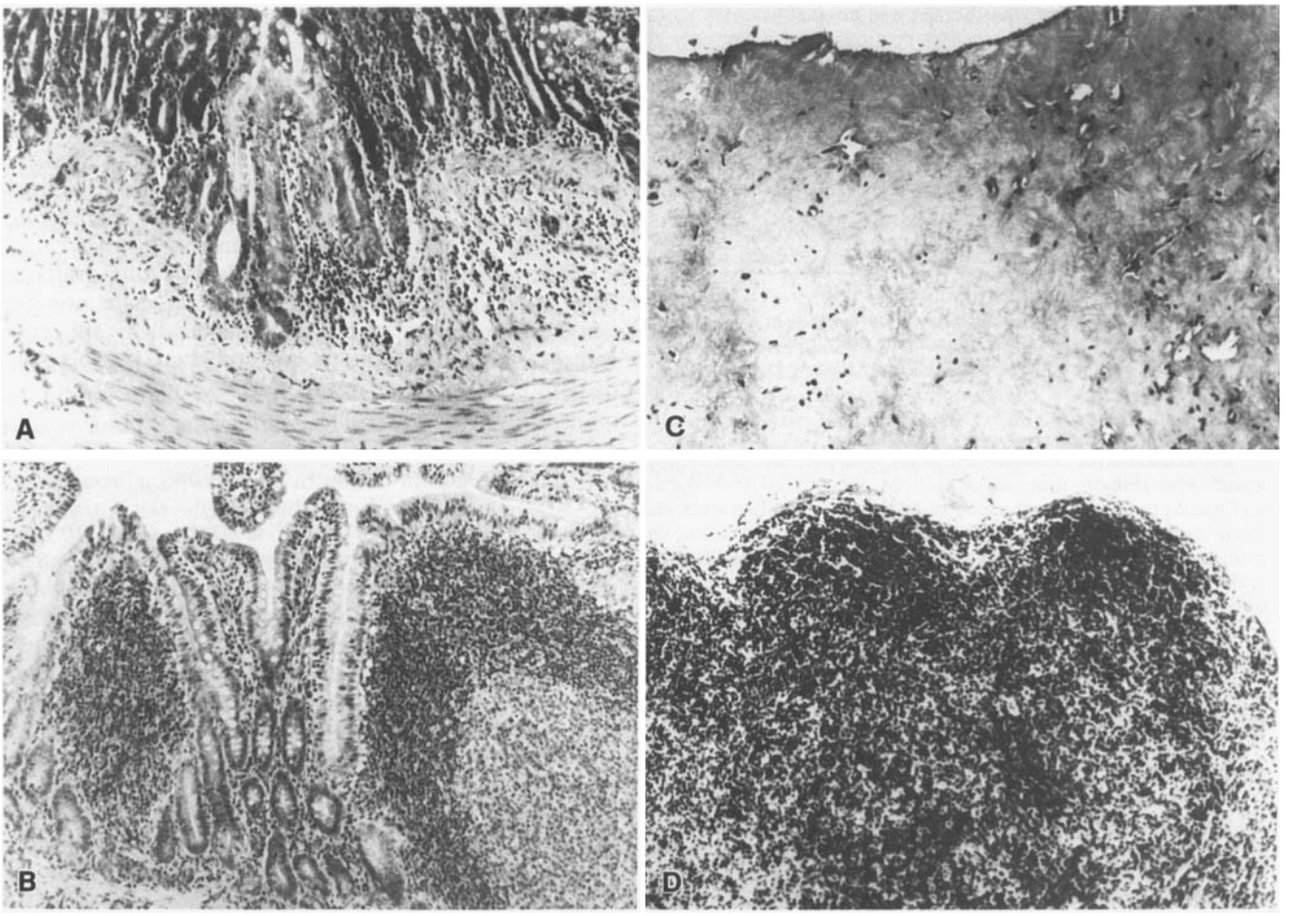

Fig. 1 A–D Sections of the small bowel and the mesenteric lymph nodes were obtained from syngeneically and allogeneically grafted animals (initially treated with CyA or FK 506) over 1 year after transplantation and stained with hematoxilin and eosin: **A** fibrosed Peyer's patch of a CyA-treated allograft (x40); **B** normal Peyer's patch of a syngeneic graft (x40); **C** fibrosed and scarred mesenteric lymph node of an FK 506-treated allograft; **D** normal mesenteric lymph node of a syngeneic graft (x40)

normal mucosal and mesenteric structures, including normal mesenteric vessels (data not shown).

In the allografted animals ($n = 5$ for groups 2 and 3), slight blunting of intestinal villi alternated with a normal villous architecture was observed. The mucosa and submucosa were free of cellular infiltrates. A histological equivalent of Peyer's patches could not be detected. In some areas, the submucosa appeared thickened and was covered by blunted villi. These areas were interpreted as residues of former Peyer's patches. The mesenteric lymph nodes of the allografts were totally fibrotic and, in some instances, mild inflammation of these residual nodes was observed (Fig. 1). The mesentery of the allografts appeared scarred and fibrotic in some areas. In 50% of the animals, mild or moderate arteritis of the mesenteric vessels, consisting of an obliterative arteriolopathy, was noticed (Fig. 2), often adjacent to normal mesenteric vessels. None of the other tissues in any of the groups demonstrated any histological abnormalities. In particular, there was no evidence of regional mononuclear infiltrates or lymphoid depletion, suggestive of an ongoing or prior graft-versus-host reaction, detected in the skin, liver, spleen, or the recipient's own mesenteric lymph nodes (data not shown). Altogether, the histological features observed closely resembled the picture of mild, chronic, intestinal allograft rejection.

Immunoperoxidase studies

To further evaluate the morphology of the intestinal grafts, frozen sections of the intestine were stained with a panel of monoclonal antibodies. The use of the anti-LEW class II antibody (L-21-6) on sections of the syngeneic grafts ($n = 4$) resulted in staining of numerous mononuclear cells within the Peyer's patches, the lamina propria, and approximately 50%–60% of the mononuclear cells in

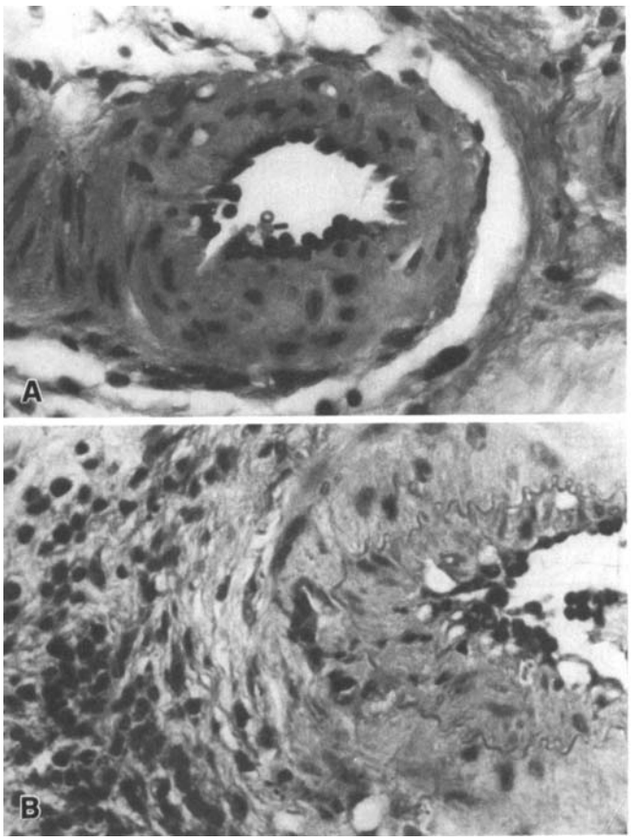

Fig. 2 A, B Sections of small mesenteric vessels from allografted animals (initially treated with CyA or FK 506) over 1 year after transplantation and stained with hematoxilin and eosin: **A** CyA-treated animal. Note the signs of mild rejection consisting of lymphocytic attachment and mild endothelitis (x400); **B** FK 506-treated animal. Note the signs of moderate rejection consisting of lymphocytic infiltration around an arterial vessel with infiltration of the vessels wall and severe endothelitis (x400)

the donor mesenteric lymph nodes. In constrast, the intraepithelial lymphocytes, the endothelial cells, and the mucosal glands were all negative.

When sections of the intestinal allografts ($n = 4$ for groups 2 and 3) were stained with L-21-6 (anti-LEW MHC class II antibody), a mucosal staining pattern similar to that seen in syngeneic grafts was evident, whereas the mesenteric lymph nodes, because of the cellular depletion, showed only little and irregular staining. The inflammatory infiltrates in allograft mesenteric lymph nodes, mesenteries, and arteries stained positively, indicating that they were derived from recipient (LEW) cells (Fig. 3).

The use of the anti-RT1^n antibody (anti-BN class I) on sections of the allografts showed staining of the endothelial cells and of the small bowel epithelium ($n = 4$ for groups 2 and 3). No staining of the mononuclear cells in the lamina propria or Peyer's patches was observed (Fig. 4).

Staining for the T-helper cells and other accessory cells (with monoclonal antibody W3/25) in the syngeneic grafts ($n = 4$) revealed a normal distribution of W3/25 + cells in the lamina propria and Peyer's patches but no staining of intraepithelial lymphocytes. In the graft mesenteric lymph nodes, the para- and interfollicular areas contained positive cells, whereas the follicles themselves were negative and no staining was observed in the mesentery.

The distribution of W3/25 + positive cells in the bowel of both allograft groups ($n = 4$ for groups 2 and 3) was similar to that of the syngeneically grafted animals, with the exception that more positive staining was evident in the lamina propria of half of the animals in both groups. Because the Peyer's patches and the mesenteric lymph nodes were fibrotic and scarred, they contained only a few mononuclear cells and no consistent staining pattern was observed. However, where obliterative arteriolopathy with focal arteritis was evident, W3/25 + cells were detected among the infiltrating cells (Fig. 3).

Staining for T suppressor/cytotoxic cells (with monoclonal antibody O × 8) in the syngeneic grafts ($n = 4$) showed only a few O × 8 + cells in the lamina propria and the Peyer's patches and rather distinct staining of the intraepithelial lymphocytes. The follicles of the mesenteric lymph nodes stained strongly positive and a small number of positive cells was found in the interfollicular areas. No staining was observed in the mesentery.

The distribution of O × 8 + cells in the allografts given CyA or FK 506 therapy ($n = 4$ for both groups) was similar to that of the syngeneic grafts, although fewer positive cells were identified. An examination of the allograft mesenteric lymph nodes and the mesentery revealed only a few O × 8 + cells because of depletion and fibrosis of these structures. Like W3/25 + cells, O × 8 + cells were found among the infiltrate at sites where an obliterative arteriolopathy with focal arteritis was observed (Fig. 3).

To determine whether the allografts contained IgA-secreting cells, a monoclonal antibody directed against the heavy chain of IgA (MARA-1) was utilized. A normal pattern (few positive cells in the Peyer's patches, marked staining of the lamina propria, and a stain lining the mucosal surface) of staining was observed in both the syngeneic ($n = 4$) and the allogeneic grafts ($n = 4$ for both groups), with the exception of the depleted, involuted Peyer's patches of the allografts (Fig. 3). Staining of the allograft sections with the mAb O × 33 (anti-B cell) confirmed the findings with the anti-IgA antibody but, as expected, no mucosal surfache stain was observed (data not shown).

Sections of the spleen from both syngeneically and allogeneically grafted animals demonstrated a normal distribution of cells and staining for each of the various antibodies utilized (data not shown).

In summary, the immunoperoxidase studies demonstrated that: (a) the small bowel allografts were repopulated with recipient class II + cells; (b) the endothelial and epithelial cells stained positive for donor class I; (c) a nor-

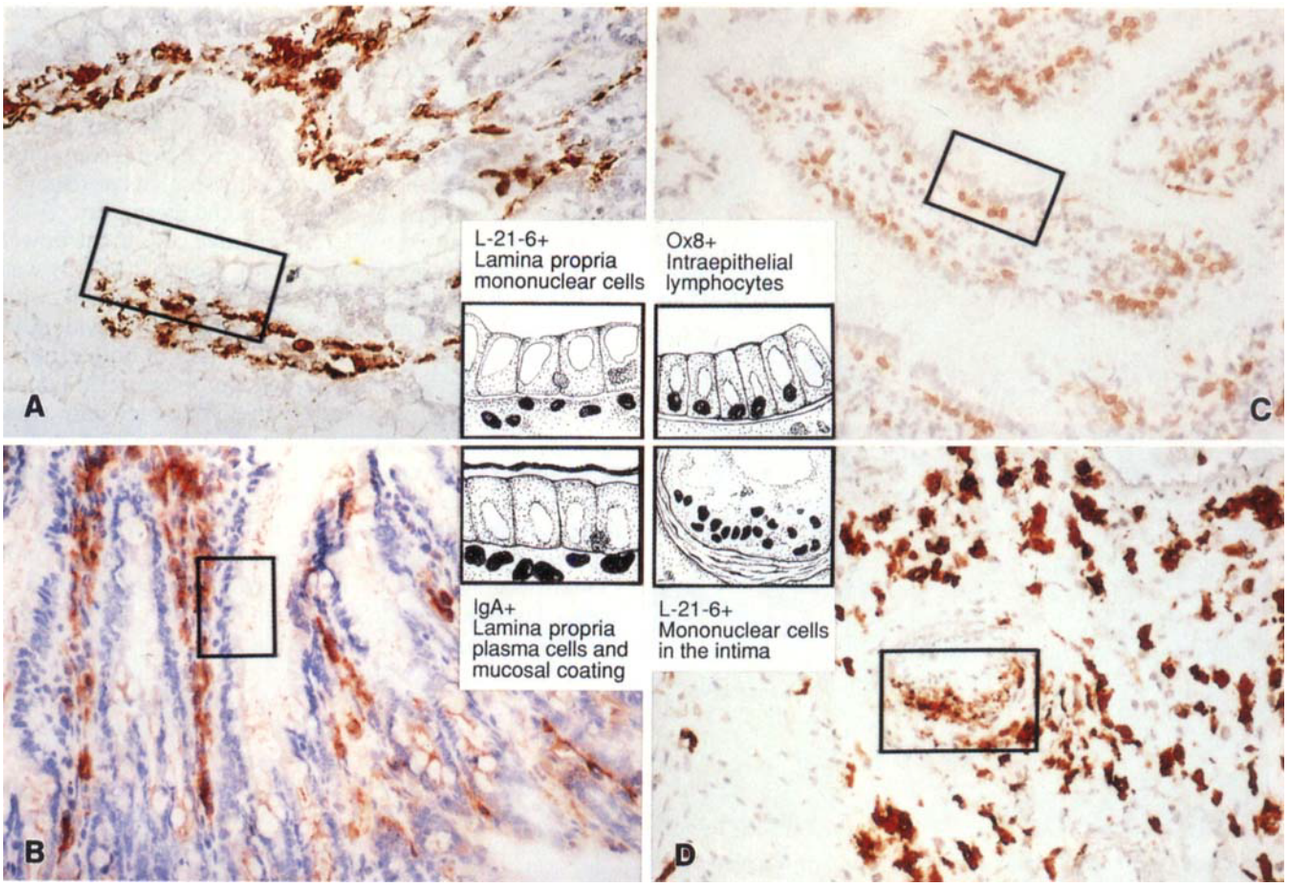

Fig. 3 A–D Sections of the small bowel allografts were obtained from the recipients (initially treated with CyA or FK 506) over 1 year after transplantation, immediately frozen, and stained with various monoclonal antibodies (mAb) using standard immunoperoxidase techniques: **A** section from an FK 506-treated allograft stained with mAb L-21-6 (anti-recipient MHC class II; x64). Note the repopulation of the lamina propria with the recipient MHC class II + cells without evidence of an infiltrative process; **B** section from an FK 506-treated allograft stained with mAb MARA-1 (anti-IgA heavy chain; x40). Note the normal distribution of the IgA + plasma cells in the lamina propria and the stain coating the mucosal surface; **C** section from a CyA-treated allograft stained with mAb Ox8 (anti-T suppressor/killer cells; x64). Note the normal distribution of the Ox8 + intraepithelial lymphocytes; **D** section of the mesentery from a CyA-treated allograft stained with mAb L-21-6 (anti-recipient MHC class II; x64). Note the recipient MHC class II + inflammatory infiltrate in the mesentery and in the wall of the inflamed artery

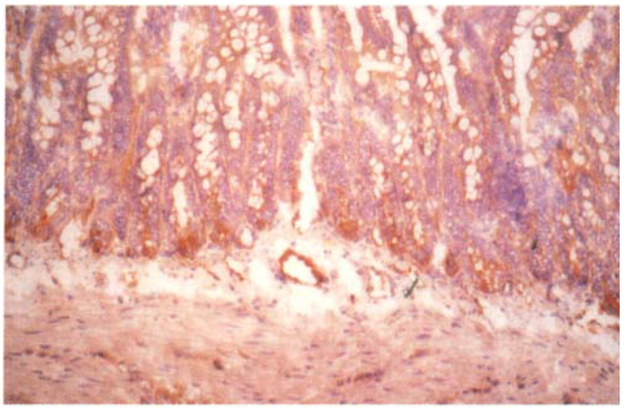

Fig. 4. Sections of the small bowel allograft (initially treated with CyA) were obtained from the recipient over 1 year after transplantation, immediately frozen, and stained with anti-$RT1^n$ monoclonal antibody, using standard immunoperoxidase techniques (x40). Note the positive staining of the endothelial and epithelial cells

mal distribution of W3/25 + cells, O × 8 + cells, O × 33 + cells, and IgA + cells was present in the small bowel allografts, with the exception of the fibrosed Peyer's patches and mesenteric lymph nodes; and (d) recipient class II +, W3/25 +, and O × 8 + cells were involved in the arteritis found in the mesenteric lymph nodes and in the mesentery of the allografts.

Discussion

This study confirms the effectiveness of both CyA and FK 506 as immunosuppressive therapies in averting acute cellular rejection of small bowel allografts and the development of GVHD in a rat model of small bowel transplantation. No detrimental side effects of either CyA or FK 506 were observed, with the exception of a transiently reduced food intake for 2–3 days beyond the course of therapy (data not shown), resulting in a minor, transient weight loss. This observation has also been made by others [12]. All animals in both allograft groups recovered from the operation and from the effects of the initial immunosuppressive therapy, gained weight, and stayed healthy for over a year following transplantation. Importantly, renal and hepatic function remained intact, no abnormalities in the hematologic profiles were observed, and functional evaluation of the allografts revealed that maltose absorption profiles and the ability to absorb nitrogen and fat were not impaired as compared to either syngeneically grafted animals or age-matched normal controls. Infectious complications (wound infections, intra-abdominal sepsis, pneumonia) were rare occurrences and were no more common in the immunosuppressed animals than in those not receiving such therapy.

Morphological evaluation of the mucosa of allografts from both the CyA- and FK 506-treated rats demonstrated an intact mucosal architecture without focal crypt inflammation (cryptitis) or cellular infiltrates around arterioles, both indicative of acute cellular rejection [11]. Moreover, immunohistochemical studies demonstrated a normal distribution of T_h helper cells (in the lamina propria), T_c suppressor/killer cells (predominantly intraepithelial lymphocytes), and B cells (in the lamina propria) in the allografts. Furthermore, numerous mononuclear cells (with the exception of the intraepithelial lymphocytes which, unlike lamina propria mononuclear cells, do not express class II in the Lewis rat [2]) in the lamina propria stained positively for the recipient class II antigen, suggesting rather strongly that a repopulation with recipient lymphoid cells had occurred. This observation confirms the recent report by Murase and colleagues, who found a recipient-type lymphoid repopulation in FK 506-treated multivisceral and small bowel grafts 3–6 months after transplantation [13]. Furthermore, our study indicates that this recipient-type repopulation is stable after it has developed and that it is not dependent upon the immunosuppressive therapy used, as it was seen in both the CyA- and FK 506-treated groups.

The finding that IgA + plasma cells are normally distributed in the lamina propria of the allografts and that secretory IgA is layered on the luminal surface of the epithelial cell suggests the presence of an intact secretory mucosal immune system. Xia and Kirkman [18] have studied the local immune response of heterotopic allografts (rejection was controlled with CyA) 1, 2, 3, and 4 weeks after transplantation by examining the host's response to cholera toxin instilled into the transplanted gut lumen. They reported a significantly blunted response in terms of the specific anticholera toxin antibody response, whereas the total IgA production did not differ from that of controls [18]. Whether a specific sIgA production (e.g., in response to cholera toxin) is detectable in long-surviving recipients of small bowel allografts needs further examination.

While the mucosal/epithelial structures were intact and functionally competent, Peyer's patches and mesenteric lymph nodes of the allografts were atrophic, hypocellular, and contained an inflammatory infiltrate. In addition, an obliterative arteriolopathy with arteritis was observed in both the mesentery and the mesenteric lymph nodes of the allografts. Because these findings were not observed in the recipients of syngeneic grafts treated with either CyA or FK 506, these changes are most likely the result of chronic graft rejection observed earlier [10]. Immunohistochemical study of the mononuclear cells located in these inflammatory foci demonstrated that these cells are of recipient origin. This represents strong evidence of an ongoing rejection process. Rejection-induced fibrosis of the mesenteric lymph nodes and Peyer's patches are also likely to be responsible for the lack of recipient-type repopulation in these tissues since Murase et al. noted that if the immunosuppressive treatment is continued, these structures are not obliterated but maintained and repopulated [13]. It is difficult to explain why the graft mucosa was seemingly spared from rejection-induced tissue damage while the mesenteric arteris and lymph nodes became subject to this process. Almost certainly, the mechanisms responsible for this observation are complex and beyond the scope of this particular study.

Finally, an understanding of the phenomenon of long-term functional survival following limited immunosuppressive therapy should have important implications for clinical intestinal transplantation. In a previous study using the same experimental set-up, we noted that 1–1.5 years after discontinuation of the initial immunosuppressive therapy, the systemic immunological response of allografted animals to the grafted and to a third party antigen, as judged by mixed lymphocyte cultures, cytotoxicity assays, and skin grafting, is not different from that of syngeneically grafted animals. This result indicates that systemic tolerance or clonal deletion is not the reason for the long-term survival, and this led us to hypothesize that

the phenomenon of long-term survival in this model may be a local process [10].

The present study suggests that potent immunosuppressive therapy is of great importance in the early post-transplant period, at a time when complex donor-recipient leukocyte trafficking and graft repopulation occur [4], but that it may not be so critical later on when lymphoid repopulation of the graft has occurred. Inadequate therapy early during the period of graft repopulation results in graft rejection and systemic allostimulation [14, 15, 17], which is probably initiated in the lymphoid tissues of the intestinal graft by infiltrating recipient cells. Prevention of this early alloreactivity by the use of potent immunosuppression results in long-term graft function associated with lymphocytic repopulation of the graft by recipient cells. Nonetheless, an indolent, clinically inapparent form of chronic rejection ensues in target structures not readily accessible by intestinal biopsy, such as the mesenteric vessels. Therefore, it is likely that a true assessment of the integrity of long-term intestinal grafts will require information from a variety of monitoring techniques, only one of which is intestinal biopsy. These other modalities may include an endoscopic survey for ulcers and laparoscopic evaluations of the graft mesentery for thickening, as well as possible angiography to detect the presence of an obliterative arteriolopathy.

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