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Alimentary Tract

Donor-derived bone marrow transfusion produces mixed chimerism and promotes a Th2 shift in Th1/Th2 balance in rat heterotopic small bowel transplantation

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ABSTRACT

Background and aim: In this study, we investigated immunomodulatory effects of donor-derived bone marrow transfusion in rat heterotopic small bowel transplantation.
Methods: Rat heterotopic segmental small bowel transplantation models (male Brown Norway to female Lewis) were established. The recipients were randomly divided into control group (pute small bowel transplantation), tacrolimus group (small bowel transplantation plus oral tacrolimus) and small bowel transplantation plus oral tacrolimus and intraportal infusion of donor-derived bone marrow cells group. We investigated the survival time, graft pathologic injuries and rejection grade by haematoxylin–eosin staining, serum IL-2 and IL-10 detection by enzyme labelled immunosorbent assay after small bowel transplantation. The recipients mixed chimerism were observed by detecting sex-determining region of Y chromosome gene in blood, liver, spleen and intestine by using real-time polymerase chain reaction and fluorescence in situ hybridization.
Results: Bone marrow cells group showed a superior survival than the other groups, accompanied by milder pathologic injuries and lower rejection grade, decreasing serum IL-2 and increasing serum IL-10. The recipient chimerism rate in blood, liver, spleen and intestine in bone marrow cells group was significantly higher than the other groups.
Conclusion: Transfusion of donor-derived bone marrow cells via portal vein induces mixed chimerism in rats after small bowel transplantation, which may promote a Th2 shift in Th1/Th2 balance and facilitate the induction of immune tolerance.

1. Introduction

Small bowel transplantation (SBTx) is a life-saving option for patients with intestinal failure who fail on total parenteral nutrition (TPN) and those who have developed irreversible liver failure by TPN [1]. SBTx was hindered by technical and immunologic complications at the early stage. In recent years, the outcome of SBTx has been improved on account of surgical advances, control of acute cellular rejection, and a decrease in lethal infections. The 1, 3, and 5 years survival rate of SBTx in 2005 improved at 57%, 61% and 47%, respectively, according to pooled data (http://optn.transplant.hrsa.gov) [2]. The 1-year survival rate of patients is >90% at experienced centres nowadays [3]. However, rejection and sepsis are still the common causes of death and graft loss in SBTx patients at present because of the intense immune response of SBTx. Exploiting a more effective immunosuppressive and immunomodulatory strategy has become the core of current research.

Immune tolerance is the ideal goal for all transplantation, which may eliminate rejection and avoid side effects of immunosuppressant (i.e. infection, tumour and renal failure). Mixed chimerism is characterized by a state of co-existence of both donor and host hematopoietic cells, which can achieve immune tolerance post transplantation [4]. This approach is an outstanding strategy as tolerance induction which has been successfully shown in nonhuman primate studies and in clinical pilot trials [5]. Stable mixed chimerism was made by bone marrow cells transfusion plus

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optimum dose of immunosuppressant in organ transplantation [6]. The similar phenomenon was seen in SBTx. Donor-derived lymphocytes were found in recipient lymphatic tissue in rat SBTx which had achieved long survival with tacrolimus (FK506) administration [7,8]. Preoperative donor-derived blood transfusion plus low dose of ciclosporin prolonged intestinal graft survival through augmenting chimerism in porcine SBTx [9]. However, another study showed opposite result suggesting that modulation of immune response with short-course immunosuppression and a single or multiple donor specific bone marrow infusion did not improve allograft or recipient survival [10]. Therefore, the effect of simultaneous donor derived bone marrow transfusion with SBTx needs further studied.

Considering the significance of the relationship between mixed chimerism and immune tolerance, we hypothesized that simultaneous donor derived bone marrow transfusion with SBTx would producing mix chimerism and prolong recipient survival. Our objectives were (1) to establish stable mix chimerism in rat SBTx moldel, (2) to observe the beneficial effects of simultaneous donor derived bone marrow transfusion on the histopathological structural integrity, grades of rejection, recipient survival rates, and levels of serum IL-2 and IL-10.

2. Materials and methods

2.1. Animals

Inbred male Brown Norway (BN) and female Lewis rats weighing 260–300 g were obtained from Beijing Medical Animal Center, China. Rats were provided with *ad libitum* access to standard chow and tap water, and housed in a 12-h light/dark cycle specific pathogen free animal facility. The experimental protocol was approved by the Ethical Review Committee for Animal Experiments at Sun Yat-sen University (Guangzhou, China).

2.2. Small bowel transplantation

Allogeneic heterotopic SBTx in rats (BN-Lewis) were performed by using the cuff technique as previously described [11,12]. All surgical procedures were performed in a sterile field under sevoflurane anaesthesia. Briefly, we harvested a segmental small bowel with the attached vascular pedicles consisting of superior mesenteric artery (SMA) and portal vein (PV). Revascularization was accomplished by end-to-side anastomosis between donor SMA and recipient infrarenal aorta and cuffed end-to-end anasmosis between donor PV and recipient left renal vein after the removal of left kidney.

2.3. Donor marrow cells isolation and transfusion

BN rats were sacrificed and their bone marrow cells were isolated by flushing the tibias and femurs. Red blood cells were removed by usage of red cell lysis buffer. The remaining mononuclear bone marrow cells were diluted in 0.85% saline solution at a final density of 2.5×10^8 cells/ml. Trypan blue exclusion testing showed more than 95% cell viability [13]. The donor marrow cells were transfused into the recipients via PV to induce the mixed chimerism before abdominal closure.

2.4. Experimental groups

The experimental SBTx protocol was comprised of 3 groups: control group, pure SBTx without tacrolimus (FK506) administration ($n = 10$); FK506 group, SBTx with FK506 administration ($n = 10$); BM-PV group, SBTx with oral FK506 plus donor derived bone marrow transfusion via PV ($n = 10$). FK506 was purchased from Astellas Pharma (Tokyo, Japan), and administrated at a dose of 1 mg/kg/day by oral gavage in day 0–5 post transplantation.

2.5. Histology

Tissues harvested from rats were fixed in 10% formalin at different time points. Fixed tissues were embedded in paraffin, sectioned at 5 μm thicknesses, and stained with haematoxylin and eosin. The evaluation of the histological grade of injuries was based on three parameters from 0 to 2: the percentage of injured villi, the relative villus height within the mucosa, and the total mucosal thickness, giving rise to maximum score of 6 [14,15].

2.6. Real-time polymerase chain reaction (PCR)

Real-time PCR for sex-determining region of Y chromosome (SRY) gene was used to determine the concentration of male cells in the samples (blood, liver, spleen and intestine). Recipients were sacrificed on day 7 and day 60 post transplantation, and their peripheral blood, spleen, liver and recipient derived intestine samples were collected. The genomic DNA of peripheral blood, liver, spleen and intestine were extracted with Genome DNA Extraction Kit (Sangon Biotec, Shanghai, China), according to the manufacturer's instruction. PCR mixture was prepared using SYBR Premix Ex Taq^{TM} (TaKaRa, Dalian, China). And the primers of 5′-AAGTCAAGCGCCCCATGA-3′ (sense) and 5′-TGAGCCAACTTGTGCCTCTCT-3′ (antisense) were applied for PCR [16]. The reactions were performed using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems Inc., USA). The thermal cycler was configured as the following: incubation (95 °C, 10 min), 40 cycles of denaturation (95 °C, 15 s), and annealing and extension (60 °C, 1 min). The genomic DNA from the male BN rats and female Lewis rats were mixed at 100%, 20%, 4%, 0.8% and 0.16% of male BN rat DNA, respectively. The mixed DNA samples were then used to establish standard curves.

2.7. Fluorescence in situ hybridization (FISH) assays

Recipient spleen, liver and recipient derived intestine were collected on day 7 and day 60 post transplantation for FISH assays. FISH was performed on paraffin-embedded tissue sections as previously described by using the rat Y FISH kit (TaKaRa, China) according

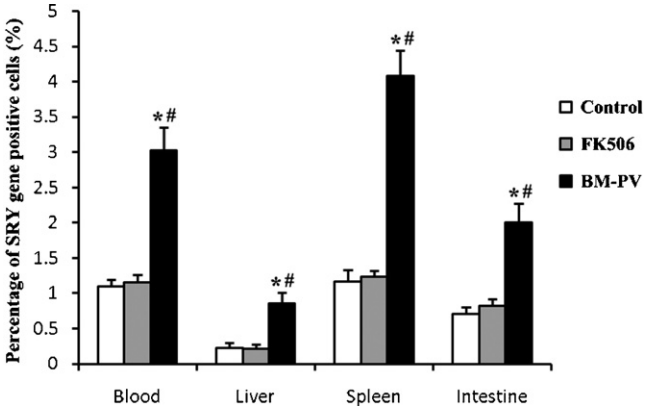

Fig. 1. Mix chimerism is induced by simultaneous donor derived bone marrow transfusion with small bowel transplantation (SBTx) in rats by real-time PCR analysis. Real-time PCR analysis for sex-determining region of Y chromosome (SRY) in various female Lewis recipient tissues (blood, liver, spleen and intestine) at day 60 after received male BN intestinal grafts in control group, tacrolimus (FK506) group, and SBTx plus oral FK506 and intraportal infusion of donor-derived bone marrow cells (BM-PV) group. Data were expressed as the mean ± SD (*$p < 0.05$, control group vs BM-PV group; #$p < 0.05$, FK506 group vs BM-PV group; $n = 5$).