Transpl Int (1997) 10: 45–50

ORIGINAL ARTICLE

Göran Kurlberg
Nils Lycke
Eva Haglind

Migration of host and donor T cells in small bowel transplantation

Received: 28 December 1995
Received after revision: 5 June 1996
Accepted: 11 July 1996

G. Kurlberg (✉) · E. Haglind
Department of Surgery,
Sahlgrenska University Hospital,
S-413 45 Göteborg, Sweden
Fax: + 46 31 417 364

N. Lycke
Department of Medical Microbiology
and Immunology,
University of Göteborg,
S-413 45 Göteborg, Sweden

Abstract In this study migration of host and donor $CD4^+$ and $CD8^+$ T cells in a fully allogeneic model was described and compared with the migration pattern in a graft-versus-host reaction (GVHR) model, where the T-cell traffic in the graft served as a physiological control. Heterotopic small bowel transplantations were performed in a rat model, with animals being sacrificed on postoperative days (POD) 2, 3, 4, 5, and 7. Graft and host mesenteric lymph nodes were harvested, homogenized, and stained with monoclonal antibodies against MHC class I, $CD4^+$, and $CD8^+$ antigens. The host and donor T cell migration patterns were studied using a double-staining flow cytometric technique. We found that during the development of rejection, the normal physiological circulation of graft and host T cells was disrupted. In the graft of the allogeneic model, a shift from host cell to graft cell dominance occurred on POD 3–4. This change in migration pattern coincided in the host with a 6 % peak in graft cell infiltration, which disappeared on POD 7. These patterns of T-cell migration may be further explored for diagnostic purposes.

Key words Small bowel transplantation, host T cells, rat · Host T cells, small bowel transplantation, rat · T-cell migration, small bowel transplantation

Introduction

The small intestinal transplant contains a large and fully competent lymphoid compartment, the gut-associated lymphoid tissue (GALT). As in bone marrow transplantation, the transplanted small bowel is both a target for rejection and a platform for graft-versus-host disease. Difficulties in controlling these reactions have severely hampered the development of clinical small bowel transplantation. Although extensively studied, the cellular basis for immune responses in organ transplantation, i.e., rejection, graft-versus-host reaction (GVHR), and graft tolerance, remains only partially understood.

As a rule, in solid organ allografts, most lymphocytes will be of host origin, and their presence is used diagnostically as a sign of rejection. However, this diagnostic method is not applicable to the intestinal allograft since lymphoid cells may be of either host or donor origin. This is another reason why it is important to understand lymphocyte trafficking in intestinal allografts and to determine whether there are distinct patterns of lymphocyte migration that precede graft rejection.

Several experimental studies have been conducted to identify the lymphocyte infiltration patterns in small bowel transplantation [1–7, 11–13, 16, 17]. These studies have focused on elucidating mechanisms leading to rejection and GVHR. Such information may be used for therapeutic as well as diagnostic purposes. Earlier studies have reported heavy infiltration of host cells in the untreated intestinal transplant during the 1st postoperative days prior to rejection [3, 4]. Even under immunosuppressive treatment, graft lymphocytes will eventually be replaced by lymphoid cells of host origin in the

tolerized transplanted organ [6]. Therefore, finding host lymphocytes in the intestinal allograft may not, by itself, preclude survival of the allograft. Studies of cell phenotypes have indicated that the majority of cells in the graft mucosa during rejection are macrophages and that only small numbers of T cells are present [3, 4]. However, in organ transplantation, the T lymphocytes have been held responsible for allograft recognition and for playing a decisive role as mediators of rejection and GVHR and in the development of tolerance. Primarily, the $CD4^+$ T helper cells have been found to react to alloantigen and to develop into effector T cells, inducing cytotoxic T lymphocyte (CTL) and delayed type hypersensitivity (DTH) responses against the transplanted tissue [14].

A shortcoming of earlier studies of cellular migration patterns has been the lack of techniques to simultaneously identify type (e.g., CD 4^+ T cell, B cell) and origin (host or donor) of the cells [4]. Recently, several double-staining techniques have been introduced that allow a more detailed analysis of both phenotype and haplotype of all cells that may be involved in rejection and GVHR in small bowel transplantation [16].

The aim of the present study was to analyze the migration of host and donor T lymphocytes following small bowel transplantation. We compared the "physiological" T-cell traffic in the graft of a semiallogeneic, GVHR model with that of a fully allogeneic transplant model. The patterns of migration of both $CD4^+$ and $CD8^+$ T-cells were recorded and evaluated in relation to graft rejection and GVHR.

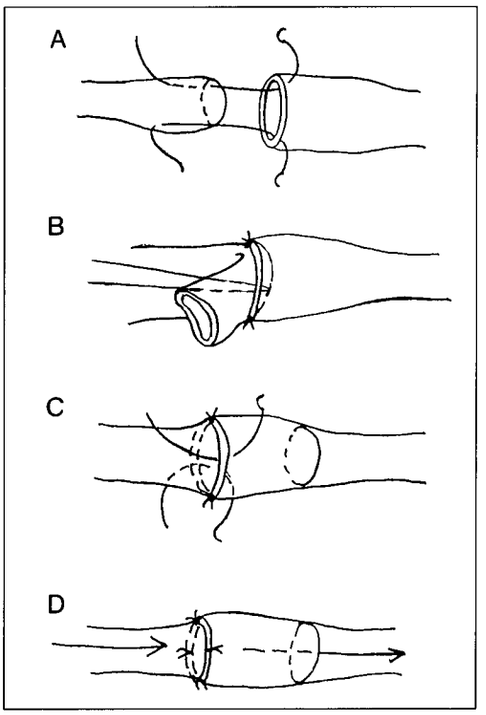

Fig. 1 A–D Construction of the sleeve anastomosis. The receiving vessel has to be of the same size or bigger than the feeding vessel. The two vessels that are to be anastomosed are dissected free, clamped, dilated, and irrigated with heparinized normal saline: **A** Two sutures, at a distance of 180 degrees, are passed the adventia of the feeding vessel and than passed from inside out through the receiving vessel; **B** The sutures are tied and the feeding vessel stump is folded and pointed toward the surgeon; **C** The feeding vessel is then tucked into the receiving vessel; **D** Another two sutures are passed to avoid leakage

Materials and methods

Animals

Inbred PVG and DA rats with MHC class I antigen haplotype $RT1A^c$ and $RT1A^a$, respectively, as well as (PVG × DA) F_1 hybrids, weighing 150–200 g at the time of surgery, were used. The animals were purchased from B&K (Stockholm, Sweden) and conditioned for at least 1 week before the experiments. They were kept under standardized conditions and provided with water and standard pellets ad libitum.

Both national and European guidelines for animal experiments were adhered to. The Local Ethics Committee approved the experiments.

Transplantation model

The animals were anesthetized with 80 mg/ml chloral hydrate (0.4 ml/100 mg body weight) given intraperitoneally (i.p.). Heterotopic, vascularized small intestinal transplantation, common in studies of immune reactions, was carried out [3, 4, 10, 17]. The donor small intestine was harvested on a pedicle of the superior mesenteric artery and portal vein. The vascular bed was immediately flushed with heparinized saline at room temperature, while the intestinal lumen was flushed with normal saline at room temperature. The intestinal graft was kept on iced, normal saline until transplantation (i.e., 30–40 min). The warm ischemia time was of the same magnitude. The left renal vessels of the recipient were dissected free and anastomosed to the donor superior mesenteric artery and portal vein using a microsurgical technique previously described by Lauritzen in 1978 [9] and modified to suit the present small bowel transplantation model (Fig. 1). The oral end of the intestinal graft was closed with a ligature, while the aboral part of the transplant was anastomosed to the native distal ileum in an end-to-side fashion, as described by Preissner et al. [15]. The native ileum remained unresected. The rate of technical failures (postoperative bleeding, aspiration pneumonia) in the series was less than 15 %.

Survival rates and macroscopic appearance

A preliminary study of small bowel transplantations was performed to establish that the combinations of rat strains chosen were fully incompatible. The mortality rates after small bowel transplantation in syngeneic (DA-to-DA) and allogeneic (PVG-to-DA) models were determined. We also studied the survival rates in a group of allografted animals given cyclosporin (Sandimmun; Sandoz, Basel, Switzerland), 15 mg/kg body weight per day,

Table 1 Clinical outcome of allogeneic and syngeneic small bowel transplantations *(SBT)* in the pilot study of the rat model. The GVHR diagnosis was determined by macroscopic examination of the animals (skin rash on palms, soles, and/or ears, sometimes erythroderma, general malaise) and simultaneous lack of signs of rejection. Acute rejection was diagnosed by inspection of the transplanted intestines (signs of acute inflammation). (*Group,* mode of transplantation, *CyA,* cyclosporine A, *n,* number of transplanted animals)

Group	CyA treatment	*n*	Survival (days)		
			Died of rejection		GVHR
PVG × DA SBT	None	3		4, 6, 8[a]	
PVG × DA SBT	15 mg/kg b.w.	8	> 40	6[a], 6[a], 21[a], 35[a]	7, 7, 8
DA × DA SBT	None	4	> 40 × 4		

[a] Animals sacrificed because of diseased state

dissolved in 1 ml Intralipid (Pharmacia, Uppsala, Sweden) by oral gavage for 30 days. The diagnosis of rejection and GVHR was based on clinical grounds and macroscopic appearance on autopsy. Distention of the graft, thickening and redness of the intestinal wall, as well as swelling and even necrosis of mesenteric lymph nodes were regarded as signs of acute rejection. GVHR was characterized by the presence of general malaise and redness of ears, nose, and paws [8]. All rats that died on or before the 3rd postoperative day (POD) were regarded as technical failures and excluded from the study.

Experimental groups

Fully allogeneic transplantations were performed using PVG rats as donors and DA rats as recipients. The animals were sacrificed on POD 2 ($n = 4$), 3 ($n = 2$), 4 ($n = 2$), 5 ($n = 2$), and 7 ($n = 3$).

A semiallogeneic, GVHR model was established by using PVG rats as donors and (PVG × DA) F1 hybrids as recipients. In this transplantation model, host T cells will not recognize the graft as non-self and, therefore, no rejection will occur. Thus, the host cell infiltration of the graft will not be due to allorecognition and an incompatible MHC phenotype but rather to the normal physiological circulation of lymphocytes following transplantation [1]. The semiallogeneic model was regarded as a "physiological", or nonrejectional, control to the model of full rejection. These rats were sacrificed on POD 2 ($n = 3$) and 7 ($n = 3$).

Analysis of haplotype, phenotype, and distribution of $CD4^+$ and $CD8^+$ T cells

Host and graft small bowel were excised. Specimens of graft and host mesenteric lymph nodes (MLN) were collected and single cell suspensions of lymphocytes were prepared. Phenotypic analysis using a fluorescence-activated cell sorter (FACS; Becton Dickinson, Martin View, Calif., USA) was performed. Mesenteric node lymphocytes were labeled with monoclonal antibodies directed against the MHC class I antigens $RT1A^a$ and $RT1A^c$, respectively, as well as with antibodies against the $CD4^+$ and $CD8^+$ T cell markers (Serotec). Live gates were set on lymphocytes by forward and side scatter. The representation of graft and host-derived $CD4^+$ and $CD8^+$ T cells was presented on dot plots.

The percentage of $CD4^+$ and $CD8^+$ T cells of graft or host origin out of the total number of $CD4^+$ or $CD8^+$ cells was calculated from the frequencies determined by dot plot analysis of donor and recipient MLN. In the allogeneic model, the analysis was straightforward, but in the GVHR model, the following calculation had to be performed. The host (PVG X DA) $CD4^+$ and $CD8^+$ T-cells were recognized by the $RT1A^a$ antigen. The percentage of graft cells (i.e., PVG, MHC class I antigen $RT1A^c$) was calculated using the formula 100 – the percentage of host ($RT1A^a$) $CD4^+$ or $CD8^+$ T cells. The means of the calculations were plotted on line graphs to visualize the different migration patterns in the fully allogeneic and GVHR models.

The ratio of graft T cells to host T cells (both $CD4^+$ and $CD8^+$ cells) in the graft MLN on POD 2 and 7 was used to statistically analyze the differences in host cell infiltration between the rejectional ($n = 4$) and the physiological ($n = 3$) models.

The ratio of $CD4^+$ to $CD8^+$ T cells was determined by the formula percentage of $CD4^+$/percentage of $CD8^+$ cells. The ratios for the rejectional, graft ($n = 4$), and host ($n = 4$) T cells, physiological, graft ($n = 3$), and host ($n = 3$) T cells, and the graft-versus-host reactional, graft ($n = 3$), and host ($n = 2$) T cell models were compared for the specimens from POD 2 and 7.

Nomenclature

In this study, the graft of the allogeneic model was regarded as the rejectional model. The graft of the GVHR transplant combination was regarded as the nonrejectional or physiological model, while the host was regarded as the GVHR model.

Statistical analysis

The T-cell migration was defined as the percentage of graft or host $CD4^+$ and $CD8^+$ T cells out of the total number of $CD4^+$ or $CD8^+$ T cells and is given as mean ± SEM. The ratio of graft to host T cells was analyzed using the Wilcoxon signed rank test. An analysis of variance (Anova, Statview) was applied to the ratio of $CD4^+$ to $CD8^+$ T cells. In all analyses, a P level below 0.05 was regarded as a statistically significant difference.

Results

Survival rates in the pilot study

All animals in the syngeneic group, none in the group without cyclosporin therapy, and only one of eight allografted and cyclosporin-treated animals survived (Table 1), confirming the high incompatibility of the donor/recipient combination.

Macroscopic appearance of small bowel transplants in the experimental groups

On macroscopic observation, the allografted MLN showed signs of edema starting on POD 2. The acute inflammation progressed to necrosis of the nodes on POD 7. In contrast, the MLN of the host tissues and of the GVHR graft did not show any macroscopic signs of inflammation.

T-lymphocyte distribution in graft MLN

In the MLN of the rejectional model, host cells constituted 70 % of the T-cell population during the first 3 POD. Between POD 3 and 4, we observed a strong shift in the proportion of host and donor T cells, with a 90 % graft cell predominance in the MLN. This redistribution between host and graft T cells lasted throughout the study period (Fig. 2). The shift to graft T-cell dominance occurred at the time when signs of rejection became macroscopically evident. In the non-rejectional model, 90 % of the $CD4^+$ and 70 %–80 % of the $CD8^+$ cells on POD 2 as well as on POD 7 were of host origin (Fig. 3). Thus, in the non-rejectional situation, we did not observe any alteration in the graft/host T-cell ratio of the graft between POD 2 and 7.

The differences in T-cell migration between the rejectional and physiological models illustrated in Figs. 2 and 3 were confirmed in the statistical analysis. The mean ratio of graft T cells to host T cells in the rejectional model was 0.430 while the corresponding ratio in the physiological model was 0.399 on POD 2. In contrast, on POD 7 the corresponding numbers were 16.01 and 0.242 ($P < 0.05$).

T-lymphocyte distribution in host MLN

In the host MLN of the allogeneic model, more than 95 % of the T cells were of host origin (Fig. 4). The T cells of donor origin made up 3 % of the cells during the first 2 POD, with a slight increase to 6 % on POD 4. After this time graft lymphocytes decreased to less than 1 % or were undetectable in the host. The peak frequency of graft lymphocytes in host MLN coincided with the shift in the T-cell distribution of the graft MLN and the time when acute rejection became manifest. In the host MLN of the GVHR model, the proportion of graft T cells was 10 %–20 %, with a higher frequency of $CD4^+$ cells on POD 7 (Fig. 5).

Proportion of $CD4^+$ versus $CD8^+$ T cells

Migration of host and donor $CD4^+$ and $CD8^+$ T cells followed the same pattern in the rejectional model. In the GVHR model, there was some variation in the $CD4^+$ and $CD8^+$ T-cell frequencies (Figs. 2–5). However, no statistically significant differences were found between the ratio of CD4 to CD8 T-cell frequencies in the rejectional, physiological or GVHR model on POD 2 and 7. The means of the ratios ranged from 2.0 to 4.8 on POD 2 and from 2.7 to 5.6 on POD 7.

Discussion

Rejection and GVHR are still major obstacles to successful clinical small bowel transplantation [18]. Although the central role of T-cells has been identified in allogeneic reactions, the exact mechanisms leading to graft survival are not completely understood. The present study of $CD4^+$ and $CD8^+$ T-cell migration in graft and host MLN during the 1st postoperative week in a fully allogeneic and a GVHR model attempted to describe the cellular mechanisms behind rejection. We found that during the first 3 POD, the migration pattern of host and donor T cells in the graft MLN, characterized by complete dominance of host cells, was similar in the rejectional and physiological models. In contrast, starting on POD 4, there was a dramatic decline in the proportion of host cells in the rejecting graft that was not seen in the physiological model (Figs. 2, 3). This difference in T-cell migration pattern was shown to be statistically significant.

Our results are at variance with studies by Ingham Clark et al. [4, 7], who found a high proportion of host cells in graft MLN of the allogeneic model throughout the 7 POD period. One possible explanation for the difference in outcome between the two studies may be that whereas we focused on $CD4^+$ and $CD8^+$ T cells, they did not distinguish between T cells and other mononuclear cells of host origin. We used double-staining techniques, detecting the phenotype as well as whether the lymphocytes were of host or graft origin. The present study determined the frequencies of $CD4^+$ and $CD8^+$ T cells and did not establish absolute numbers of infiltrating cells. Hell et al. [3] demonstrated that the total number of T cells in the allograft declined at the end of the 1st postoperative week, while there was an increase in the number of macrophages.

In the present study, the difference in host MLN migration between the allogeneic and the GVHR model was that, in the latter 10 %–20 % of the T cells in host MLN were of graft origin compared to the very few cells – nearly 0 % on POD 7 – in the allogeneic model. There was also an increase in the frequency of graft-derived $CD4^+$ T-cells on POD 7 as compared to POD 2 in the

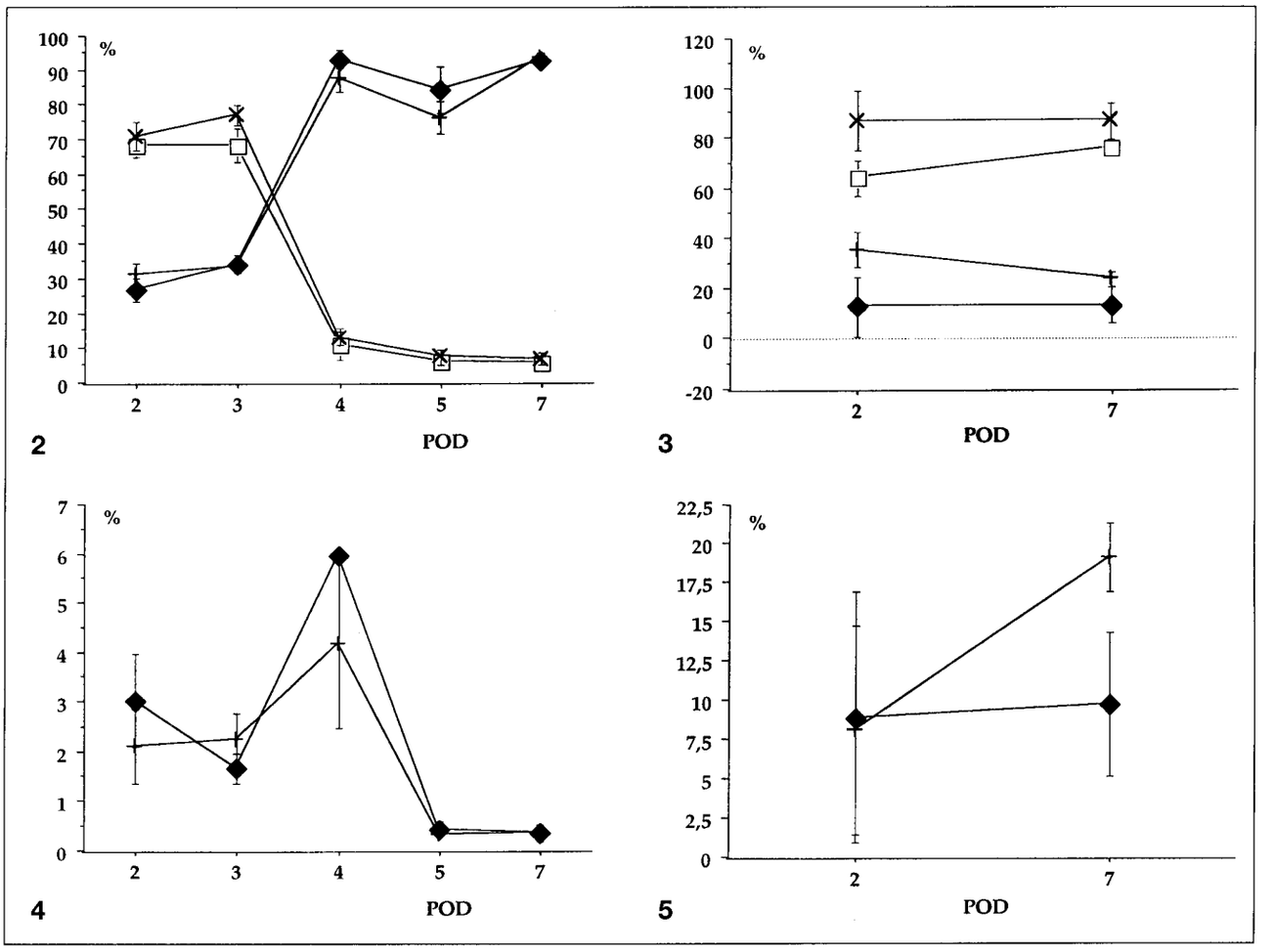

Fig. 2 Pattern of T-cell traffic in graft MLN of the allogeneic model during the 1st postoperative week with percentage of graft or host $CD4^+$ and $CD8^+$ cells out of the total number of $CD4^+$ and $CD8^+$ T cells in the MLN; —|— graft $CD4^+$; —◆— graft $CD8^+$; —□— host $CD4^+$; —×— host $CD8^+$

Fig. 3 Pattern of T-cell traffic in graft MLN of the GVHR model during the 1st postoperative week with percentage of graft or host $CD4^+$ and $CD8^+$ cells out of the total number of $CD4^+$ and $CD8^+$ T cells in the MLN; —|— graft $CD4^+$; —◆— graft $CD8^+$; —□— host $CD4^+$; —×— host $CD8^+$

Fig. 4 Pattern of graft T-cell traffic in host MLN of the allogeneic model during the 1st postoperative week with percentage of graft $CD4^+$ and $CD8^+$ cells out of the total number of $CD4^+$ and $CD8^+$ T-cells in the MLN; —|— graft $CD4^+$; —◆— graft $CD8^+$

Fig. 5 Pattern of graft T-cell traffic in host MLN of the GVHR model during the 1st postoperative week with percentage of graft $CD4^+$ and $CD8^+$ cells out of the total number of $CD4^+$ and $CD8^+$ T-cells in the MLN; —|— graft $CD4^+$; —◆— graft $CD8^+$

GVHR model (Figs. 4, 5). Our data on T-cell migration patterns of host MLN, both in the allogeneic and GVHR models, were more in line with the results of other investigators [11, 13, 16].

The dramatic shift in the graft MLN from host to graft T-cell predominance, as well as the disappearance of graft cells from the host in the allogeneic model of our study, may be used as a diagnostic tool of graft rejection. Fine needle aspiration of lymph nodes and sampling of peripheral blood to look for the migration patterns of graft lymphocytes in the host have been advocated as realistic means of diagnosing rejection. We are currently attempting to develop double-staining techniques for immunohistochemical analysis of cell trafficking into the intestinal mucosa. Via endoscopy, specimens for such an analysis can easily be obtained from the transplanted small bowel, making it possible to establish whether this particular migration of T cells into the transplant can be used as a diagnostic tool of rejection.

In the fully allogeneic model, both $CD4^+$ and $CD8^+$ T cells followed similar migration patterns throughout

the study. The ratio of $CD4^+$ to $CD8^+$ T-cells ranged from 2.0 to 5.6, and no particular pattern was observed when comparing rejectional, physiological, and GVHR models on POD 2 and 7. This may indicate that $CD4^+$ and $CD8^+$ T cells are both involved in the rejectional process. At some variance with this result, Webster et al. [16], using flow cytometric studies of lymphocytes in peripheral blood of allogeneically transplanted rats, observed a decrease in the graft $CD4^+$ to $CD8^+$ T-cell ratio from POD 1 to POD 5.

In conclusion, this study has shown that during the development of rejection, the normal physiological migration of graft and host T cells was disrupted. We found that preceding rejection, a shift from host to graft T-cell dominance in the grafted tissues coincided with a peak in graft T-cell infiltration of the host on POD 4. These patterns of T-cell migration may be further explored for diagnostic purposes.

Acknowledgements The authors would like to thank Ms. Karin Schön and Ms. Harriet Törnquist for their excellent technical assistance in the laboratory. The study was supported by grants from the Göteborg Medical Society and the Swedish Medical Research Foundation (project no. 10398).

References

1. Grover R, Ingham Clark CL, Pockley AG, Wood RFM (1992) Host cell infiltration of graft tissues in a non-rejecting model of small bowel transplantation. Br J Surg 79: 451
2. Grover R, Lear PA, Ingham CC, Pockley AG, Wood RF (1993) Method for diagnosing rejection in small bowel transplantation. Br J Surg 80: 1024–1026
3. Hell K, Gundlach M, Schmidt P, Hansmann ML, Schroeder P, Deltz E (1990) Immunohistochemal analysis of immunocompetent cells involved in small bowel rejection in the rat. Transplant Proc 22: 2461–2462
4. Ingham Clark CL, Cunningham AJ, Crane PW, Wood RFM, Lear PA (1990) Lymphocyte infiltration patterns in rat small bowel transplants. Transplant Proc 22: 2460
5. Ingham Clark CL, Crane PW, Cunninghan AJ, Wood RFM, Lear PA (1992) An immunosuppressive regimen that reduces graft cell emigration following small bowel transplantation. Transplant Proc 24: 1130
6. Ingham Clark C, Price B, Crane P, Lear P, Wood R (1992) Persistence of allogeneic cells in graft and host tissues after small bowel transplantation. Br J Surg 79: 424–426
7. Ingham Clark CL, Grover R, Pockley AG, Wood RFM (1993) Delayed-start cyclosporine immunosuppression reduces graft cell migration after small bowel transplantation. Transplantation 56: 1284–1286
8. Johnsson C (1994) Graft-versus-host-reaction and rejection after experimental small-bowel transplantation. (Dissertation), Uppsala University
9. Lauritzen C (1978) A new and easier way to anastomose microvessels. Scand J Plast Reconstr Surg 12: 291–294
10. Lear PA (1990) Experimental models in small-bowel transplantation. Transplant Proc 22: 2441–2442
11. Lear PA, Cunningham AJ, Crane PW, Wood RFM (1989) Lymphocyte migration patterns in small bowel transplants. Transplant Proc 21: 2881–2882
12. Lear PA, Cunningham AJ, Ingham Clark CL, Crane PW, Wood RFM (1990) What role for passenger leucocytes in small bowel allografts? Transplant Proc 22: 2463
13. Lear PA, Ingham Clark CI, Crane P, Pockley G, Wood RFM (1993) Donor cell infiltration of recipient tissue as an indicator of small bowel allograft rejection in the rat. Transpl Int 6: 85–88
14. Nickerson P, Steurer W, Steiger J, Zheng Xi, Steele AW, Strom TB (1994) Cytokines and the TH1/TH2 paradigm in transplantation. Curr Opin Immunol 6: 757–764
15. Preissner WCH, Schroeder P, Gundlach M (1986) Small bowel transplantation. Experimental and clinical fundamentals. In: Deltz E, Thiede A, Hammelman HK (eds) Springer, Berlin, Heidelberg, New York, 2–6
16. Webster GA, Bowles MJ, Karim MS, Wood RFM, Pockley AG (1994) Characterisation of blood lymphocyte subsets following rat small bowel transplantation. Transplant Proc 26: 1554
17. Webster GA, Bowles MJ, Karim MS, Wood RFM, Pockley AG (1994) Light scatter characteristics of peripheral blood lymphocyte subsets: a means of monitoring rat small bowel transplant recipients. Transplant Proc 26: 1555
18. Wood RFM (1992) Small bowel transplantation. Br J Surg 79: 193–194