Transpl Int (2000) 13: 1–11

ORIGINAL ARTICLE

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Functional, histological, and inflammatory changes in chronically rejecting small bowel transplants

Received: 19 January 1999
Accepted: 19 July 1999

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Abstract Our aim was to develop a model of chronic rejection (CR) in small bowel allografts, and to study the changes occurring in these grafts. Small bowel transplantation was performed using the DA to AS rat strain combination. Short-term (5 mg/kg intramuscular, from days − 2 to + 9), or long-term cyclosporin treatment (5 mg/kg, 3 times a week until day 50) was given to prevent acute rejection. Controls were untreated allografts, DA isografts with and without cyclosporin, and normal DA and AS rats. They were followed for 50 and 100 days after transplantation. Recipients of a syngeneic graft lost weight during the first week after transplantation, but started to regain weight and kept growing thereafter. Histology showed normal bowel architecture with normal mesenteric lymph nodes and Peyers patches. Vigorous acute rejection occurred in the untreated allografts. Animals had persistent weight loss, and were killed between 6–13 days after transplantation. No clinical signs of graft-versus-host disease were seen. Histology showed end-stage acute rejection. In both cyclosporin-treated allografted groups the postoperative course was as in the isografted animals. However, all animals had histologic signs of CR by 50 and 100 days after transplantation. Changes were most prominent in the mesentery. Serositis with increased vascularity, inflammation with sclerosis, and patchy myointimal proliferation with endotheliialitis of the mesenteric vessels were found. Changes in the bowel were patchy and included some thickening of the muscle coat, crypt hyperplasia, scattered necrotic cells in the crypts, slight blunting of villi and loss of goblet cells. Infiltrating cells in the mesentery and bowel consisted mainly of CD 4^+ cells, CD 8^+ T-cells and monocytes/macrophages. Lactulose-mannitol urinary excretion ratio was significantly increased in short-term cyclosporin treated allografts at days 50 and 100 post-transplant. Serum albumin levels were significantly lowered in this group at both time points examined. We developed two models in which CR occurs after small bowel transplantation. Long-term cyclosporin treatment delayed the development of CR, since functional abnormalities were only seen in the animals that were treated with short-term cyclosporin.

Introduction

Small bowel transplantation (SBT) is, potentially, an improved treatment modality for patients suffering from irreversible short-bowel syndrome and who are currently fed using total parenteral nutrition (TPN). In specialized centers, SBT has evolved over the last decade from a cumbersome procedure with unacceptably high morbidity and mortality to a more acceptable therapy for those with permanent intestinal failure. Patients that qualify for SBT are those with irreversible dependency on TPN and a poor long-term prognosis related to progressive difficulties with the administration of TPN (venous access), catheter associated sepsis, and liver failure [1, 4].

A major problem hampering the long-term success of SBT is vigorous acute rejection, requiring high doses of immunosuppression. A major improvement in the results of SBT came in the early 1990-ies when the powerful immunosuppressant tacrolimus replaced cyclosporin. Although the outcome of SBT is still mainly determined by acute rejection and lethal infections, the long-term results are nevertheless improving [1]. Because of this, new problems that arise in the longer term are encountered. Of these, dysmotility, eating disorders, post transplant lymphoproliferative disease, late acute rejection episodes, and chronic rejection (CR) are of special concern.

Little is currently known about CR following SBT, although some experimental and clinical data show that CR may develop after SBT [12, 21]. The clinical characteristics reported on CR are diarrhoea and weight loss. Histologically, total villous atrophy, apoptosis of crypt cells with inflammatory infiltrate, and arteriosclerosis of the mesenteric vessels are described [7, 17].

The aim of the present study is to develop a model in which CR of the small bowel allograft would develop in a reproducible manner that would allow us to study the changes occurring in those grafts.

Materials and methods

Animals

Inbred adult male Dark Agouti (DA) ($RT1^{av1}$) and Albino Surgery (AS) ($RT1^{1}$) rats were obtained from Monash Animal Services, Victoria, Australia. All experimental procedures involving animals conformed to the National Health and Medical Research Council Code of Practice and were approved by Monash Medical Centre Animal Ethics Committee B and the Monash Standing Committee on Ethics in Animal Experimentation.

Small bowel transplantation

SBT was performed as described previously [24]. In brief, the total small bowel, from the Ligament of Treitz to the terminal ileum was transplanted on a vascular pedicle consisting of the superior mesenteric artery and portal vein. In the recipient, end-to-side anastomoses were made between the recipient infra-renal aorta and donor superior mesenteric artery, and recipient caval donor and portal vein. After resection of the recipient small bowel, the graft and recipient remnant bowel were anastomosed end-to-end.

Experimental design

The following groups were studied: 1) DA to DA, no treatment, 2) DA to DA, long-term cyclosporin (lCsA), 3) DA to AS, no treatment, 4) DA to AS, short-term cyclosporin (sCsA), and 5) DA to AS, long-term CsA. sCsA treatment consisted in administering 5 mg/kg intramuscularly from day − 2–+ 9 relative to transplantation. lCsA treatment consisted in administering 5 mg/kg intramuscularly three times a week until day 50. Normal DA or AS rats were used as controls. Animals were killed 50 and 100 days after transplantation, or when they demonstrated 30% weight loss.

Functional parameters

Serum albumin was measured using the bromocresol green method on a Cobias bio autoanalyser. Results are given as mean ± sd in g/l. Lactulose mannitol urinary excretion test: Animals were fasted during the day. 400 mg/kg D-lactulose and 100 mg/kg of D-mannitol were dissolved in 1 ml of water. This solution was given orally by gavage. Each animal was placed in a metabolic cage, and urine was collected overnight. The amount of urine produced was recorded, and samples were stored at − 20 °C until analysis. Both lactulose [22] and mannitol [18] concentrations (mmol/l) were measured colorimetrically on a Cobias bio autoanalyser. The results are expressed as mean ± sem lactulose : mannitol ratio.

Macroscopic appearance of the graft

The small bowel graft, its mesentery, the Peyers patches, mesenteric lymph nodes, and spleen were inspected for signs of CR at the time of organ retrieval. Samples of the graft were prepared for histology and immunohistochemistry.

Histology

Samples of graft ileum were fixed in 10% neutral formalin. After dehydrating and embedding in paraffin, 3–4 μm thick sections were cut and stained with hematoxylin and eosin (H&E). They were scored by two observers. The incidence of the following features was recorded for each of nine high power fields (400 ×); Blunting of villi and loss of goblet cells, crypt hyperplasia and crypt cell necrosis, muscularis thickening, cellular infiltration, vascular involvement (i.e. myointimal proliferation, endothelialitis, adherence of leucocytes to the endothelium, and perivascular infiltration), and sclerosis in the mesentery. Scores of 0, 1, 2, or 3 were assigned if features were observed in 0, 1/3, 2/3, or all fields respectively. Results for each feature were added, and histologic grades were assigned as follows: 0 points = none (0), 1–3 points = very mild (grade 1), 4–6 points = mild (grade 2), 7–9 points = moderate (grade 3), and 10–12 points = severe (grade 4).

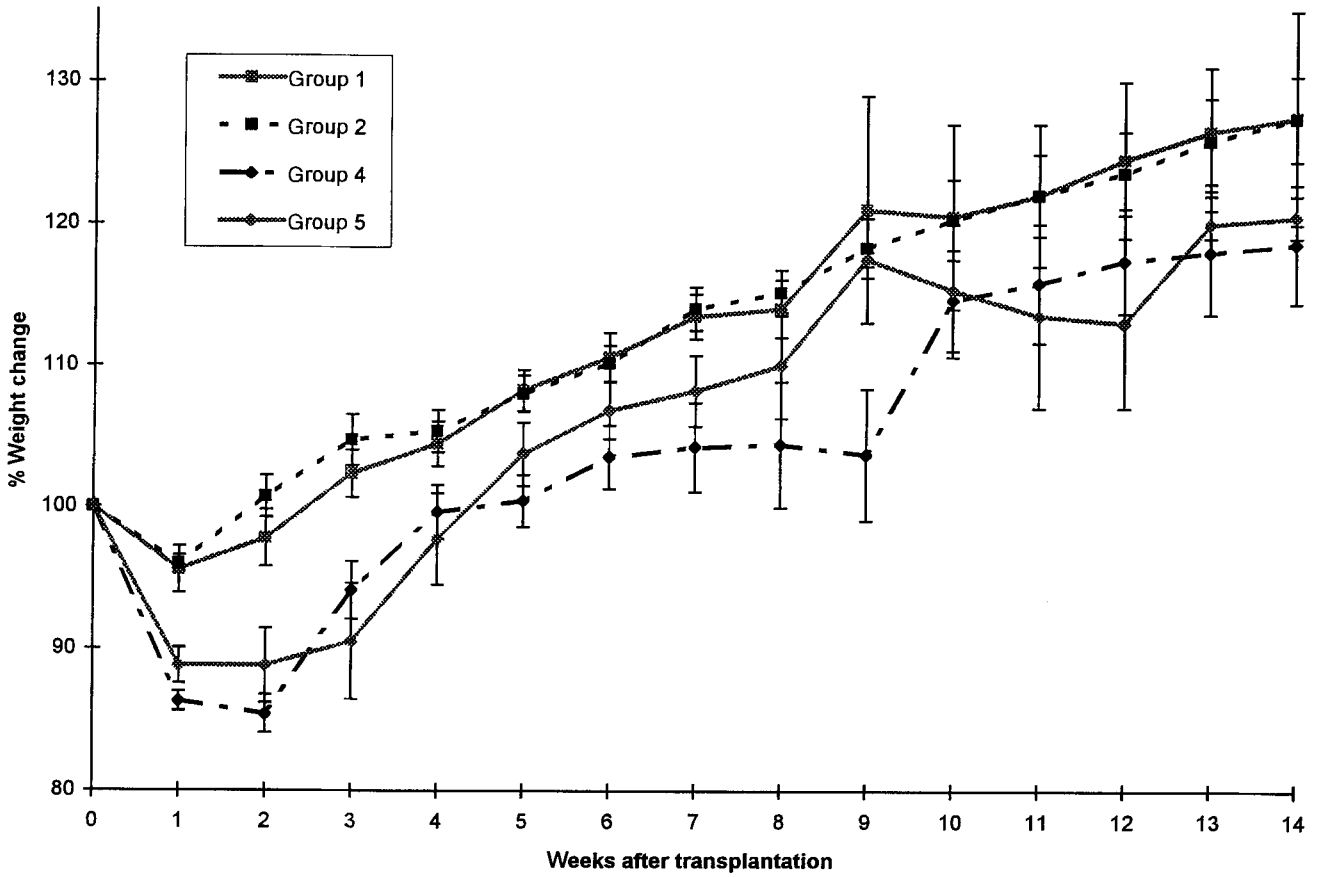

Fig. 1 Weight changes after small bowel transplantation

Immunohistochemistry

Tissues were embedded in OCT and snap frozen. Cryostat sections of 7 μm thick were cut. These sections were fixed in PLP and stained using a three-layer peroxidase technique [27]. In brief, sections were pre-incubated for 10 min in 10% Normal Swine Serum (NSS; Dako, Christchurch, New Zealand)/10% Fetal Calf Serum (FCS) in phosphate buffered saline (PBS) with 0.01% NaN_3 (Az) to avoid non-specific binding of the primary antibodies. Slides were drained and incubated at room temperature for 1 h with primary mouse antibody diluted in 1% NSS/1% FCS with 0.01% Az. They were washed 1 × in PBS for 5 min, taken through graded alcohols, and incubated at 4 °C for 10 min in methanol/0.03% H_2O_2 to block endogenous peroxidase activity. Thereafter they were reversed through graded alcohols and washed 2 × in PBS, Rabbit anti-mouse-peroxidase conjugate (Dako) was applied, and then they were incubated for 30 min at room temperature.

After washing 1 × in PBS, the third step, swine anti-rabbit-peroxidase conjugate (Dako), was applied, after which they were incubated for 30 min at room temperature. Sections were then washed in PBS 2 ×, and colour was developed using metal-enhanced DAB substrate (Pierce-Rockford). Finally, slides were counterstained with Harris' haematoxylin, dehydrated, and covered using glass slips.

The following mouse-anti-rat monoclonal antibodies were used: OX-1 (CD45), OX-8 (CD8), W3/25 (CD4), OX-6 (MHC class II), OX-33 (B-cells), ED-1 (monocytes, free and tissue macrophages), ED-2 (tissue macrophages), ED-3 (lymphoid macrophages) (ED-1, 2, and 3 are a kind gift of dr C. D. Dijkstra, Amsterdam, The Netherlands), and 3.2.3. (NK cells).

The %-area OX-1 positive cells was measured using a "Video Pro" image analysis system. OX-8, W3/25, ED-1, OX-33 and 3.2.3 were counted using a graticule at a magnification of 400X. Six separate fields were measured, corresponding to an area of 0.1 mm^2. For the submucosa, an area corresponding to 0.02 mm^2 was measured and then multiplied by 5. The other markers were scored semiquantitatively.

Statistics

Statistical analyses of the nutritional parameters, histology and immunohistochemistry were carried out with the Kruskal Wallis analysis of variance, followed by a Mann-Whitney U-test, using SPSS for Windows. A P-value < 0.05 was considered statistically significant.

Results

Postoperative recovery

Recipients of a syngeneic graft (groups 1, and 2) lost weight during the first week post transplant, but started

to regain weight and kept growing thereafter. Untreated allografts demonstrated vigorous acute rejection. Animals had persistent weight loss and developed diarrhoea from day 4 onwards. They were killed between days 6 and 13 after transplantation. No signs of graft-versus-host disease were seen. Histopathological analysis showed end stage acute rejection.

CsA treated allografts (groups 4 and 5) seemed to lag behind in growth. No statistically significant differences between allografted, and syngeneically transplanted animals were demonstrable however, since the variation between the animals in the different groups was considerable (figure 1). Since no diarrhoea or weight loss was observed at the time points studied, these models may be considered as representing subclinical CR.

Nutritional parameters

Serum albumin levels were significantly lowered in sCsA treated animals at both timepoints as compared to normal AS rats (28.1 ± 4.9 and 27.2 ± 2.3 vs. 34.8 ± 0.8 g/l, $p = 0.02$ and $p = 0.006$, respectively). At day 50, the lactulose-mannitol urinary excretion ratio in sCsA allografts was significantly higher than that in lCsA allografts and normal DA controls (figure 2). Thereafter, the ratio further increased. Hundred days after engraftment, the sCsA allografts had significantly higher ratios than the lCsA treated ones, isografts and normal DA controls. There were no significant differences between control rats and syngeneically transplanted groups. CsA treatment in the isografts did not affect the excretion of both urinary lactulose and mannitol. The lactulose-mannitol excretion significantly correlated to the histological degree of chronic rejection ($r = 0.35$, $P = 0.047$, $n = 33$).

Macroscopic appearance of the grafts

Fifty and 100 days after transplantation, isografts showed a normal macroscopic bowel architecture with normal mesenteric lymph nodes and Peyers patches. Signs of CR were observed in 67% of sCsA treated animals and 33% of lCsA treated animals by 50 days, and all sCsA and 67% of lCsA animals by 100 days post transplant.

Macroscopic signs were: sclerosis and enlargement of the MLN, sclerosis of the mesentery and serosa, thickening of the bowel wall, splenomegaly, and enlargement of the cecum (both 1.5–3 times their normal size). In most allografts the Peyers patches were atrophied, sometimes to a degree that they were no longer visible. In other allografts, Peyers patches had become enlarged and hemorrhagic. In advanced CR, the MLN had become a large, sclerotic and sometimes hemorrhagic mass. The mesentery and serosa were rigid due to the extensive sclerosis.

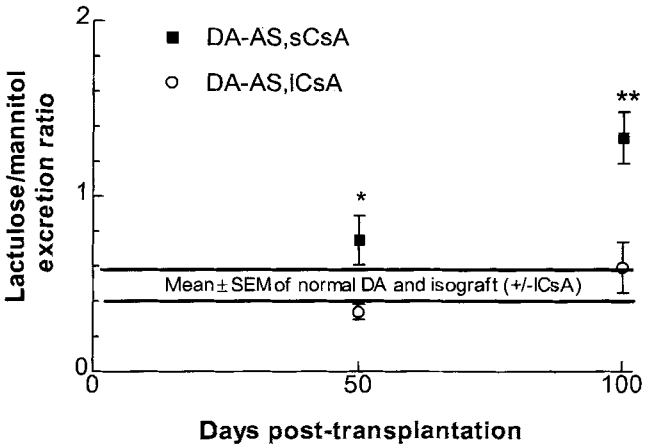

Fig. 2 Intestinal function of small bowel transplants was measured by the urinary excretion of orally administered lactulose and mannitol. The ratio lactulose/mannitol was calculated and results are expressed as mean ± SD. ** $P < 0.05$ vs DA-AS, lCsA and DA-DA at the same timepoint, and vs DA-AS, sCsA at day 50; * $P < 0.02$ vs DA-AS, lCsA and DA at the same timepoint. Normal DA, DA-DA +/− lCsA did not differ, and are depicted as 'normal range bar'

None of these abnormalities were found in the syngeneic groups.

Histology

At 50 days post transplant, a pericapillary infiltrate in the mesentery was usually the first indication of CR (figure 3). Other early features were cellular depletion and active infiltration of the graft MLN.

Serositis with increased vascularity, florid mesenteric inflammation with sclerosis, and patchy myointimal proliferation with endothelialitis of the mesenteric vessels were found later in the course of CR. Changes in the bowel were patchy and included some thickening of the muscle coat, crypt hyperplasia, scattered necrotic cells in the crypts, sclerosis between the crypts, slight blunting of the villi, and loss of goblet cells (figure 3). Apart from an increase in mononuclear cells, the lamina propria between the crypts also contained mast cells and eosinophils. The degree of CR could vary from very mild to moderate within a group (Table 1 a). At day 50, lCsA treated animals had significantly less chronic damage compared to sCsA treated animals (1.3 ± 0.5 vs. 2.8 ± 0.5, $p = 0.001$, Table 1 b). However, this difference disappeared by day 100 posttransplant. None or very mild changes were seen in the syngeneically transplanted grafts (figure 3).

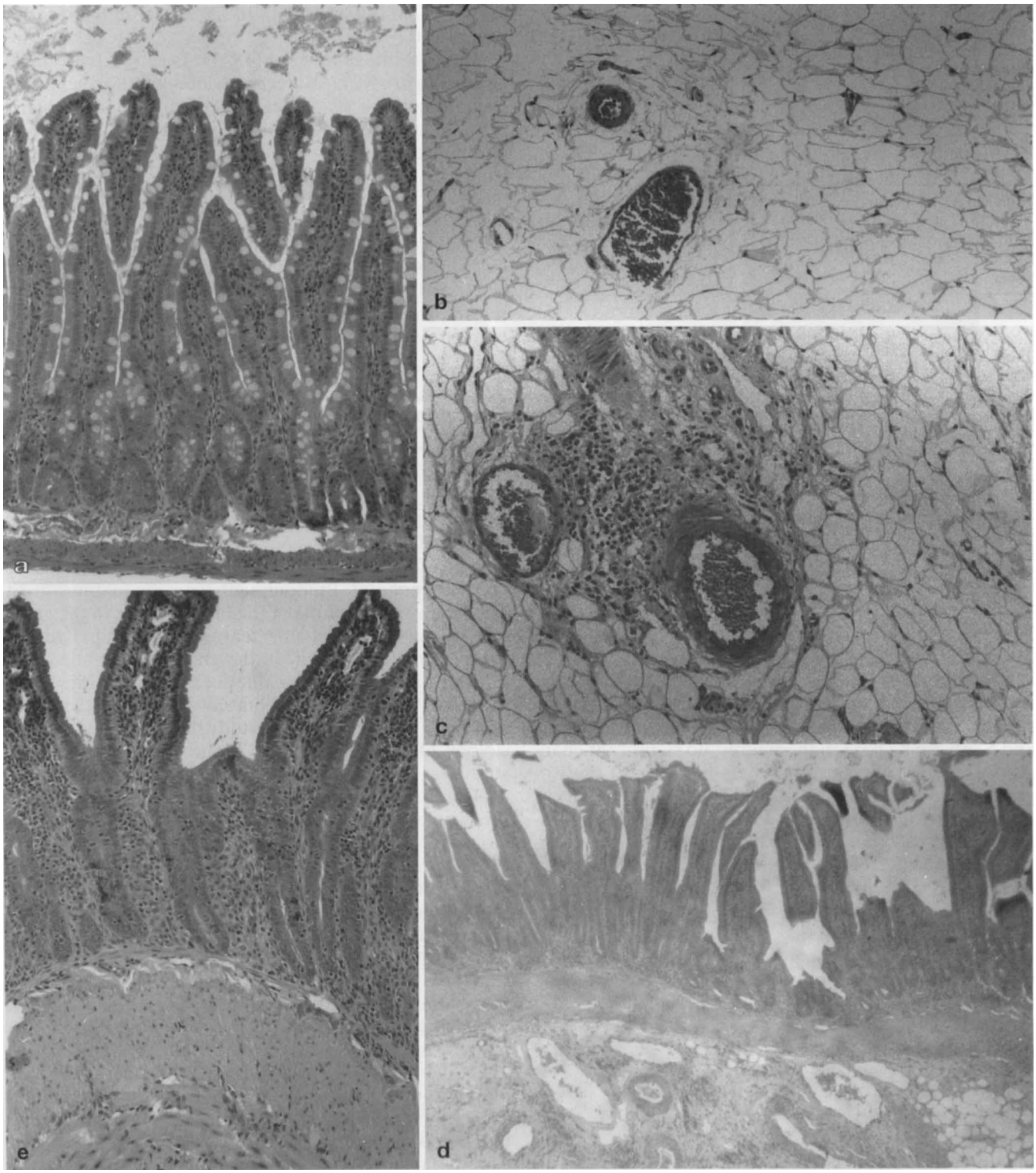

Fig. 3 **a** DA to DA isograft 100 days post transplant showing normal bowel architecture. **b** Mesentery of the graft shown in Fig. 3 a. **c** Pericapillary infiltrate in the mesentery of a long-term cyclosporin treated allograft at day 50 posttransplant with mild chronic rejection. **d** Allograft treated with long-term cyclosporine at day 100 post transplant showing moderate chronic rejection with blunting of villi, loss of goblet cells, crypt hyperplasia, hypertrophy of the muscle coat, and sclerosis of the mesentery. **e** High-power magnification of an allograft treated with short-term cyclosporine at day 50 post transplant showing moderate chronic rejection with blunting of villi, loss of goblet cells, crypt hyperplasia and thickening of the muscularis

Table 1a Histopathological analysis of chronic rejection after small bowel transplantation. Percentage of animals per group with a chronic rejection grade. Histopathological chronic rejection grades were assigned according to the severity of the lesions (see: materials and methods)

	Short-term CsA		Long-term CsA	
Grade ↓/ day →	Day 50 ($n = 9$)	Day 100 ($n = 5$)	Day 50 ($n = 6$)	Day 100 ($n = 6$)
None (0)	0	0	0	0
Very mild (1)	0	20	67	17
Mild (2)	33	40	33	50
Moderate (3)	67	40	0	33
Severe (4)	0	0	0	0

Table 1b Chronic rejection grades after small bowel transplantation. Histopathologic rejection grades were assigned according to the severity of the lesions (see materials and methods). * Short-term CsA vs. long-term CsA at day 50, $P = 0.001$

Group	Histopathological rejection grade, mean ± sd
Short-term CsA day 50	2.7 ± 0.5
Short-term CsA day 100	2.2 ± 0.8
Long-term CsA day 50	1.3 ± 0.5*
Long-term CsA day 100	2.2 ± 0.7

Analysis of infiltrating cells

The numbers, types, and distribution of leucocytes in the different compartments of the ileum (ie. villi, crypts, submucosa, and muscularis) are depicted in figures 4 and 5. As can be seen in the figures, no significant changes were detected in cell numbers in either of the isograft groups when compared to normal DA rat controls. In some isografts however, a focal infiltrate around single crypts was seen. This was never observed in non-transplanted controls. In general, in the mucosa of small bowel grafts, the highest density of cells was found at the base of the crypts, showing a gradual decrease up the crypt-villus axis.

In allografts, the number of infiltrating cells ($CD45^+$) was significantly increased. These consisted mainly of $CD4^+$-, $CD8^+$ T-cells and $ED\text{-}1^+$ macrophages. Long-term CsA treatment delayed the infiltration of the allografts, since at day 50 significantly less cells were present in the lCsA – than in sCsA treated animals. $CD4^+$ cells were present in constitutively high numbers in normal ileum and isografts (figure 4.3 a). A significant increase was observed in the crypts of allografts at both time points. $CD8^+$ cells were significantly increased in the crypts and muscularis of sCsA treated allografts, and in the crypts of lCsA treated allografts at day 100. Changes in number of $ED\text{-}1^+$ macrophages were evident in all compartments, with a significant increase in the crypts and submucosa. In the muscle layer, which is normally devoid of $ED\text{-}1^+$ cells, a significant influx was observed at day 100.

The absolute number of $OX\text{-}33^+$ B-cells was low in all groups (figure 5). No differences among the groups were found except for a significant increase in the muscularis of sCsA treated allografts at day 100. The number and distribution of NK cells was comparable in all groups (figures 4.6 b and 5). $ED\text{-}2^+$ tissue macrophages were markedly increased in the submucosa and muscularis of allografted animals. Many cells were situated at the border between the longitudinal and circular muscle layers, i.e. in close association with the myenteric plexus. Very low numbers of $ED\text{-}3^+$ lymphoid macrophages were seen in the crypts, submucosa, and muscularis. These numbers were comparable in all groups studied.

Discussion

Chronic rejection is the major determinant that hampers long-term graft survival of solid organ grafts [3, 10]. With the improving results after seen after SBT [1], CR will also emerge as an obstacle to long-term survival. Although its pathophysiology seems in part similar in different types of grafts, organ specific features are also apparent. Predominant aspecific features are a thickening of the intima of the blood vessels in the graft, and fibrosis [25]. Specific features are unique to the anatomy and physiology of the graft, and include tubulointerstitial damage in kidneys [23], loss of bile ducts in liver grafts [25], and blunting of villi in the small bowel [12]. As we wished to study the specific pathophysiology of CR following SBT, the aim of the present study was to develop a model for CR after SBT.

The macropathological findings of our study are on the whole comparable to the findings published previously by Langrehr et al. [12], namely: enlargement of the MLN and involvement of the Peyers patches, fibrosis of the mesentery, and, in a later stage, the serosa. Histological findings were quite similar also: cryptitis, blunting of villi, loss of architecture and cellular depletion of the MLN. Loss of goblet cells was not reported. In their study, however, these changes progressed rapidly after the discontinuation of CsA, whereas we found that CR had developed in all animals 50 days posttransplant, but did not rapidly progress over the next 50 days. This difference may be explained by the fact that we killed the animals at the time points of evaluation and did not take consecutive biopsies of the graft, as did Langrehr et al. These successive laparotomies and biopsies of the graft cause multiple episodes of wound healing with the release of growth factors that may accelerate fibrotic changes in the graft and evoke multiple adhesions.

No histological, and only very minimal inflammatory changes were found in the isografted groups. There was

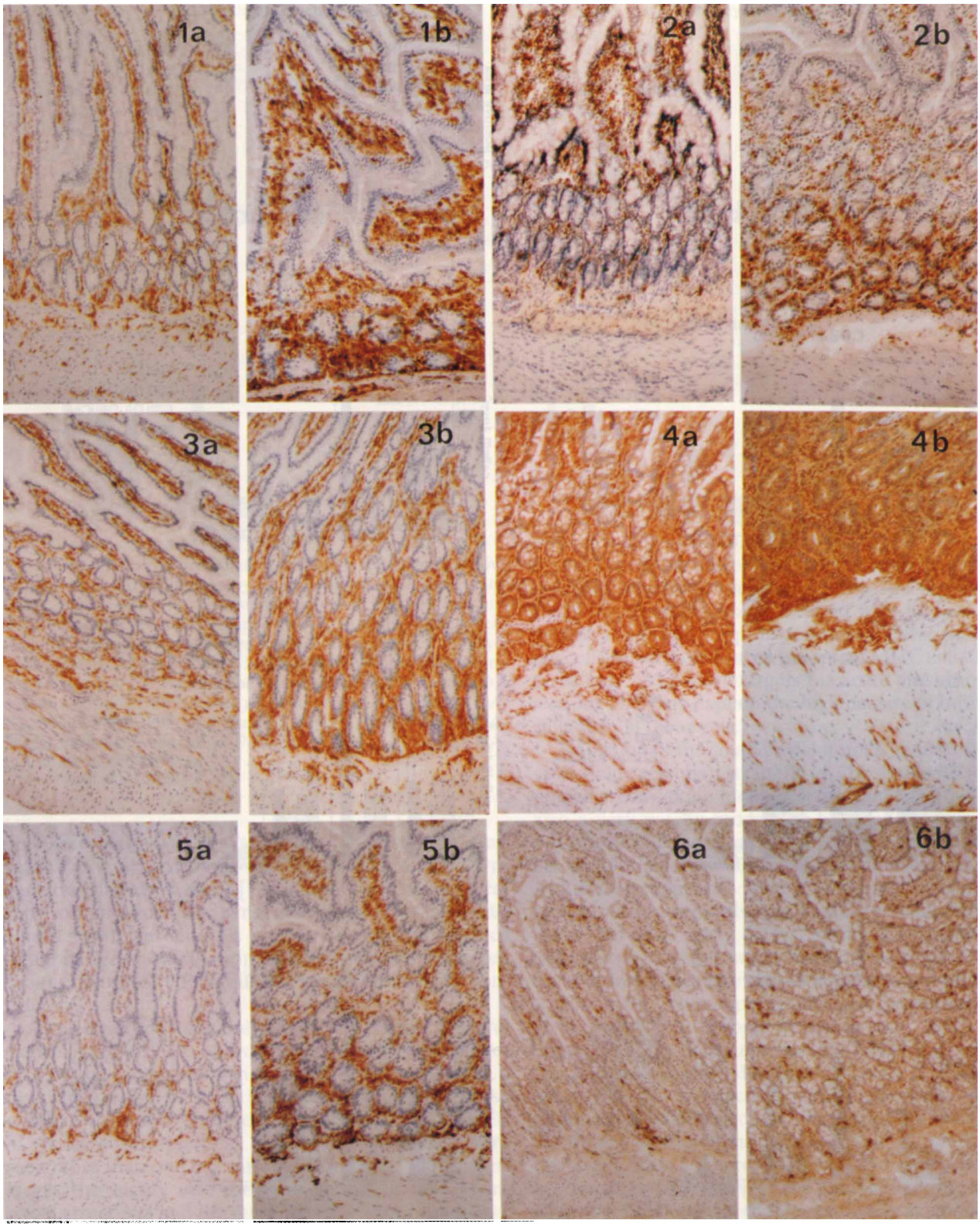

Fig. 4 Distribution of **1** CD45 (OX-1), **2** CD8 (OX-8), **3** CD4 (W3/25), **4** MHC class II (OX-6), **5** macrophages (ED-1), and **6** NK cells (323) in isografts **a** and allografts **b** 100 days post transplantation

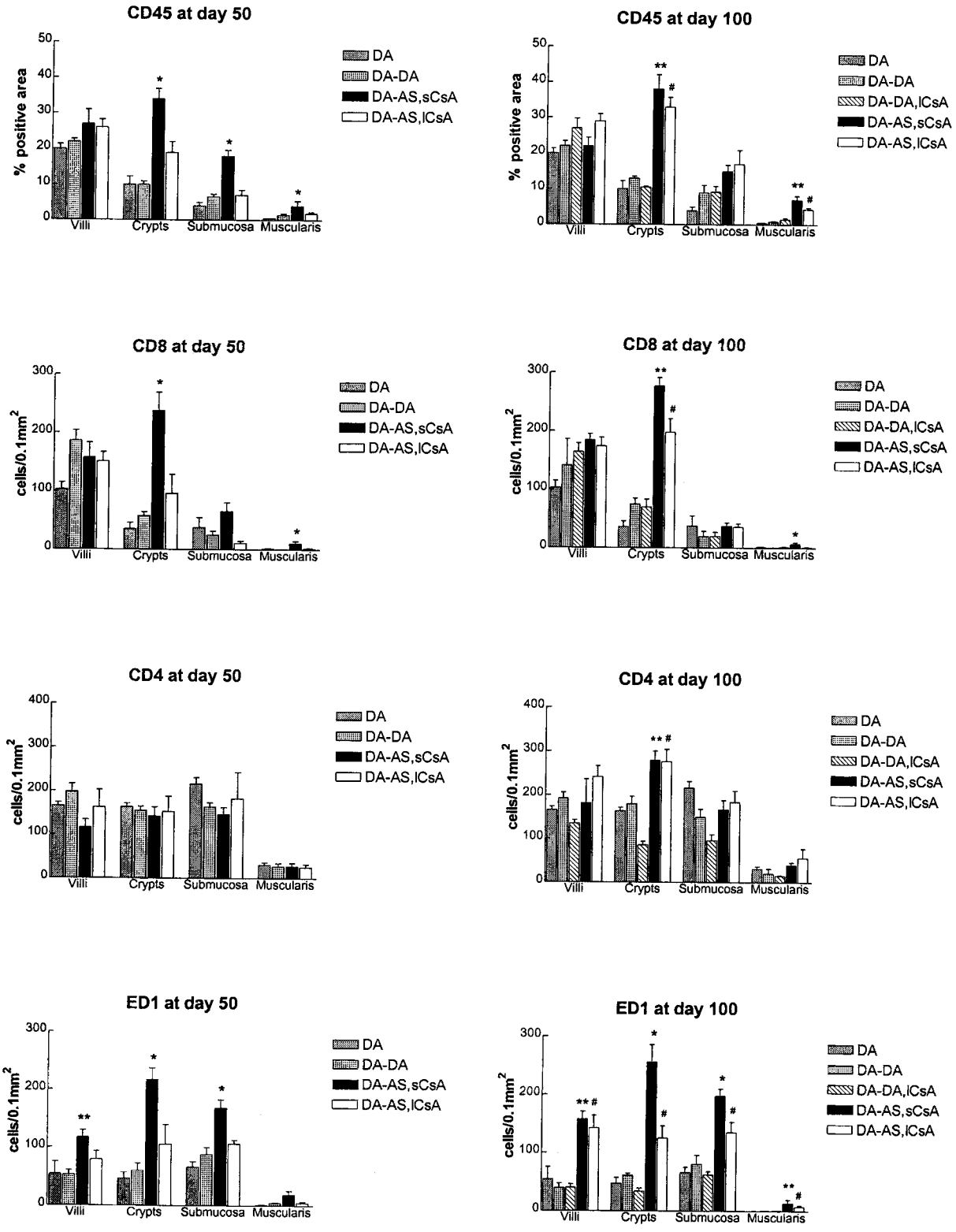
CD45 at day 50
% positive area
DA
DA-DA
DA-AS,sCsA
DA-AS,lCsA
Villi
Crypts
Submucosa
Muscularis
CD45 at day 100
% positive area
DA
DA-DA
DA-DA,lCsA
DA-AS,sCsA
DA-AS,lCsA
CD8 at day 50
cells/0.1mm2
CD8 at day 100
cells/0.1mm2
CD4 at day 50
cells/0.1mm2
CD4 at day 100
cells/0.1mm2
ED1 at day 50
cells/0.1mm2
ED1 at day 100
cells/0.1mm2

Fig. 5

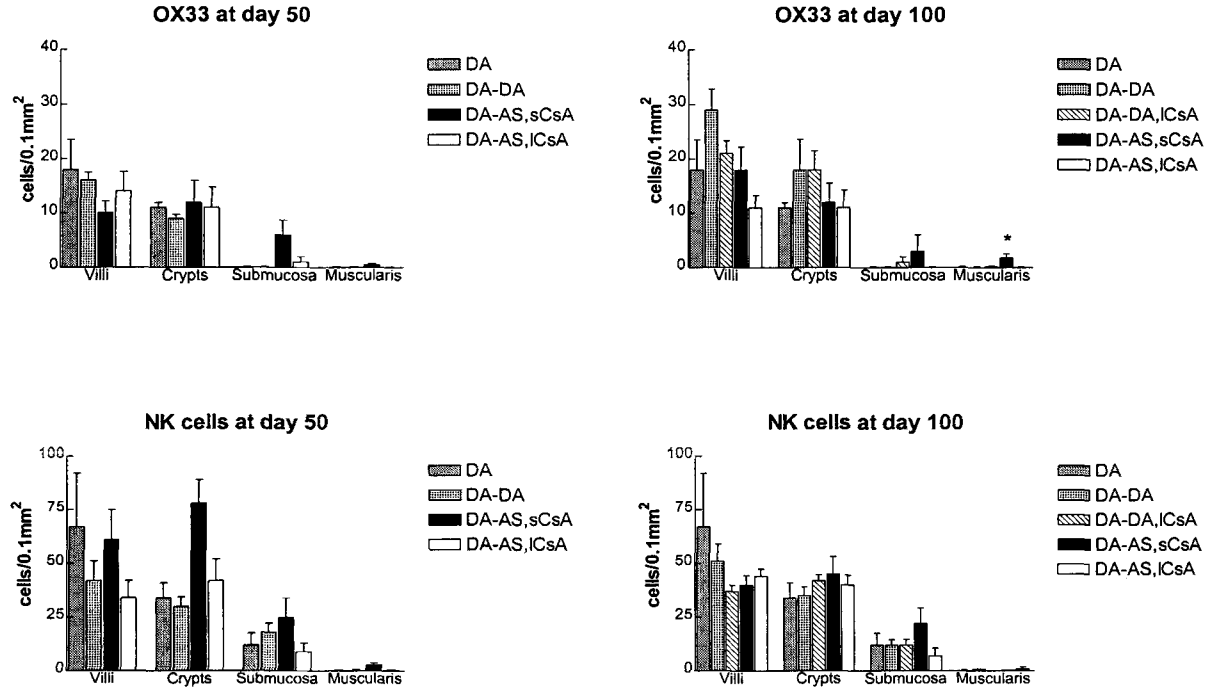

Fig. 5 Cell infiltration in normal, isografted, and allografted bowel. The increased infiltration by $CD45^+$ leucocytes in the chronically rejecting allografts consisted mainly of T lymphocytes and $ED1^+$ macrophages. CsA therapy delayed the influx of these cells in the allografts. B lymphocytes and NK cells appeared to play a minor role in chronic rejection. Isografts with or without CsA therapy had comparable numbers of mononuclear cells as normal DA controls. Results are expressed as mean ± SEM. * $P < 0.05$ vs DA-DA and DA-AS, lCsA at the same timepoint; ** $P < 0.05$ vs DA-DA at the same timepoint; # $P < 0.05$ vs DA-DA, lCsA and DA-DA at the same timepoint

no difference between CsA-treated or untreated animals. This is in accordance with previous findings in syngeneic small bowel grafts [12, 16], but contrasts with recent observations in syngeneic kidney transplants, where significant changes may be seen in the absence of alloantigenic stimulation [30], and shows that the pathophysiology of CR in different organs may vary considerably.

The earliest histologic signs of CR in our small bowel transplants were loss of architecture and cellular depletion of the MLN and Peyers patches together with a pericapillary infiltrate of the mesenteric vessels. These changes were also observed in allogeneic SBT models in which no morphologic or functional changes were seen more than one year after grafting [13]. This underscores the highly immunogenic nature of these lymphoid structures and the mesentery.

Mucosal ischemia may be important in the development of CR since arteriosclerosis, which may lead to ischemia, is one of the characteristics observed in this study. The small bowel has an enormous regenerative potential after ischemic damage [19]. This regeneration starts in the crypts which respond with an increase in proliferating cells (crypt hyperplasia). These proliferating cells, which constitutively express MHC class II antigens, may be primary targets for acute and chronic rejection. This may explain the high density of infiltrating cells seen in the crypts.

Rejection of the small bowel is not a generalised process. Acute rejection is patchy [4, 20], and may be easily missed when taking biopsies. Here we show that CR is also a very patchy process, in one and the same graft both normal appearing bowel and severe lesions may be found. This may also explain the variation found within the groups with respect to histologic score, numbers of infiltrating cells, and lactulose: mannitol excretion. Therefore, early detection of CR in human SBT will be possible only when histology is combined with immunohistochemistry and functional evaluation.

This is the first comprehensive phenotypic analysis of cells involved in chronic rejection after small bowel transplantation. The increase in $CD45^+$ leucocytes in the graft was mainly due to an increase in T-lymphocytes and $ED\text{-}1^+$ macrophages. These cells are also dominant in acute rejection episodes of the small bowel in humans [2, 8]. Pericryptic T-cell infiltration preceeds histologic overt acute rejection and seems an early marker for acute rejection [2]. The focal pericryptic infiltration with $CD8^+$ cells in the present study again indicates that the crypts are an early target for both alloantigen-independent (syngeneic grafts) and alloantigen-dependent destruction. How these infiltrating cells damage

the enterocytes is not clear. However, in several suspected immune-mediated small intestinal disorders, crypt hyperplasia and villus atrophy are observed. It has been shown recently that activated macrophages can directly inhibit the proliferation of rat intestinal epithelial cells. Mediators produced by macrophages may be involved, and both nitric oxide and TNFα have been shown to be involved in this cytostatic effect [11]. Eosinophils and mast cells were observed in the allografts also. These cells are found in inflammatory diseases of the bowel, such as celiac disease [14] and Crohn's disease [6]. Their possible contribution to CR in our model is intriguing, and deserves further study. In the muscle layer of allografts, significant infiltration with macrophages (both ED-1$^+$ and ED-2$^+$) was seen. These cells may contribute to the thickening of the muscle wall observed, and to the loss of contractile activity – as others report [15] – by secreting cytokines and by activating the myocytes and cells in the myenteric plexus to produce cytokines, which may exacerbate the process. B lymphocytes and NK cells did not appear to be involved in the development of chronic rejection: There was no significant difference in the numbers of OX-33$^+$ B cells and 3.2.3$^+$ NK cells in the different groups. This is in contrast to the findings by Bauer and co-workers who observed a complete loss of B cells in the mucosa [9] and an increase of NK cells [28] of chronically rejecting small bowel grafts.

Histological changes in the graft were accompanied by loss of graft function. Although we found no statistically significant differences in growth between iso- and allografts, serum albumin was significantly lower in the group treated with short-term CsA. Serum albumin is an important nutritional parameter and lowered serum levels indicate that the small bowel graft was unable to digest normally and/or absorb nutrients. The lactulose-mannitol test is a widely accepted method to test intestinal permeability [29]. Lactulose is normally not absorbed by the intestinal mucosa but under pathologic conditions, it transfers paracellularly and is subsequently secreted in the urine. Mannitol is normally absorbed transcellularly and excreted in the urine. Under pathologic conditions its absorption is impaired, and its excretion diminished. The urinary excretion ratio therefore is a sensitive measure for mucosal integrity. In our model, lactulose-mannitol ratio was significantly increased in sCsA-treated allografts, which indicates a compromised epithelial integrity with transcellular leakage. In addition, we hypothesise that the loss of goblet cells, which produce the constituents of the overlying mucus layer, results in loss of this layer. Although the role of mucus is not fully understood, there is increasing evidence that both glycoproteins and trefoil peptides present in mucus play a role in mucosal repair after injury [5]. The loss of integrity of the epithelium, together with the loss of goblet cells may contribute to the progression of mucosal atrophy by further impairing its capability to respond to injury.

Although it is known that CsA has a profound effect on small bowel function even in normal animals [26], the changes we found seem not attributable to CsA toxicity, since function and morphology of isografts treated with CsA was normal.

Long-term CsA treatment delayed the development of CR somewhat. At 50 days posttransplant, 67% of the lCsA group had very mild CR and none had moderate CR. In the sCsA group this was reversed. However, 100 days posttransplant (ie, 50 days without CsA in the lCsA group and 93 days in the sCsA group) this difference had disappeared. Nonetheless, the mucosal integrity in the sCsA allografts, as defined by lactulose/mannitol excretion, further deteriorated by day 100, compared to the allografts treated with long-term CsA.

These findings show we have developed two rat models in which CR develops in a reproducible manner. Histological and inflammatory changes are accompanied by loss of function only in short-term CsA treated animals. Clinical signs of CR were not present at the time points studied, representing subclinical CR. Long-term CsA treatment delays the development of CR, but is unable to prevent it. Further studies using this model are currently done to determine the role of growth factors in the pathophysiology of CR following intestinal transplantation.

Acknowledgements R.W.F.de Bruin was supported by a grant from the Ter Meulen Fund, Royal Netherlands Academy of Arts and Sciences.

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