Biochemical and Biophysical Research Communications 436 (2013) 400–405

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Generation of mouse models for type 1 diabetes by selective depletion of pancreatic beta cells using toxin receptor-mediated cell knockout ☆

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ARTICLE INFO

Article history:
Received 21 May 2013
Available online 6 June 2013

Keywords:
Transgenic mice
Diabetes
Diphtheria toxin
Allogeneic transplantation
Xenogeneic transplantation

ABSTRACT

By using the toxin receptor-mediated cell knockout (TRECK) method, we have generated two transgenic (Tg) murine lines that model type 1 (insulin-dependent) diabetes. The first strain, C.B-17/Icr-*Prkdc*^{scid}/*Prkdc*^{scid}-*INS*-TRECK-Tg, carries the diphtheria toxin receptor (hDTR) driven by the human insulin gene promoter, while the other strain, C57BL/6-*ins2*(BAC)-TRECK-Tg, expresses hDTR cDNA under the control of the mouse insulin II gene promoter. With regard to the C.B-17/Icr-*Prkdc*^{scid}/*Prkdc*^{scid}-*INS*-TRECK-Tg strain, only one of three Tg strains exhibited proper expression of hDTR in pancreatic β cells. By contrast, hDTR was expressed in the pancreatic β cells of all four of the generated C57BL/6-*ins2*(BAC)-TRECK-Tg strains. Hyperglycemia, severe ablation of pancreatic β cells and depletion of serum insulin were observed within 3 days after the administration of diphtheria toxin (DT) in these Tg mice. Subcutaneous injection of a suitable dosage of insulin was sufficient for recovery from hyperglycemia in all of the examined strains. Using the C.B-17/Icr-*Prkdc*^{scid}/*Prkdc*^{scid}-*INS*-TRECK-Tg model, we tried to perform regenerative therapeutic approaches: allogeneic transplantation of pancreatic islet cells from C57BL/6 and xenogeneic transplantation of CD34^{+} human umbilical cord blood cells. Both approaches successfully rescued C.B-17/Icr-*Prkdc*^{scid}/*Prkdc*^{scid}-*INS*-TRECK-Tg mice from hyperglycemia caused by DT administration. The high specificity with which DT causes depletion in pancreatic β cells of these Tg mice is highly useful for diabetogenic research.

1. Introduction

Conditional *in vivo* depletion of lineage-specific cells can be achieved through administration of diphtheria toxin (DT) to TRECK-Tg mice that carry a human diphtheria toxin receptor (hDTR) cDNA transgene driven by a tissue-specific promoter. Unlike conditional knockout mice, cell depletion is initiated by DT administration; therefore, the effects of the depletion can be observed in the same Tg strain by comparing cohorts pre- and post-administration of DT [1]. Moreover, the administration of DT to TRECK-Tg mice causes depletion in hDTR expressing cells and therefore specifically eliminates these cells without eliciting an inflammatory response. We succeeded in generating the first TRECK-Tg mouse model for the specific ablation of hepatocytes in the mouse liver under the control of the albumin promoter [2]. This technique has been successfully applied to generate several models for human diseases [2–4] and has revealed several previously unknown *in vivo* cellular functions [5,6].

Type 1 diabetes (T1D) is a major disease that has garnered considerable attention for both children and young adults. T1D is a severe autoimmune disease, and its onset is known to be caused by an immunological destruction of pancreatic β cells, the insulin-producing islet cells [7]. In humans and mice, the etiology of T1D, including the natural diabetogenic agents [8,9] and pathogenic processes that result in T1D, has not been well characterized [10–12]. By contrast, artificial diabetogenic procedures in mice, e.g., alloxan- or streptozotocin-induced T1D models [13,14], are well established, and these models have been widely used for developing drugs for T1D and for pathogenic studies of diabetes-related complications. However, the onset incidences in both

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0006-291X/$ - see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.114

models vary with different mouse strains, sex, administration dose, breeding, etc. In particular, sex- [15] and/or strain differences [16–19] strongly influence the incidence of onset. Therefore, it is difficult to establish optimal conditions for the onset of the alloxan- or streptozotocin-induced T1D. Furthermore, side effects of the drugs, including alloxan- or streptozotocin-induced nephropathy [20] and streptozotocin-induced hepatopathy [21,22], are severe and make model generation difficult.

To overcome the drawbacks of drug-induced T1D models, we generated the Tg mice expressing the hDTR under control of the human *INS* and mouse *ins2* promoters by the TRECK method [2]. hDTR is expressed exclusively on the surface of target cells in the TRECK-Tg mice. Without administration of DT, the Tg mice maintain a healthy condition. Once DT is administered to the Tg mice, the lineage of cells expressing hDTR is severely depleted. We have successfully generated two lines of *ins*-TRECK-Tg mice with different vectors or strains. These lines include C.B-17/Icr-*Prkdc*^{scid}/*Prkdc*^{scid} (SCID)-*INS*-TRECK-Tg mice and C57BL/6 (B6)-*ins2*(BAC)-TRECK-Tg mice. All of the Tg mice were hyperglycemic 3 days after DT administration. Immunohistochemical analysis revealed that hyperglycemia was caused by the depletion of pancreatic β cells. Furthermore, hyperglycemia was cured by the administration of insulin. Ultimately, our evidence supports that these TRECK-Tg mice may be used to model T1D.

2. Materials and methods

An expanded Section 2 is in the online data Supplementary material

2.1. Construction of transgenes

2.1.1. Plasmid vector

Transgene was constructed, as shown previously [2], with the 1.9 kbp human insulin promoter region, rabbit β-globin intron, human HB-EGF L148S/P149T mutant cDNA [23] and the polyadenylation signals of rabbit β-globin and SV40 (Fig. 1A). The 3.8 kbp DNA fragment was excised by double digestion with SphI and XhoI and purified using the QIAquick gel extraction kit (QIAGEN, Valencia, CA, USA) and the Wizard DNA Clean-Up System (Promega, Madison, WI, USA).

2.1.2. BAC vector

A recombinant BAC clone was generated using a recombineering method with galK selection [24]. A mouse BAC clone that contained *ins2* (RP23-92L23) was purchased from BACPAC Resources Center, Children's Hospital Oakland Research Institute (Oakland, USA). To perform the first replacement, the *ins2* coding region in RP23-92L23 was replaced by *galK* as previously described to generate BAC/*galK*. Then, the *galK* insert was replaced by the *hDTR* cDNA to generate BAC/*hDTR* (Fig. 1B).

2.2. Transplantation of pancreatic islets from C57BL/6 mice

Langerhans islets were prepared from the C57BL/6 pancreas. Briefly, intraductal perfusion of pancreases was performed using HBSS containing 1.5 mg/ml collagenase P (Roche, Indianapolis, USA). Langerhans islets were separated by centrifugation using Histopaque-1077 (Sigma–Aldrich, St. Louis, USA) and isolated by hand picking under a dissecting microscope. Two hundred islets were transplanted beneath the renal capsule.

2.3. Transplantation of human umbilical cord blood-derived cells

We have been approved the ethics committee of The Tokyo Metropolitan Institute of Medical Science (approved no. 17-2-3) and the Tokyo Cord Blood Bank (07-11-02) to use human umbilical cord blood. Mononuclear cells in the cord blood were isolated on Ficoll Paque (GE healthcare, Pittsburgh, USA) and processed further to isolate CD34^{+} cells by using a magnetic cell sorter direct CD34 progenitor cell isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Seven days

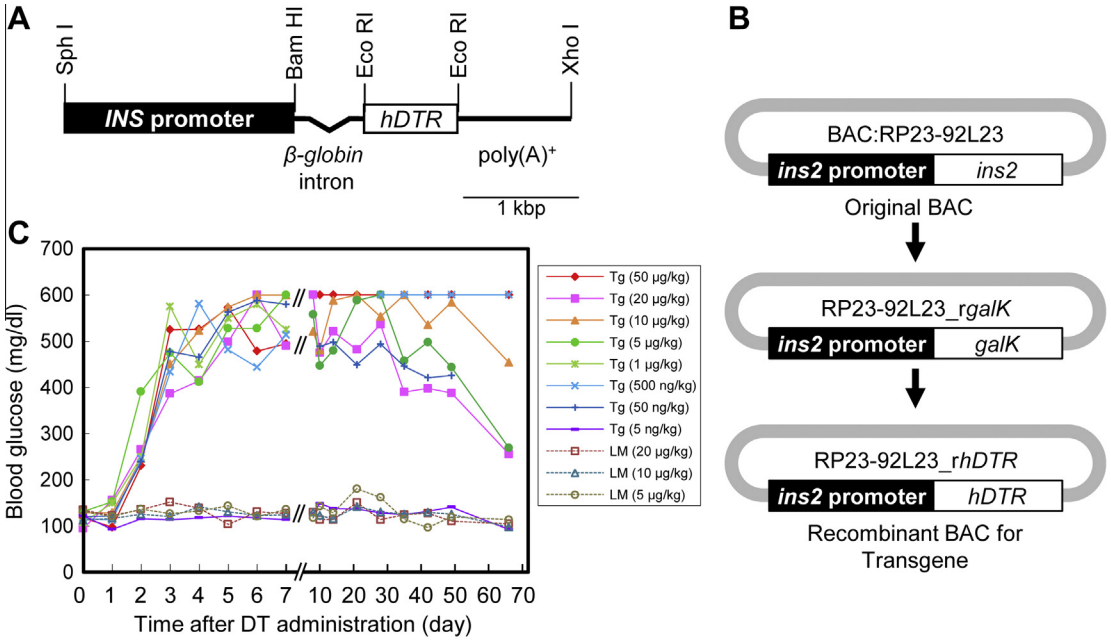

Fig. 1. Generation of a transgene for *ins*-TRECK-Tg mice. (A) Construct of a plasmid-based transgene. The *alb* promoter of the original TRECK cassette was replaced with a SphI/BamHI fragment of the human insulin (*INS*) promoter [2]. (B) Generation of a BAC-based transgene. The DNA fragment from the first exon to the poly(A) signal of a BAC clone carrying the complete mouse *ins2* gene (RP23-92L23) was replaced by the galK cassette. The resultant recombinant BAC (RP23-92L23_r*galK*) was replaced again by the hDTR cassette to finally obtain a recombinant *hDTR* BAC. Only the scaffold structures of the BAC clones are shown. (C) Induction of hyperglycemia in SCID-*INS*-TRECK-Tg mice by DT administration. Mice were intraperitoneally administered the indicated doses of DT at day 0. Then, blood glucose levels were measured. Tg and LM stand for transgenic mice and their littermates, respectively. Values over 600 mg/dl, which is the upper limit of the blood glucose meters, were indicated as 600 mg/dl.