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CD4+ and CD8+ T cell subset distribution in the blood of small-bowel-grafted rats: modification during graft-versus-host disease

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Abstract We studied the modifications of blood T cell distribution following small-bowel allografting in rats under different experimental conditions. Group 1: ACI ($RT1^a$) rats were used as small-bowel donors for ACI × Wistar ($RT1^y$) F_1 hybrid rats (WAF_1) in which graft-versus-host disease (GVHD) developed. Group 2: WAF_1 rats were used as small-bowel donors to ACI rats which developed rejection. Group 3: WAF_1 rats received small bowel from ACI rats hyperimmunized for 10 days (by grafting them with WAF_1 skin) and GVHD developed. Group 4: Wistar rats received small bowel from ACI rats hyperimmunized for 10 days (by Wistar skin) and bidirectional GVHD and rejection were assured. A second set of the same groups which were continuously administered with cyclosporine (15 mg/kg per day s.c. for 15 consecutive days) was also studied. Recipient peripheral blood lymphocytes, obtained at 7 and 15 days following small-bowel transplantation, were stained with monoclonal antibodies anti-rat CD4 and CD8 and then analyzed in an automated flow cytometer. A significant major reduction of $CD4^+/CD8^+$ T cell ratios was shown in rats that developed simultaneous GVHD and rejection with respect to ungrafted rats.

Key words Small bowel transplantation · Rats · Peripheral blood lymphocytes · $CD4^+/CD8^+$ ratios

Introduction

We previously observed that cyclosporine (CsA) treatment (consecutively for 15 days, s.c.) was efficient in delaying graft-versus-host disease (GVHD) and rejection in rats [1]. However, modifications induced by CsA on blood T cell subsets during GVHD and rejection have not been evaluated. Since $CD45^+$ CD4 T cells are considered potent in GVHD [2, 3], we studied the modifications of $CD4^+$ and $CD8^+$ cell content during GVHD or rejection, or simultaneous GVHD and rejection, in the model of small-bowel transplantation in rats. In fact, small bowel allotransplantation in rats induces rejection (like other organ allotransplantations in the same animal combinations) and GVHD owing to the presence of a large amount of lymphoid tissue in the grafted intestine [4, 5]. T cell traffic in graft mesenteric lymph nodes of the allogenic or GVHD model in the postoperative week following rat small-bowel transplantation has been previously studied by analyzing host and donor T cell lymph node migration [6].

Since interest has centered on small-bowel transplantation [7] and in consideration of the development of GVHD following bone marrow transplantation in humans, we studied the T cell variations related to GVH, host-versus-graft (HVG), or both by analyzing the $CD4^+/CD8^+$ ratios in the blood of recipient rats. We also studied the effect of CsA treatment in modifying such ratios, in view of the possible identification of the usefulness of these values for the diagnosis of GVHD or rejection.

Materials and methods

Animals

Adult male ACI ($RT1^a$) rats, Wistar ($RT1^v$) rats and ACI × Wistar F_1 hybrids (WAF_1) weighing about 250 g (donors) and 300 g (recipients) were used. The animals were purchased from Charles River Italia (Como, Italy). They were kept under standardized conditions and provided with water and standard pellets ad libitum. National and European guidelines for animal experiments were adopted.

Experimental groups

We studied the following experimental groups. Group 1: ACI rats were used as small-bowel donors and WAF_1 as recipients, and unidirectional GVHD developed. Group 2: WAF_1 hybrids were used as small-bowel donors and ACI rats as recipients which developed rejection. Group 3: ACI rats, hyperimmunized by grafting them with WAF_1 skin for 10 days, were used as small-bowel donors and WAF_1 as recipients and GVHD alone developed. Group 4: ACI rats, hyperimmunized by grafting them with Wistar skin for 10 days, were used as donors of small bowel and Wistar rats as recipients, and bidirectional GVHD and rejection were assured. A second set of the same groups of rats was used to study the effect of CsA treatment. CsA (a gift from Sandoz, Basel, Switzerland) was administered at a dose of 15 mg/kg per day s.c. for 15 consecutive days. It was mixed with Tween 80 and 96% ethanol in physiological saline to achieve the final concentration of 2 mg/ml.

GVHD and rejection

The diagnosis of GVHD was based on clinical signs (wasting, hair loss, as previously indicated, [8]) and on macroscopic appearance at autopsy. Rejection was characterized by graft distention and thickening of the intestinal wall. At the death of the rat, the grafted small bowel and the recipient' own were removed and used for histological examinations to confirm the clinical findings.

Surgical techniques

Hyperimmunization of donors was induced by grafting them with recipient skin as previously reported [9]. Heterotopic small-bowel transplantation was performed according to the method described by Monchik and Russell [10]. Briefly, the entire donor small bowel was harvested with its vascular pedicles consisting of the aortic stump containing the superior mesenteric artery and the portal vein. End-to-side anastomoses were made between the donor aorta and the host infrarenal aorta and between the portal vein of the donor and the host vena cava using 9/0 and 8/0 nylon monofilament. Finally, a duodenostomy and an ileostomy were created by suturing the small bowel to the abdominal wall using 5/0 silk.

$CD4^+$ and $CD8^+$ T cell separation

The rats were exsanguinated at the level of the aortic bifurcation. Peripheral heparinized blood lymphocytes were isolated by Lympholite-Rat (Cederlane, Ontario, Canada) gradient centrifugation, according to Böyum [11]. Adherent cells were removed by plastic adherence. The lymphocytes were suspended in cold phosphate-buffered saline (ICN, Milan, Italy), stained with phycoerythrin-conjugated monoclonal antibodies to CD4 (W3/25) or CD8 (MRC OX8; Serotec, Oxford, UK), and incubated for 20 min on ice. After washing twice with phosphate-buffered saline, the cells were subjected to flow cytometric analysis using a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif., USA).

Statistical analysis

Student's two-tailed test was used for comparison of parametric sample means. Variance analysis (ANOVA) was applied to the ratio of $CD4^+$ to $CD8^+$ T cells. Significance was assumed when $P < 0.05$.

Results

GVHD

Recipients of groups 1 and 3 developed GVHD alone. Figures 1 and 2 show the Kaplan-Meier survival curves for groups 1 and 3, respectively, in the absence or in the presence of CsA treatment. GVHD developed in 14–19 days in group 1 and in 18–25 days in group 3. Treatment with CsA delayed GVHD to 25–40 and 30–45 days, respectively.

Rejection

ACI rats receiving the small bowel from WAF_1 hybrids (group 2) developed rejection alone. Treatment with CsA significantly delayed small-bowel rejection (90.2 ± 4.3 days as opposed to 8.9 ± 1.4 in untreated recipients).

Simultaneous GVHD and rejection

Wistar rats receiving the small bowel of ACI rats bearing 10-day-old Wistar skin grafts (group 4) survived for 7.1 ± 2.2 days. When CsA was administered, the survival of recipient rats was significantly prolonged to 22.3 ± 3.1 days.

$CD4^+$ to $CD8^+$ T cell ratios

Table 1 shows the $CD4^+/CD8^+$ T cell ratios, determined by measuring the percentage of $CD4^+$/percentage of $CD8^+$ T cells in rat blood present at 7 and 15 days following small-bowel graft. All the results represent the mean ± SEM of ten experiments, except at the 15th day for group 1 without CsA treatment (one rat died at the 14th day) and for groups 2 and 4 untreated rats, which died after the 7th and before the 15th postoperative day. All the $CD4^+/CD8^+$ T cell ratios were signifi-

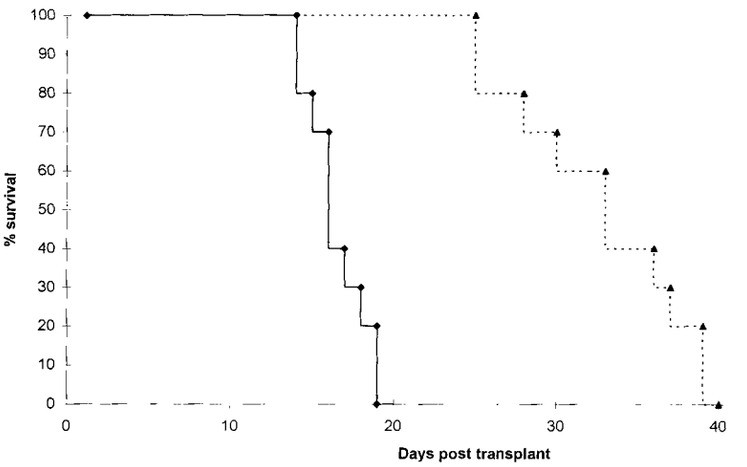

Fig. 1 Percentage survival of ACT × Wistar F_1 (WAF_1) hybrid rats following small-bowel transplantation from ACI (group 1) and graft-versus-host disease (GVHD) development. *Solid line* control, *broken line* cyclosporine (CsA) treatment

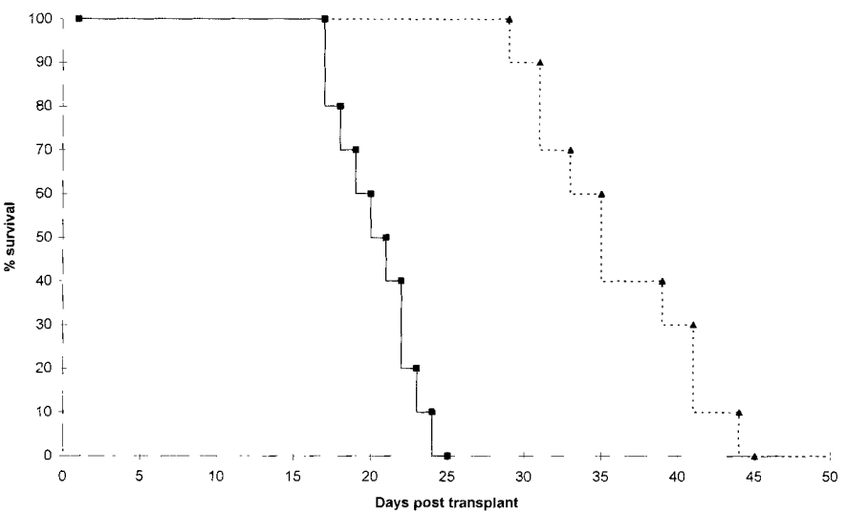

Fig. 2 Percentage survival of WAF_1 hybrid rats following small-bowel transplantation from hyperimmunized ACI (group 3, bearing 10-day-old WAF_1 skin grafts) and GVHD development. *Solid line* control, *broken line* CsA treatment

Table 1 $CD4^+$ to $CD8^+$ T cell ratios measured in blood of control or grafted rats at the 7th and 15th (when alive) day following small-bowel transplantation (*CSA* cyclosporine, *WAF_1* ACI × wistar F_1 hybrids)

	Control	Seven days postgraft	Fifteen days postgraft
ACI	1.94 ± 0.14	–	–
WAF_1	2.03 ± 0.09	–	–
Wistar	1.98 ± 0.12	–	–
Group 1	–	1.24 ± 0.11*	1.25 ± 0.08*
Group 1 + CsA	–	1.31 ± 0.13*	1.33 ± 0.09*
Group 2	–	1.44 ± 0.07	–
Group 2 + CsA	–	1.15 ± 0.04**	1.62 ± 0.07
Group 3	–	1 ± 0.09	1.24 ± 0.02*
Group 3 + CsA	–	1.08 ± 0.05*	1.27 ± 0.10*
Group 4	–	0.84 ± 0.04***	–
Group 4 + CsA	–	0.92 ± 0.06***	1.04 ± 0.06***

$P < 0.05$ versus control: * versus WAF_1; ** versus ACI; *** versus Wistar

cantly lower in grafted recipients than in the same ungrafted rats.

$CD4^+$ to $CD8^+$ T cell frequencies were measured in the blood of surviving control and grafted rats at the 7th and 15th day following small-bowel transplantation. In group 1 the ratio values, which were not significantly modified by CsA treatment, did not vary at the 15th day with respect to 7th day following the operation. In group 3, hyperimmunization of donors (the same combination as group 1), which failed to more rapidly induce GVHD, induced a significant reduction of $CD4^+$ T cells and of the $CD4^+/CD8^+$ T cell ratio with respect to group 1. CsA treatment did not correct such results until the 15th postoperative day. In group 2, rejection processes induced a significant reduction of $CD4^+$ and $CD8^+$ T cells with respect to ungrafted rats, with a not significant major reduction in $CD4^+$ than in $CD8^+$ cells. Such data were amplified by CsA treatment until the

7th postoperative day. In group 4, in which simultaneous GVHD and rejection were developed, the maximal reduction of $CD4^+/CD8^+$ T cell ratios was observed, especially in the absence of CsA treatment, due to a dramatic reduction of both cell subsets of lymphocytes with major losses of $CD4^+$ with respect to $CD8^+$ T cells.

Discussion

$CD4^+$ T cell immunodeficiency in GVH-reactive mice possibly due to a thymic functional defect has been previously observed [12]. Following small-bowel rat transplantation, a decrease in the $CD4^+$ to $CD8^+$ T cell ratio was observed by day 5 posttransplant [13] in recipients, both allografted and isografted. Moreover, before the rejection, there was a shift from host-to-graft T cell dominance in grafted small bowel coincident with a peak in graft T cell infiltration of the host by day 4 following the operation [6]. Considering of the usefulness of such evaluations for diagnostic purposes in GVH and HVG reactions, we studied the modifications of $CD4^+$ and $CD8^+$ T cell levels in the rat small-bowel transplantation model. Our results showed that rats which developed GVHD alone had a significant reduction in $CD4^+$ cells. The reduction was more significant when the donors were hyperimmunized by grafting with the recipient skin (group 3 with respect to group 1). However, hyperimmunization delayed the induction of GVHD. Treatment with CsA increased the survival of GVH-reactive rats, but failed to significantly restore the $CD4^+/CD8^+$ ratios with respect to untreated animals, both at 7 and 15 days posttransplant. In rats that developed rejection (group 2), a minor reduction (not significant) in $CD8^+$ with respect to $CD4^+$ cells was observed, and CsA amplified such a result at the 7th day following transplantation. The reduction was accompanied by an important reduction of $CD4^+$ and $CD8^+$ T cells following small-bowel transplantation. The decrease in lymphocytes is possibly due to involvement of $CD4^+$ and $CD8^+$ as effector cells in alloreactivity [14]. CsA treatment significantly increased rat survival. Surviving treated rats had a significant increase in $CD4^+/CD8^+$ ratios at the 15th day following operation with respect to the 7th day. Rats that developed both GVHD and rejection of the small bowel from hyperimmunized rats (group 4) also developed the maximal reduction of $CD4^+/CD8^+$ ratios, in the absence of CsA treatment, at 7 and 15 days following the operation. Such results are in accordance with the significant reduction of $CD4^+$ T cells observed in the blood of group 1 and group 3 rats that developed GVHD alone. However, the presence in group 4 of simultaneous GVHD and rejection enhanced the reduction of $CD4^+$ T cells observed in all the other groups [1–3].

Our data seem to suggest that the prior determination of the $CD4^+/CD8^+$ T cell blood ratio may help to predict the events that follow small-bowel transplantation in rats and could be correlated with rat survival. In fact, when GVHD occurred, there was a severe and significant reduction in the $CD4^+/CD8^+$ T cell ratio, that was more elevated when hyperimmunized donors were used, at the 7th day following the operation and was due to an important $CD4^+$ T cell decrement. CsA treatment in such groups [1, 3] caused a delay in rat death, but did not improve $CD4^+/CD8^+$ T cell ratios. A reduction in the $CD4^+/CD8^+$ T cell ratio was thus related to the presence of GVHD and a sure death due to GVHD development, despite the efficacy of CsA treatment in improving rat survival. In grafted animals that developed rejection alone, $CD4^+/CD8^+$ T cell ratio reduction was less evident than in rats displaying GVHD. In fact, rejection was related to a significant reduction in $CD4^+$ T cells and also in $CD8^+$ T cells, owing to the use of both subsets as effectors of donor small-bowel rejection. However, prolongation of graft survival induced by CsA treatment determined a recovery of $CD4^+/CD8^+$ T cell ratios to values lower than those of untreated rats. Elevated $CD4^+/CD8^+$ T cell ratios were thus related to a long survival of rats that developed rejection. The maximal reduction of $CD4^+/CD8^+$ T cell ratios occurred when both GVHD and rejection developed, due to a more elevated loss of $CD4^+$ than of $CD8^+$ T cells. Such a condition was correlated with a very poor survival rate. We believe that the $CD4^+/CD8^+$ T cell ratios in our experimental model of small-bowel transplantation are useful for predicting animal survival and could be applied in the diagnosis of GVHD or rejection in humans.

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