



BASIC RESEARCH

Formation of microchimerism in rat small bowel transplantation by splenocyte infusion

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CONCLUSION: Donor splenocyte infusion combined with CsA decreases remarkably the rejection and prolongs the survival time after rat small bowel transplantation.

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Key words: Microchimerism; Splenocyte infusion; Immunologic tolerance; Small bowel transplantation

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Abstract

AIM: To investigate the effect of donor splenocyte infusion combined with cyclosporine A (CsA) on rejection of rat small bowel transplantation (SBT).

METHODS: Male Sprague-Dawley (SD) rats and female Wistar rats weighing 230-270 g were used as donors and recipients respectively in the study. Heterotopic small bowel transplantation was performed. The rats were divided into three groups: group one receiving allotransplantation (SD→Wistar), group two receiving allotransplantation (SD→Wistar) + donor splenocyte infusion, group three receiving allotransplantation (SD→Wistar) + donor splenocyte infusion + CsA followed by CsA 10 mg/kg per day after transplantation, in which recipient Wistar rats were injected with 2×10^8 SD splenocytes 28 d before transplantation, and treated with CsA after transplantation. Finally, the specific DNA fragment of donor Y chromosome was detected in recipient peripheral blood and skin by PCR. The survival time after small bowel transplantation was observed. Gross and histopathological examinations were performed.

RESULTS: The survival time after small bowel transplantation was 7.1 ± 1.2 d in group 1, 18.4 ± 3.6 d in group 2 and 31.5 ± 3.1 d in group 3. The survival time was significant longer ($P < 0.01$) in group 3 than in groups 1 and 2. The gross and histopathological examination showed that the rejection degree in group 3 was lower than that in groups 1 and 2.

INTRODUCTION

Small bowel transplantation (SBT) has become an accepted therapy for intestinal diseases in patients who require total parenteral nutrition^[1,2]. Because of the rich lymphatic tissue in small bowel and its mesentery, the mesenteric lymph nodes and lymphatic plexus are transplanted along with the small bowel. Thus, small bowel transplantation has a more severe immune rejection compared with other organ transplantations^[3]. Immune rejection is the leading cause of failure in small bowel transplantation^[4-7]. Although the results of SBT have been dramatically improved during the past few years, the major impediment to success in SBT is still acute rejection (AR). The key steps toward a successful transplantation are therefore to attenuate immune reactions and to induce immune tolerance to grafts.

The spleen is an immunologically privileged organ. The incidence rate and degree of rejection after spleen transplantation are much lower than those of other solid organ transplantations. Splenocyte chimerism has been successful in inducing tolerance in acute and chronic rejection liver transplant models^[9]. Both in experimental study and in clinical practice, splenocytes (including lymphocytes, dendritic cells, Kupffer cells, *etc*) play an important role in immune tolerance induction. Although splenocyte chimerism can effectively decline the immune reactions in organ transplantation, but whether the administration of spleen cells to recipients has the same effect in SBT is uncertain. Some parameters were tested to confirm the anti-rejection effect of splenocyte infusion

combined with CsA on rat small bowel transplantation in this study.

MATERIALS AND METHODS

Animal preparation

Healthy inbred male Sprague-Dawley (SD) rats as donors, and inbred female Wistar rats as recipients, weighing 250 ± 20 g, provided by Medical Experiment Animal Center, Harbin Medical University, were housed in standard animal facilities, and fed with commercially available rat chow and tap water *ad libitum* for 1 wk before test to acclimatize to the laboratory. The donor and recipient were paired according to the similar body weight.

Rat small bowel transplantation

All procedures were performed under inhalation anesthesia with ether. The entire small bowel from the ligament of Treitz to the ileocecal valve was isolated with the superior mesenteric artery on a segment of aorta and portal vein^[14]. After donor systemic heparinization (300 U), the graft was perfused with 20 mL of cold lactated Ringer's solution *via* the aorta. The lumen was also washed in 20 mL of the same solution. In the recipient, end-to-side vascular anastomoses were performed between the graft aorta and recipient infra-renal aorta and between the graft portal vein and recipient inferior vena cava with 10-0 sutures using the standard microsurgical technique. The superior extremity of the transplanted small bowel was ligated and a distal small bowel stoma was performed on the left abdominal wall. Animals that died within 3 d were considered as technical failures and excluded from data collection^[10-12].

Spleen cell preparation

SD rats were sacrificed by decapitation. Spleens were collected and kept on ice in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with antibiotics (100 units/mL penicillin and 100 μ g/mL streptomycin). The spleens were disrupted in the medium by pressing spleen fragments between two glass slides. Cell suspensions were filtered through cotton gauze and washed three times with RPMI 1640 medium. Viable nucleated cells were counted and adjusted usually to 2.0×10^7 /mL.

Experimental groups and postoperative care

The rats were divided into three groups: group 1 as allotransplantation group (SD \rightarrow Wistar, $n = 10$), group 2 as allotransplantation (SD \rightarrow Wistar) + donor splenocyte infusion, group 3 as allotransplantation (SD \rightarrow Wistar) + donor splenocyte infusion + CsA, with CsA 10 mg/kg per day after transplantation, in which recipient Wistar rats were injected with 2×10^8 SD splenocytes 28 d before transplantation and treated with CsA after transplantation. Animals were fed with only sugar water (7 g/d) on d 1, rat chow and water on d 2 and thereafter. The rats' mental state, appetite, and ejection liquid of small bowel stoma were also observed.

Analysis of microchimerism

Microchimerism in peripheral blood and skin of Wistar rat recipients was assessed by PCR. Blood and skin were

collected, and genomic DNA was isolated from buffy coat with a DNA extract kit and from skin by the proteinase k-phenol-chloroform method. Microchimerism was detected by specific primers for the donor Y chromosome. PCR was performed with 100 ng of the DNA template, 18 pmol of forward (5'- CGT GGA GAG CGC AAG TT -3', p1) and reverse primers (5'- GTC GCT GTT TCT GCT GTA GTT A -3', p2). The primers were designed to distinguish donor DNA from recipient DNA, and yielded a visible PCR product of 154 bp with the donor DNA template under ethidium bromide fluorescence. The 50 μ L reaction contained 10 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.001% gelatin (Perkin- Elmer, Foster City, CA), 50 g/L BSA, 0.2 mmol/L dNTPs, and 1 unit of Amplitaq DNA polymerase (Perkin-Elmer). The cycling conditions were: at 94°C for 4 min, followed by 35 cycles at 94°C for 60 s, at 55°C for 60 s, at 72°C for 60 s and a final extension at 72°C for 8 min in a GeneAmp PCR System 2400 (Perkin Elmer). The products were separated on 1.5% agarose gel containing ethidium bromide.

Histopathological analyses

Rats' small bowel allografts were excised from stoma or by laparotomy and fixed in 10% formalin. The fixed tissue was paraffin embedded, and tissue sections were stained with hematoxylin and eosin (HE). Rejection was graded histologically according to the phase of acute intestinal rejection established by Rosemurgy *et al*^[13] and Sudan *et al*^[14]. The sections were graded for tissue injury using a scale of 0 (none) to 7 (severe) based on the following criteria: 0: normal mucosa; 1: development of subepithelial (Gruenhagen's) spaces at villus tips; 2: extension of the subepithelial space with moderate epithelial cell lifting from the lamina propria; 3: massive lifting down sides of villi, some denuded tips; 4: denuded villi, dilated capillaries; 5: disintegration of the lamina propria; 6: crypt layer injury; 7: transmucosal infarction. All histological analyses were performed in a blinded fashion to avoid bias.

Graft survival

All recipients were followed up by visual inspection and submitted to autopsy soon after they died. Graft survival time was defined as the time until death of recipient due to immune rejection.

Statistical analysis

All data were analyzed by Student's *t* test and expressed as mean \pm SD. $P < 0.05$ was considered significant and $P < 0.01$ very significant.

RESULTS

Gross observation

The rats awaked soon after operation. In allotransplantation group (group 1), the rats presented various degrees of lethargy, anorexia hair disorder, unresponsiveness to outside stimulation and body weight loss. Intestinal graft was pale. Intestinal lumina was enlarged with massive adhesion and gradually aggravated, accompanying mass purulent discharge. Intestinal perforation occurred in some severe cases. In allotransplantation + donor splenocyte

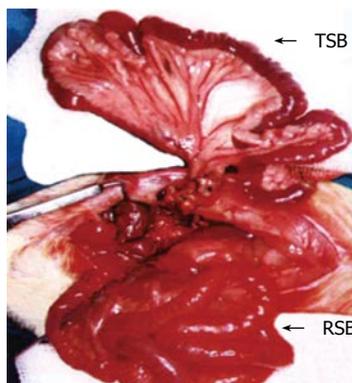


Figure 1 Heterotopic small bowel transplantation in rats. The graft vasculature was anastomosed. TSB: transplanted small bowel; RSB: recipient's small bowel.

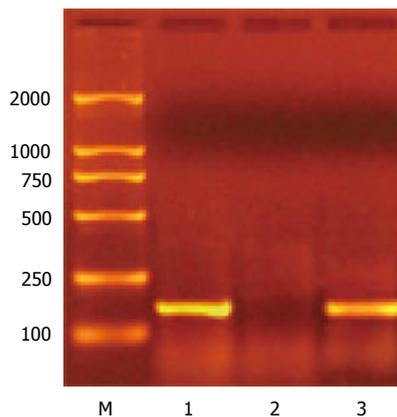


Figure 2 Agarose gel electrophoresis of PCR products. M: 2 Kb DNA ladder; lane 1: PCR amplification bands from normal SD rats; lane 2: PCR amplification bands from allotransplantation group rats; lane 3: PCR amplification bands from allotransplantation + splenocyte infusion group rats.

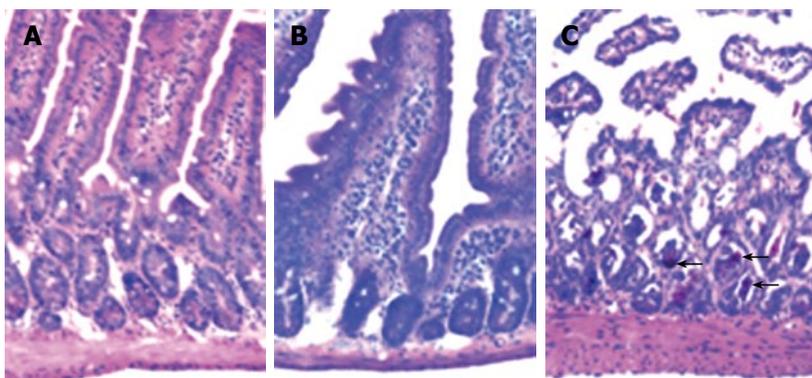


Figure 3 Histopathological findings in different groups after SBT. **A:** Few histopathological changes in the normal tissue (group 3); **B:** Intact villi and some infiltrates in allografts obtained from donor splenocyte recipients (group 2); **C:** Inflammatory infiltrates throughout the intestine and extensive apoptosis (arrows) and ulceration in the transplanted intestine (group 1).

Table 1 Survival time after small bowel transplantation in rats (mean \pm SD)

Group	<i>n</i>	Survival time (d)
group 1	10	7.1 \pm 1.2
group 2	10	18.4 \pm 3.6
group 3	10	31.5 \pm 3.1 ^b

^b*P* < 0.01 vs groups 1 and 2.

Table 2 Histologic grading of graft after small bowel transplantation in rats (mean \pm SD)

Group	<i>n</i>	Histologic grading
group 1	10	6.4 \pm 2.81
group 2	10	5.1 \pm 1.06
group 3	10	3.2 \pm 1.67 ^a

^a*P* < 0.05 vs groups 1 and 2.

infusion group (group 2), the rats were vigorous, sensitive to outside stimulation, and low-grade adhesion occurred 7 d after transplantation. In allotransplantation + donor splenocyte infusion + CsA group (Group 3), the rats were vigorous, and sensitive to outside stimulation, and low-grade adhesion occurred 6 d after transplantation (Figure 1).

Donor cell chimerism

Male donor cell chimerisms in recipients were determined by analyzing metaphase spreads for the presence of donor Y chromosomes. Y chromosomes were found in rats of the spleen cell infusion group but not in rats of allotransplantation group (Figure 2).

Survival time after transplantation

The survival time after small bowel transplantation was 7.1 \pm 1.2 d in group 1, 18.4 \pm 3.6 d in group 2, and 31.5 \pm 3.1 d in group 3. The survival time was significant longer (*P* < 0.01) in group 3 than in groups 1 and 2 (Table 1).

Histopathologic examination

Few histopathological changes and inflammatory infiltrate were detected in rats of group 3 (Figure 3A). After allotransplantation + splenocyte infusion treatment, some histopathological features of rejection were found, villi were intact but showed some blunting, crypts had no necrosis and lymphocyte infiltrate was minimal. A significant decrease in AR changes was observed in grafts of recipients treated with donor splenocyte infusion (Figure 3B). In group 1, severe rejection was characterized by complete villus flattening, epithelial apoptosis, and transmural cellular infiltrate (Figure 3C, Table 2).

DISCUSSION

In clinical practice, immune rejection induced by organ transplants necessitates the use of potent immunosuppressive drugs. However, excessive dosage of immunosuppressive agents may result in severe side effects, such

as hypertension, hepatic and/or renal toxicity. Moreover, prolonged usage of immunosuppressants often leads to severe infection and increased susceptibility to malignant tumors, thus critically affecting the health of recipients. It is therefore imperative to assess the protective effect of immune tolerance on organ transplantation. Recently, it has been reported that donor-derived multilineage hemopoietic cell microchimerism is a prerequisite for tolerance induction in organ allograft recipients^[15,16]. In an effort to augment the natural microchimerism that occurs following organ transplantation, adjunctive perioperative donor spleen cell infusion should be considered in conventionally immunosuppressed human organ transplant recipients^[17,18]. It was reported that donor spleen cell infusion can enhance liver allograft survival in humans^[19] because of the lack of recipient type antigen presenting cells (APC)^[20]. In our study, the survival time of the rats that received infusion of donor spleen cells combined with CsA was significantly longer compared to those that did not receive it.

The spleen is an immunologically privileged organ. The rejection incidence rate and degree of spleen transplantation are much lower than those of other solid organs. The spleen cell infusion can induce donor-specific transplantation tolerance and transient microchimerism. In our study, SD splenocyte chimerism was established in Wistar rats. SB grafts remained normal and were well vascularized. By contrast, the bowel of control animals showed AR.

The survival time was significant longer (31.5 ± 3.1 d *vs* 7.1 ± 1.2 d, $P < 0.01$) in group 3 than groups 1 and 2. The rejection degree in group 3 was lower than that in group 1. Rats in group 3 had better histological structures than rats in group 1 (3.2 ± 1.67 *vs* 6.4 ± 2.81 , $P < 0.05$), suggesting that donor spleen cell transfusion combined with cyclosporine A is a simple and practical method to suppress rejection of small bowel transplantation in clinical practice.

In conclusion, donor-specific tolerance induced by splenocyte chimerism prevents AR in an experimental model of highly immunogenic SB allografts. Central tolerance through splenocyte chimerism can be used to study the immune tolerance mechanisms. Further work is needed to reveal if chimerism is induced by spleen cell transfusion.

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