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Induction of tolerance and prolongation of islet allograft survival by syngeneic hematopoietic stem cell transplantation in mice

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ABSTRACT

Backgrounds: Syngeneic or autologous hematopoietic stem cells transplantation (HSCT) has been proposed to treat autoimmune diseases because of its immunosuppressive and immunomodulatory effects, which can also contribute to posttransplant antirejection therapy. In this study, we explored the tolerogenic effect of syngeneic HSCT on prolonging islet allograft survival.
Methods: C57BL/6 mice received syngeneic HSCT plus preconditioning with sublethal irradiation. Then islets of BALB/c mice were transplanted into the renal subcapsular of C57BL/6 mice after chemically induced into diabetes.
Results: HSCT mice exhibited improved islet allograft survival and increased serum insulin compared to control mice. Islet allografts of HSCT mice displayed lower level lymphocyte infiltration and stronger insulin staining than control mice. T cells of HSCT mice proliferated poorly in response to allogeneic splenocytes compared to control mice. Mice appeared reversed interferon-γ (IFN-γ)/interleukin-4 (IL-4) ratio to a Th2 immune deviation after syngeneic HSCT. The percentage of $CD8^+$ T cells was lower, while percentage of $CD4^+CD25^+Foxp3^+$ T regulatory cells (Tregs) was higher in HSCT mice than control mice. HSCT mice showed higher percentage of $CTLA\text{-}4^+$ T cells and expression of CTLA-4 mRNA than control mice. Targeting of CTLA-4 by intraperitoneal injection of anti-CTLA-4 mAb abrogated the effect of syngeneic HSCT on prolonging islet allograft survival, inhibiting activity of T cells in response to alloantigen, promoting Th1 to Th2 immune deviation and up regulating $CD4^+CD25^+Foxp3^+$ Tregs.
Conclusions: Syngeneic HSCT plus preconditioning of sublethal irradiation induces tolerance and improves islet allograft survival in fully mismatched mice model. Th1 to Th2 immune deviation, increased $CD4^+CD25^+Foxp3^+$ Tregs and up-regulation of CTLA-4 maybe contribute to the tolerogenic effect induced by syngeneic HSCT.

1. Introduction

Islet transplantation represents a curative treatment for type 1 diabetes. It improves or stabilizes diabetes-related complications, normalizes glucose homeostasis and reduces hypoglycemic episodes [1]. However, multiple islet infusions are required to sustain insulin independence [2], yet islet graft survival rates remain far below those of other solid organ grafts [3]. Although new immunosuppressive regimens have contributed greatly to increased islet graft survival, immune rejection still is the main challenge need to be overcome before islet transplantation therapy for diabetes is utilized in clinic [4]. Therefore, researchers are concentrating on the development of tolerogenic protocols to obtain islet grafts acceptance.

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment for many autoimmune diseases [5]. In recent years, the use of allogeneic HSCT has been proposed for the potential of inducing tolerance. After the engraftment of hematopoietic stem cells (HSCs) in the bone marrow, donor-derived cells migrate to the thymus of recipient, where they can promote central tolerance to donor-derived antigens [6]. Furthermore, Allogeneic HSCT has potent immunosuppressive properties and can effectively protect the grafts from injury of recipient's cytotoxic T cells, thus prolonging graft survival [7]. Based on these evidences, allogeneic HSCT represents an attractive tool for inducing tolerance toward islet grafts [8]. However, allogeneic

Abbreviations: GVHD, graft-versus-host disease; GFP, green fluorescent protein; HSCs, hematopoietic stem cells; HSCT, hematopoietic stem cell transplantation; IFN-γ, interferon-γ; IL-2, interleukin-2; IL-4, interleukin-4; MST, median survival time; MLR, mixed lymphocyte reaction; PBMCs, peripheral blood mononuclear cells; PBS, phosphate buffered saline; STZ, streptozotocin.

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HSCT needs aggressive preconditioning treatment to permit donor HSCs engraftment and usually causes treatment-related toxicity [9]. What is more, allogeneic HSCT has been associated with an increased risk of developing severe graft-versus-host disease (GVHD), which weakens the benefit of islet transplantation and results in increased mortality [10].

The mechanism of allogeneic HSCT for treating autoimmune diseases are best explained by a qualitative change in the reconstituted immune repertoire, which is described as a "resetting" of the immune system [11]. Growing evidences have suggested that autologous or pseudoautologous (using syngeneic animals as the recipient) HSCT was also effective in treatment of autoimmune diseases in numerous animal models [12,13]. In contrast to allogeneic HSCT, autologous HSCT exhibits few serious complications and little risk of GVHD [14]. Clinical trials suggested that autologous HSCT is a safe approach of treatment for patients [15]. Autologous HSCT has been proposed to treat autoimmune diseases because of its immunosuppressive and immunomodulatory effects, which can also contribute to posttransplant antirejection therapy [16]. Studies have showed that autologous HSCT enables successful engraftment of kidney allografts [17] and prolongs cardiac allograft survival [18] in animal models. Therefore, autologous HSCT maybe a potential approach of antirejection therapy for islet allografts in posttransplant.

In this study, we proposed an approach to induce immune tolerance by syngeneic HSCT in an islet transplantation model, with the goal of exploring the immunomodulatory effect of syngeneic HSCT on survival of islet allografts.

2. Materials and methods

2.1. Animals

BALB/c and C57BL/6 mice were purchased from Animal Production Centre, Medical College of Xi'an Jiaotong University, and used at 6–8 weeks of age as donors and recipients for islet transplantation. Green fluorescent protein (GFP) C57BL/6 mice were obtained from the Biomedical Institute of Nanjing University and used as donors for HSCT. All animals were cared for according to the institutional guidelines and under protocols approved by the Laboratory Animal Care and Use Committee of the university.

2.2. Isolation and transplantation of HSCs

Bone marrow cells were obtained from femurs and tibiae of GFP^+ C57BL/6 mice by flushing with phosphate buffered saline (PBS) and isolated by density-gradient centrifugation. Bone marrow cells were lineage depleted by using an EasySep Mouse Hematopoietic Progenitor Cells Isolation Kit (Stem Cell Technologies) follow the manufacturer's instruction. Unwanted cells are targeted for removal with biotinylated antibodies directed against non-HSCs and non-progenitor cells (CD3, CD11b, CD19, CD45R, Gr-1, Ter119) and streptavidin-coated magnetic particles. Labeled cells were separated using an EasySep Magnet (Stem Cell Technologies) and desired cells were poured off into a new tube. After a total body irradiation with a single dose of 8 Gy from a cobalt source, GFP- C57BL/6 mice received an intravenous injection of approximately 1×10^6 lineage-depleted cells through tail vein. Mice of anti-CTLA-4 group received HSCT combined with intraperitoneal injection of anti-mouse CTLA-4 mAb (clone 9H10, BioXCell) at a dose of 100 μg per mouse. Mice of irradiation group only received irradiation without HSCT. Mice of non-irradiation group received HSCT as described above without irradiation. Mice of control group received saline injection instead of HSCs (Table 1).

2.3. Tracking of infused HSCs

Mice were sacrificed and the main tissues were removed, snap-frozen in Tissue-Tek OCT (SAKURA) and made into cryosections. Nucleus was visualized with DAPI (Roche) staining. The sections were imaged using a fluorescent microscope (Olympus IX71) and photographs were merged with Image Pro Plus 6.0. GFP^+ cells were counted manually in ten random microscopy fields of each section and the number was expressed as mean ± SEM.

2.4. Detection of hematopoietic chimerism

Peripheral blood mononuclear cells (PBMCs) were obtained by density-gradient centrifugation from tail vein blood and stained for $CD3^+$ (T cells) and $CD45RA^+$ (B cells). Cells were incubated with PE-conjugated anti-CD3 (eBioscience) and APC-conjugated anti-CD45RA (Biolegend) for 1 h at 4 °C and resuspended with flow cytometry buffer (PBS with 1% bovine serum albumin and 0.1% sodium azide). Analysis was performed on a FASCalibur flow cytometer (Becton Dickinson) along with appropriate isotype controls. GFP^+ cells in each cell lineage were detected.

2.5. Islet transplantation

Recipient C57BL/6 mice were intraperitoneally injected with streptozotocin (STZ, Sigma) at a dose of 220 mg/kg. Non-fasting blood glucose of tail vein blood was determined using a glucose detector (Accu-Chek, Roche) after STZ administration. Diabetes was defined as blood glucose more than 350 mg/dl. Islets were isolated from BALB/c mice by using collagenase P (Roche Diagnostics) digestion and purified by density gradient separation with discontinuous Ficoll (Sigma). Three days after HSCT, recipient C57BL/6 mice received islet transplantation into the renal subcapsule (500 islets per mouse). Blood glucose was monitored every day after transplantation. Loss of islet allografts was defined as blood glucose more than 200 mg/dl for two consecutive days.

2.6. Histological and immunohistochemistry of islet grafts

Islets grafted kidney was removed, fixed in 4% paraformaldehyde, embedded in paraffin, and prepared into five-micrometer-thick sections. The sections were underwent hematoxylin-eosin staining and lymphocyte infiltration was determined by insulitis scores. Briefly, sections were analyzed by two independent observers who were blind to the experimental conditions. Each observer assessed a minimum of 20 islets per animal. Insulitis was scored as described previously [19]: grade 0, no infiltration; grade 1, peri-islet infiltration, but no intra-islet infiltration; grade 2, mild insulitis (infiltration in less than one-third of the islet area) 3, severe insulitis (infiltration in one-third to half of the

Table 1
Summary of experimental groups.

Groups	Irradiation	HSCT	Islet donor	Recipient	Anti-CTLA-4 mAb
Control	No	No	BALB/c	C57BL/6 (GFP−)	No
Irradiation	8 Gy	No	BALB/c	C57BL/6 (GFP−)	No
Non-irradiation	No	C57BL/6 (GFP^+)	BALB/c	C57BL/6 (GFP−)	No
HSCT	8 Gy	C57BL/6 (GFP^+)	BALB/c	C57BL/6 (GFP−)	No
Anti-CTLA-4	8 Gy	C57BL/6 (GFP^+)	BALB/c	C57BL/6 (GFP−)	100 μg/mouse

HSCT, hematopoietic stem cell transplantation; GFP, green fluorescent protein.