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Phenotypic and functional characteristics of intestinal intraepithelial lymphocytes during acute rejection of small intestinal allografts

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Abstract Infiltration of a transplanted organ by host lymphoid cells is the hallmark of acute rejection. However, after intestinal transplantation, physiological lymphocyte migration may lead to host cell infiltration of the graft even in the absence of rejection. It is unclear whether this lymphocyte migration also involves the intraepithelial compartment of the graft or whether infiltration there is indicative of acute rejection. We demonstrate here that host cell infiltration of the intestinal mucosa occurs both during acute rejection of a small bowel allograft and, to a lesser extent, when rejection is prevented by immunosuppression with FK506. The infiltrating host cells consisted of $CD3^+$ T cells with a predominant $CD4^-CD8^+$ phenotype resembling intraepithelial lymphocytes (IELs). Functional studies showed that the nonspecific cytolytic activity of IELs was not affected by acute rejection or by immunosuppression with FK506. These findings indicate that host cell infiltration of the intestinal mucosa does not connote an ongoing acute rejection. Furthermore, the decreased mucosal barrier function during acute rejection of intestinal allgrafts is probably not due to impaired cytolytic activity of IELs.

Key words Intestinal transplantation, acute rejection, intraepithelial lymphocytes · Acute rejection, intraepithelial lymphocytes, intestinal transplantation

Introduction

The hallmark of acute rejection after whole organ transplantation is the infiltration of the graft with recipient lymphocytes [4, 8]. However, after small bowel transplantation, the demonstration of host lymphocellular infiltration of the graft is only of limited diagnostic value since it does not necessarily indicate an ongoing acute rejection, but may be the result of physiological lymphocyte trafficking into the intestine [23]. This physiological lymphocyte migration leads to repopulation of the lamina propria, Peyer's patches, and regional lymph nodes with recipient lymphoid cells, even in the absence of acute graft rejection [23].

In contrast, the intestinal epithelium does not participate in this physiological lymphocyte migration between secondary lymphoid tissues [27]. Based on this observation, it has been suggested that the intestinal epithelium may represent a segregated and separate lymphoid compartment that is of limited access for mature peripheral lymphocytes [26, 27]. This hypothesis is supported by the finding that the lymphocyte population in the intestinal mucosa differs in its phenotype, function, and ontogeny from other lymphocyte populations including the intestinal lymphoid populations of the lamina propria and Peyer's patches [10, 26, 27].

Intraepithelial lymphocytes (IELs), which are located between the intestinal epithelial cells, consist entirely of T cells with a predominant $CD4^-CD8^+$ phenotype, whereas the majority of other peripheral T lymphocyte populations are $CD4^+CD8^-$ [10, 20]. While peripheral

T lymphocytes express almost exclusively $\alpha\beta$TCR, human and rat IELs consist of up to 30% $\gamma\delta$TCR$^+$ cells [2, 3, 26], and in mice, $\gamma\delta$TCR$^+$ cells may even account for more than 50% of IELs [5, 20, 29].

A characteristic function of IELs is their nonspecific cytolytic activity, which is believed to contribute to the mucosal barrier of the small intestine [15, 29]. This nonspecific cytolytic activity of isolated IELs can rapidly be elicited by ligation of the CD3/TCR complex [6, 21]. In contrast to their cytolytic activity, isolated IELs show only weak proliferative capabilities in response to TCR-driven signals, which are very effective proliferative stimuli in other peripheral lymphocyte populations [13, 31]. Due to these functional characteristics and their sentinel location, IELs have been hypothesized to represent a "first line of defense" in the intestinal epithelium, participating in the barrier function of the intestinal mucosa [1, 32].

Little, however is known about the role of IELs during acute rejection after allogeneic small bowel transplantation. Therefore, the present study was undertaken to investigate phenotypic and functional characteristics of IELs during acute rejection of intestinal allografts and during immunosuppressive treatment with FK506.

Materials and methods

Animals

Male ACI (RT-1^a) and Lewis (RT-1^l) rats weighing 250–300 g were purchased from Harlan Sprague Dawley (Indianapolis, Ind.) and were housed under specific pathogen-free conditions in the University of Pittsburgh animal care facilities, which are fully approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Small bowel transplantation

One-step orthotopic small bowel transplantations were performed in allogeneic (ACI → Lew) and syngeneic (ACI → ACI) combinations. Anesthesia consisted of methoxyfluorane inhalation (Pittman Moore, Mundelein, Ill.) and 25 mg/kg IP sodium pentobarbital (Abbott Lab., Chicago, Ill.).

All transplantations were performed following previously described techniques [16]. Briefly, after intravascular and intraluminal irrigation, the small bowel of the donor was isolated from the ligament of Treitz to the ileoceacal valve. Then, the graft was harvested with a vascular pedicle consisting of the superior mesenteric artery (SMA) with an aortic patch and the portal vein. The portal vein and SMA of the graft were anastomosed end-to-side to the recipient's inferior vena cava and infrarenal aorta, and the graft was placed in functional continuity by end-to-end intestinal anastomoses after the recipient's native small intestine had been removed.

Postoperative immunosuppression consisted of 2 mg/kg per day FK506, a dose that has been shown to prevent acute rejection in the fully allogeneic ACI-into-Lewis model [12, 18].

Histology

Tissue samples for histological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and evaluated using conventional light microscopy after hematoxylin and eosin staining.

Media

Lymphocytes were cultured in RPMI 1640 medium (Bio-Whittaker, Walkersville, Mass.) supplemented with 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.05 mM 2-mercaptoethanol, and 5% heat-inactivated fetal calf serum (FCS), unless otherwise indicated.

Isolation of IELs and preparation of lymph node blasts

IELs were isolated as described previously [29] with slight modifications. Briefly, the entire transplanted or native small bowel from one rat was obtained and Peyer's patches were excised from the intestinal wall. The intestine was flushed free of luminal contents, opened longitudinally, and cut into 1-cm pieces. Tissue pieces were washed three times with RPMI 1640 containing 2% FCS and stirred for 20 min at 37°C in Ca^{++}- and Mg^{++}-free phosphate-buffered saline (1 mM dithioerythriotol, 10% FCS). Tissue pieces were allowed to settle, the supernatant containing the cells was collected, and the stirring process was repeated with fresh medium. Cells were pooled, washed, and resuspended in RPMI 1640 without FCS. In order to remove cell debris and mucus, cells were passed over a loosely packed nylon wool column without incubating the cells on the column. Lymphocytes were further separated from epithelial elements by discontinuous density gradient centrifugation (44%/67% Percoll, Pharmacia, Uppsala, Sweden). After centrifugation for 30 min at 600 g at room temperature, IELs were collected from the interface and washed three times in RPMI 1640. Viability, determined by trypan blue exclusion, always exceeded 90%.

PHA lymph node blasts were prepared as follows: axillary and cervical lymph nodes were obtained from one ACI or Lewis rat and single-cell suspensions were prepared in MEM (100 U/ml penicillin, 0.1 mg/ml streptomycin). Lymph node cells were cultured in RPMI with 10% syngeneic rat serum and 2 μg/ml PHA in six-well, flat-bottom plates at a density of 2×10^6 cells/ml and were used as target cells in the cytotoxicity assay on day 2 of culture.

Phenotype analysis of IELs and splenocytes

Fluorescein isothiocyanate (FITC) mouse anti-rat CD3 (clone G4.18), FITC mouse anti-rat CD4 (OX-35), FITC mouse anti-rat CD8α (OX-8), R-Phycoerythrin (PE) mouse anti-rat $\alpha\beta$TCR (R73), and Cy-chrome-labeled streptavidin were purchased from Pharmingen (San Diego, Calif.). Unconjugated rat anti-rat RTI^a (211–4D9-1E9) was a kind gift of Dr. H.W. Kunz, Department of Pathology, University of Pittsburgh, biotinylated goat anti-rat IgG (R40015) was obtained from Caltag Laboratories (San Francisco, Calif.), and FITC mouse anti-human Leu-4, PE mouse anti-human Leu-4, and biotin mouse anti-human Leu-4 (SK7) were purchased from Becton Dickinson (San Jose, Calif.).

Staining procedures were performed at 4°C. IELs were resuspended in FACS medium (HBSS, 0.1% BSA, 0.1% sodium azide) at a concentration of 1×10^6 cells/ml, washed, and incubated for

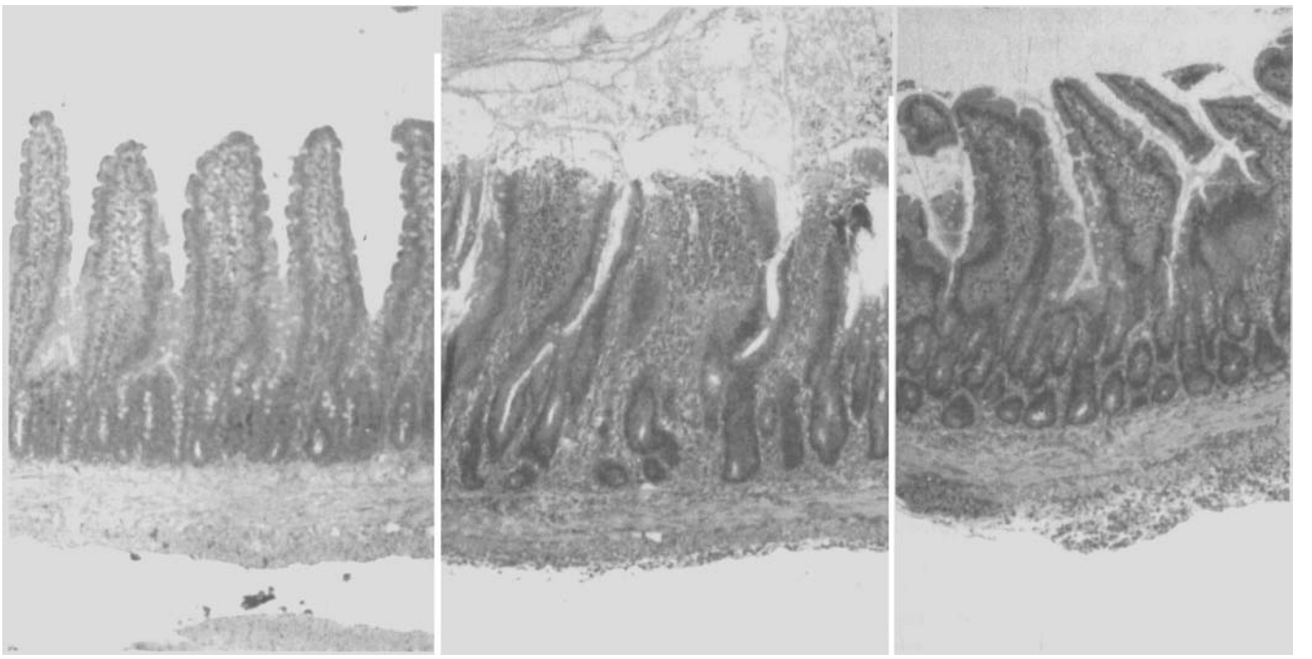

Fig. 1 Histologic appearance of orthotopic small bowel allografts on day 6 after transplantation. *Left*: Syngeneic transplant demonstrating normal intestinal architecture. *Center*: Acute rejection of a small bowel allograft showing severe lymphocytic infiltration, goblet cell loss, capillary thrombosis, and destruction of the intestinal architecture. *Right*: Small bowel allograft of an animal receiving immunosuppressive treatment with FK506 without signs of acute rejection and intact mucosal architecture (H&E, × 100)

Table 1 Percentage of infiltrating host lymphocytes in the small intestinal epithelium after allogeneic small bowel transplantation. Values are expressed as mean ± SEM of five different rats for each group

	Acute rejection	FK506 Treatment
% Host cells[a]	32.0 ± 2.5	14.6 ± 2.6*

[a] Percentage of RT-1[a] negative cells among the lymphocytes isolated from the intestinal epithelium
* $P < 0.05$ vs untreated animals

30 min with unconjugated antibody. The cells were washed twice in FACS medium and incubated with the secondary biotinylated antibody for 30 min. After two washes in FACS medium, the cells were labeled with monoclonal antibodies coupled to FITC and PE for 30 min. Cells were washed twice in FACS medium and reacted with Cy-chrome-labeled streptavidin for 10 min, followed by two washes in FACS medium. Finally, cells were fixed in 1 % paraformaldehyde solution and analyzed on a FACScan (Becton Dickinson, Mountain View, Calif.). A total of 25000 events were counted for three-color flow cytometry after dead cells and epithelial cells had been excluded by gating on forward and side light scatter. Data were analyzed using LYSIS II software (Becton Dickinson, Mountain View, Calif.).

Cytotoxicity assay

Freshly isolated IELs from allogeneically or syngeneically transplanted animals were assayed for nonspecific cytolytic activity in a lectin-mediated ^{51}Cr release assay against the murine mastocytoma cell line P815 in the presence of 0.5 μg/ml PHA. Specific target cell lysis by IELs was measured in a standard ^{51}Cr release assay against Lewis or ACI lymph node PHA blasts.

Target cells were labeled with [^{51}Cr]sodium chromate (1 mCi/ml) for 1 h at 37 °C and then washed three times in RPMI 1640. For each experiment, triplicates of 2×10^3 radio-labeled target cells were incubated in 96-well, round-bottom microtiter plates with varying numbers of effector cells in the presence or absence of PHA for 4 h (specific lysis) or 14 h (nonspecific lysis) at 37 °C. Cells were pelleted by centrifugation (10 min at 650 *g*) and supernatants were assayed for radioactivity. Percent lysis was calculated as: % lysis = [sample release cpm – spontaneous release cpm] / [total release cpm – spontaneous release cpm] × 100 %. Spontaneous release from P815 or PHA blasts was less than 10 %.

Results

FK506 treatment prevents acute rejection of small bowel allografts

Allogeneic small bowel transplantation in the highly immunogenic strain combination ACI into Lewis consistently leads to acute rejection of the transplanted organ within the 1st week after transplantation [34]. Using this strain combination, histologic evaluation of intestinal grafts on day 6 after transplantation revealed typical signs of acute rejection, such as villus sloughing, extensive mononuclear infiltration, crypt cell necrosis, goblet cell loss, and capillary thrombosis (Fig. 1). In contrast,

Table 2 Lymphocyte subset distribution of infiltrating host cells in mucosa of small intestinal allografts. Values are expressed as mean ± SEM of five different experiments for each group

	% Host cells[a]		
	Control[b]	Acute rejection	FK506 Treatment
$CD3^+$	75.6 ± 1.2	86.5 ± 2.9	84.3 ± 4.1
$CD4^+$	12.8 ± 1.0	15.6 ± 2.2	13.2 ± 0.9
$CD8^+$	69.4 ± 2.9	78.0 ± 5.1	76.5 ± 5.3
$\alpha\beta$ TCR^+	67.7 ± 2.9	70.5 ± 3.9	71.2 ± 1.7

[a] Percentage of RT-1^a negative cells expressing the particular lymphocyte marker
[b] IELs isolated from rats that received a syngeneic transplant. There was no difference in the subset distribution of IELs isolated from control animals that did or did not receive FK506 treatment

Table 3 Lymphocyte subset distribution of donor lymphocytes in the mucosa of small bowel allografts. Values are expressed as mean ± SEM of four different experiments for each group

	% Donor cells[a]		
	Control[b]	Acute rejection	FK506 Treatment
$CD3^+$	75.6 ± 1.2	75.2 ± 4.8	70.9 ± 3.3
$CD4^+$	12.8 ± 1.0	12.9 ± 3.7	9.8 ± 0.8
$CD8^+$	69.4 ± 2.9	70.7 ± 3.5	68.5 ± 2.2
$\alpha\beta$ TCR^+	67.7 ± 2.9	64.9 ± 1.0	59.6 ± 3.6

[a] Percentage of RT-1^a positive cells expressing the particular lymphocyte marker
[b] IELs isolated from rats that received a syngeneic transplant. There was no difference in the subset distribution of IELs isolated from control animals that did or did not receive FK506 treatment

none of the allografts of animals that received immunosuppressive treatment with FK506 showed signs of acute rejection by postoperative day 6, and the intestinal architecture of these grafts remained intact (Fig. 1). This is in accordance with previous reports documenting that acute rejection of small bowel grafts can be prevented by immunosuppressive treatment with FK506 [12, 18].

Small intestinal epithelium is infiltrated by host lymphocytes after allogeneic bowel transplantation

In order to analyze the extent of host cell infiltration of the small intestinal epithelium after allogeneic small bowel transplantation, cytofluorometric analysis of isolated IELs was performed on day 6 after transplantation and expression of donor MHC class I (RT-I^a) was determined. Cells expressing RT-I^a were considered to be of donor origin, whereas cells negative for RT-I^a were considered to be of host origin (Table 1).

The lymphocyte population isolated from the allograft mucosa of rats undergoing acute rejection contained more than 30% lymphocytes of host origin (Table 1). In contrast, IELs isolated from allografts of rats that received immunosuppressive treatment with FK506 contained less than 15% host-derived lymphocytes on day 6 after transplantation. Treatment with FK506 after allogeneic small bowel transplantation thereby reduced but did not prevent, host cell infiltration of the intraepithelial compartment of the graft.

Infiltrating host lymphocytes display a phenotype similar to IELs

The next experiments were performed to analyze the phenotype of the infiltrating cells in the small intestinal epithelium after allogeneic small bowel transplantation (Table 2).

FACS analysis of isolated IELs revealed that, in both FK506-treated and untreated animals, the infiltrating host-derived lymphocytes consisted mainly of $CD3^+$ T cells with a predominant $CD4^-CD8^+$ phenotype (Table 2). Interestingly, in both FK506-treated and untreated animals, up to 15% of the infiltrating host T cells in the intestinal epithelium lacked the $\alpha\beta$TCR, thus resembling the TCR expression of IELs from control animals. However, in peripheral lymphocyte populations from which the infiltrating host cells most likely emerged, $CD3^+\alpha\beta TCR^-$, which are presumably $CD3^+\gamma\delta TCR^+$ cells, accounted for less than 5% of the T cells. The infiltrating host lymphocytes in the allograft epithelium thereby displayed a subset distribution similar to the subset distribution of normal IELs but different from the subset distribution of other peripheral T cells. These phenotypic characteristics of the infiltrating host lymphocytes were detectable both in allografts undergoing acute rejection and in allografts where rejection was controlled with FK506, indicating that treatment with FK506 reduces the infiltration of the epithelium of small bowel allografts (Table 1) but has no influence on the phenotype of the host cells infiltrating the intestinal mucosa.

Phenotype of donor IELs remains unchanged during acute rejection

Using FACS analysis of lymphocytes isolated from the allograft mucosa, the phenotype of the donor-derived IELs was also determined (Table 3). The majority of IELs isolated from both FK506-treated and untreated rats on day 6 after allogeneic small bowel transplantation were $CD3^+$ T cells with a predominant $CD4^-CD8^+$ phenotype, similar to IELs isolated from control rats (Table 3). These results show that there was no detectable preferential deletion or expansion of one particular subpopulation of the donor IELs, regardless of whether the host was undergoing acute rejection or received immunosuppressive treatment with FK506 after small bowel transplantation.

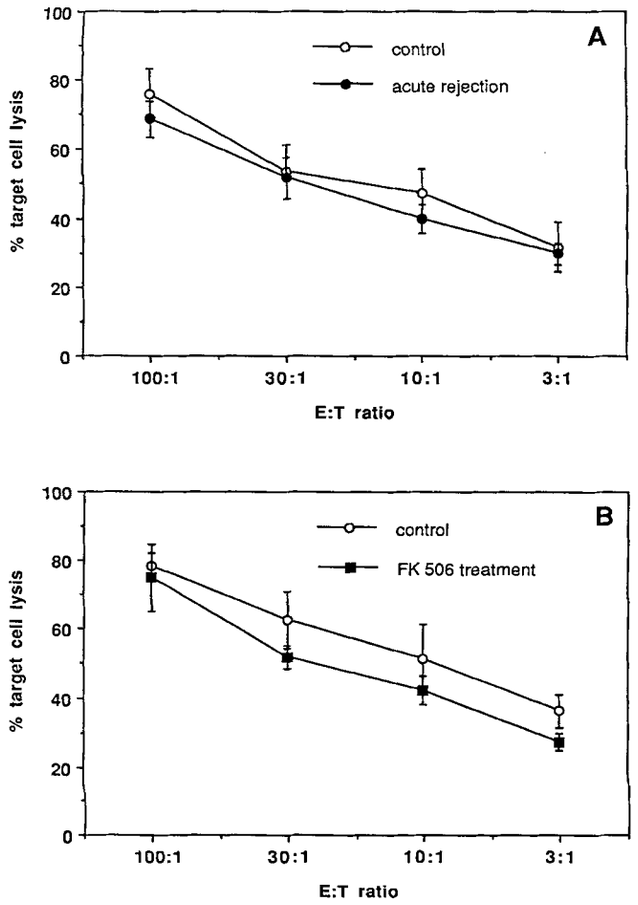

Fig. 2A, B Nonspecific cytolytic activity of isolated IELs after allogeneic small bowel transplantation. Lymphocytes were isolated on day 6 after transplantation from the allograft mucosa of animals undergoing acute rejection (-●-), from FK506-treated animals (-■-), or from syngeneic transplants (-○-). The nonspecific cytolytic activity of IELs against the murine tumor cell line P815 was tested in the presence of PHA (0.2 μg/ml) in a redirected lysis assay. Results are depicted as % target cell lysis at various effector:target ratios (mean ± SEM of three separate experiments for each group). Treatment with FK506 had no effect on the cytolytic activity of IELs isolated from control animals (data not shown)

Nonspecific cytolytic activity of IELs remains unchanged during acute rejection and immunosuppressive treatment with FK506

One important effector function of IELs is their nonspecific cytolytic activity, which can be demonstrated in vitro using a redirected lysis assay [15, 29]. Accordingly, IELs isolated from untreated control animals displayed nonspecific target cell lysis in a redirected lysis assay (Fig. 2A,B). IELs isolated from intestinal allografts undergoing acute rejection showed no significant changes in their nonspecific cytolytic activity when compared to IELs isolated from control rats (Fig. 2A). Immunosuppressive treatment with FK506 after allogeneic small bowel transplantation also had no effect on the nonspecific cytolytic activity of isolated IELs in vitro (Fig. 2B). These data show that there was no significant difference between the cytolytic activity of control IELs and IELs isolated from animals undergoing acute rejection or animals receiving immunosuppressive treatment with FK506.

IELs display specific antidonor cytolytic activity during acute rejection

Specific antidonor cytolytic activity of infiltrating host cells is believed to contribute to the tissue destruction of a transplanted organ during acute rejection [30]. Therefore, we determined the specific anti-donor (anti-ACI) cytolytic activity of isolated IELs after allogeneic small bowel transplantation (Fig. 3A). IELs isolated from allografts undergoing acute rejection displayed specific antidonor activity in a standard ^{51}Cr release assay, while IELs isolated from control ACI rats did not show any specific cytolytic activity against ACI target cells (Fig. 3A). Immunosuppressive treatment with FK506 significantly reduced the specific antidonor activity of IELs when compared to the antidonor activity of IELs isolated from grafts undergoing acute rejection (Fig. 3A). However, it is important to point out that the specific lysis of donor-derived targets in these experiments reflects only the cytolytic activity of the infiltrating host cells. Therefore, the diminished cytolytic activity of isolated IELs from FK506-treated animals does not necessarily reflect a direct immunosuppressive effect of FK506 on the function of IELs but may just as well have been due to reduced host cell infiltration of the intestinal mucosa of these animals (Table 1).

Isolated IELs display graft-versus-host activity during acute rejection

Depending on the strain combination used, the clinical course after small bowel allotransplantation may vary considerably [33, 34]. Using ACI as donor strain and Lewis as recipient, the animals typically develop severe acute rejection within the 1st week after transplantation, without symptoms of graft-versus-host disease [34]. However, IELs isolated from animals with acute rejection after small bowel transplantation displayed not only antidonor cytolytic activity (Fig. 3A), but also specific cytolytic activity against host-derived target cells (Fig. 3B). This specific antihost (anti-LEW) cytolytic activity of isolated IELs was significantly diminished by treatment with FK506 (Fig. 3B).

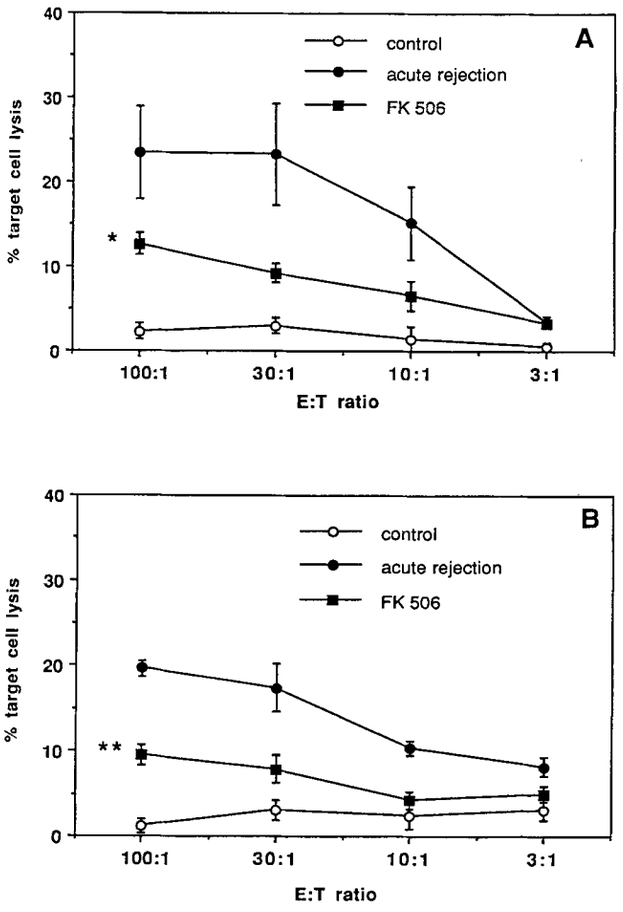

Fig. 3A Specific antidonor and **B** specific antihost cytolytic activity of IELs after allogeneic small bowel transplantation. Lymphocytes were isolated on day 6 after transplantation from the mucosa of small bowel allografts undergoing acute rejection (-●-), from FK506-treated animals (-■-), or from syngeneic transplants (-○-). The specific cytolytic activity against donor or host-derived PHA lymph node blasts was tested in a standard cytotoxicity assay. Results are depicted as % target cell lysis at various effector:target ratios (mean ± SEM of three separate experiments for each group). Treatment with FK506 had no effect on the cytolytic activity of IELs isolated from control animals (data not shown) * $P < 0.05$ vs acute rejection; ** $P < 0.01$ vs acute rejection

Discussion

The early diagnosis of acute rejection of small intestinal allografts remains difficult since the rejection process often has a patchy distribution and does not always involve all segments of the graft at the same time. Furthermore, one of the major signs of acute rejection, the infiltration of the allograft by host lymphocytes, is only of limited diagnostic value after human and rodent intestinal transplantation due to the physiological migration of host lymphoid cells into the gut-associated lymphoid system, even in the absence of acute rejection [8, 9, 23]. While infiltration of the lamina propria and submucosal layers of the intestine has been shown to be an unreliable parameter for the early diagnosis of acute rejection [14], reports concerning the diagnostic relevance of host cell infiltration of the intraepithelial compartment of the transplanted small bowel seem to be more promising. Using a rat model of small bowel transplantion, Grover et al. [8, 9] and Oberhuber et al. [25] reported that infiltration of the intestinal epithelium by host cells occurs only during rejection and could, therefore, be a diagnostic sign of acute rejection. Yet, we have demonstrated here that infiltration of the intestinal mucosa occurs both during acute rejection and, to a lesser extent, when acute rejection is averted by immunosuppressive treatment with FK506. Our observations indicate that, in rats, host cell infiltration of the intestinal epithelium does not necessarily connote an ongoing acute rejection.

The discrepancies between the studies may be explained by the different models used. In the study presented, orthotopic intestinal transplantation was performed in a fully allogeneic strain combination (ACI into LEW), and acute rejection was prevented by immunosuppressive treatment with high doses of FK506. In contrast, Oberhuber et al. [25] employed a model of accessory intestinal transplantation and used a different rat strain combination (LEW into BN). Furthermore, their immunosuppressive protocol consisted of cyclosporin A, a less potent drug for the prevention of acute rejection after intestinal transplantation than FK506 [11, 17, 18, 22]. Grover and colleagues [9] used a semiallogeneic strain combination (parent into F1) to demonstrate the lack of host cell infiltration of the allograft mucosa in the absence of acute rejection. Although acute rejection does not occur in this strain combination, the animals develop graft-versus-host disease which, by itself, may have had an effect on host cell infiltration of the graft. Furthermore, Grover and colleagues [9] evaluated host cell infiltration of the intestinal mucosa of heterotopically transplanted grafts without luminal continuity, whereas in the study presented here, the more physiological model of orthotopic small bowel transplantation was used.

We demonstrate here that the host cells infiltrating the mucosa of the small bowel allograft display a phenotype similar to IELs isolated from control animals. This subset distribution was observed both in animals undergoing acute rejection and in animals receiving immunosuppression with FK506, suggesting that the enrichment of $CD4^-CD8^+$ T cells among the infiltrating cells in the mucosa was not only due to the acute rejection process. The hypothesis that the intestinal mucosa is only of limited access for peripheral lymphocytes [27] may explain this special "organ-specific" phenotype of infiltrating cells. In accordance with this hypothesis, we have previously reported that during acute graft-versus-host dis-

ease in mice, the intestinal epithelium is also infiltrated exclusively by T cells with a predominant $CD4^-CD8^+$ phenotype [29].

The infiltrating host cells in the small intestinal mucosa resembled normal IELs from control animals, not only in their expression of CD4 and CD8, but also in their TCR expression. While peripheral T cells are almost exclusively $\alpha\beta TCR^+$, up to one-third of IELs express $\gamma\delta TCR$ instead of $\alpha\beta TCR$ [3]. Interestingly, a considerable proportion of the infiltrating host $CD3^+$ T cells in the allograft mucosa were also $\alpha\beta TCR^-$. However, it is still uncertain whether these $CD3^+$ $\alpha\beta TCR^-$, presumably $\gamma\delta TCR^+$, [3] host-derived T cells originate from immature bone marrow precursor cells, as in the development of IELs [26, 27], or whether these cells are derived from the small population of $\gamma\delta TCR^+$ peripheral T cells.

Specific antidonor cytotoxic lymphocytes are thought to contribute to the tissue destruction seen in intestinal allografts undergoing acute rejection [30]. Accordingly, the infiltrating host cells in the intestinal epithelium displayed specific antidonor cytolytic activity, which was diminished by treatment with FK506. The diminished antidonor activity of the isolated IELs might have been due to inhibition of the development of donor-specific CTL, an effect of FK506 treatment, as previously reported [24, 28]. However, treatment with FK506 at the same time reduced the host cell infiltration of the intestinal mucosa, thus reducing globally the percentage of cells capable of antidonor cytolytic activity. This reduction in the number of donor-specific CTL in the cell population isolated from the intestinal mucosa of these grafts may, therefore, partially explain the observed decrease in antidonor cytolytic activity in the FK506-treated rats.

The clinical course of animals receiving small intestinal allografts is usually dominated by the process of acute rejection [23, 34]. However, we demonstrate here that IELs isolated from animals undergoing acute rejection also show weak, specific graft-versus-host activity in addition to the specific antidonor activity mentioned above. Tissue destruction due to the graft-versus-host reaction may be hidden by the more severe, pronounced acute rejection process. As observed for the antidonor activity, the graft-versus-host activity was diminished by immunosuppression with FK506, confirming the usefulness of this drug for the treatment of small bowel transplant recipients.

Acute rejection of intestinal allografts is associated with an increased risk of septic complications, probably due to a breakdown of the intestinal mucosal barrier function [7, 19]. Interestingly, the nonspecific cytolytic activity of IELs, which is believed to be part of the intestinal barrier function [1, 32] remained unaltered during acute rejection. This indicates that impairment of the mucosal barrier during acute rejection of intestinal allografts is not due to an impaired cytolytic activity of IELs.

In conclusion, we demonstrate here, in a rodent model of intestinal transplantation, that host cell infiltration of the intestinal mucosa occurs during acute allograft rejection as well as in stable, nonrejected grafts. Despite the considerable differences between the techniques and immunosuppressive protocols of experimental transplantation and clinical transplantation, our data indicate that lymphocytic infiltration of an intestinal allograft may not be a valid diagnostic sign of acute rejection. Furthermore, we have been able to show enhanced, specific antidonor cytolytic activity in the intestinal mucosa during acute rejection of the allograft. Both acute rejection and antidonor activity were diminished by immunosuppressive therapy with FK 506, supporting the hypothesis that antidonor cytotoxic T cells play an important role in the tissue destruction of intestinal allografts during acute rejection.

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