Biomaterials 34 (2013) 5019–5024

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Inhibition of instant blood-mediated inflammatory responses by co-immobilization of sCR1 and heparin on islets

Nguyen Minh Luan, Hiroo Iwata*

Department of Reparative Materials, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-Cho, Shogoin, Sakyo-Ku, Kyoto 606-8507, Japan

ARTICLE INFO

Article history:
Received 17 January 2013
Accepted 14 March 2013
Available online 9 April 2013

Keywords:
Islet transplantation
Instant blood-mediated inflammatory response (IBMIR)
Soluble complement receptor 1 (sCR1)
Heparin
Cell surface modification

ABSTRACT

Intraportal transplantation of islets of Langerhans is followed by marked islet loss, mainly caused by instant blood-mediated inflammatory responses (IBMIR). We previously developed a method of co-immobilizing sCR1 and heparin on islets. Here we examined whether this process could reduce islet loss following intraportal islet transplantation in a syngeneic mouse model. sCR1-heparin islets or unmodified islet controls were transplanted into the livers of streptozotocin-induced diabetic mice. Transplantation of 100 and 125 sCR1-heparin islets normalized blood glucose levels in 8 of 9 (88.9%) and 9 of 9 diabetic mice (100%), respectively, whereas transplantation of 100 and 125 non-treated islets induced normoglycemia in 0 of 9 and 2 of 9 diabetic mice, respectively. Fibrin staining and plasma insulin measurements indicated that, compared to non-treated islets, sCR1-heparin islet transplantation was associated with fewer blood clots around islets, and significantly less insulin leakage from damaged islets at 1 h post-transplantation. Long-term follow-up of the sCR1-heparin islet group showed islet cells in the livers and insulin expression. In conclusion, co-immobilization of sCR1 and heparin on islets could effectively reduce islet damage by IBMIR, and might be useful to enable transplantation with only one donor and one recipient.

1. Introduction

Transplantation of islets of Langerhans (islets) is a promising method for treating type 1 diabetes mellitus [1–3]. In current clinical practice, islets are transplanted into the liver through the portal vein and patients must receive islets obtained from two or three donors to normalize blood glucose levels [4,5]. Transplantation between one donor and one recipient is not sufficient, because a large quantity of islets are destroyed soon after intraportal infusion, leaving less than 30% of islets surviving and functioning [6]. It has been proposed that this post-transplantation islet destruction is mainly due to instant blood-mediated inflammatory reactions (IBMIR) [7], wherein upon contact with blood, the islets trigger activation of the blood coagulation and complement systems. To prevent these inconvenient events, activation of these systems must be inhibited; however, systemic administration of anti-coagulants and complement inhibitors in clinical islet transplantation is impractical as this will make it difficult to control bleeding and complement activation [14].

We previously immobilized sCR1 and heparin onto islets, alternatively utilizing maleimide-PEG-lipid and affinity between sCR1 and heparin [15,16]; the immobilized sCR1 effectively inhibited activation of the complement system, and the immobilized heparin showed a strong anti-coagulant effect *in vitro*. In the present study, we examined the protective effects of these sCR1-heparin layers against IBMIR following intraportal islet transplantation in a syngeneic mouse model.

2. Materials and methods

2.1. Islet isolation

Islets were isolated from pancreases of eight-week-old male C57BL6/c mice (Shimizu Co., Japan) using a collagenase digestion method as described previously [17]. Prior to modification, islets were cultured for two days in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C under 5% CO_2.

2.2. sCR1 preparation

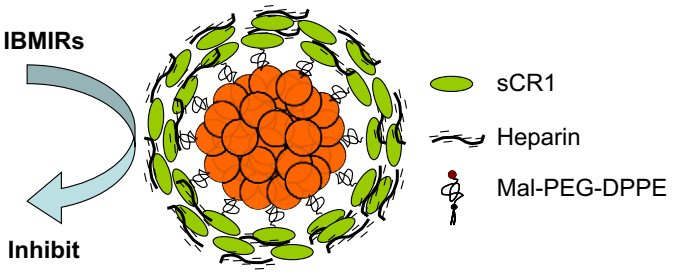
The Chinese hamster ovary (CHO) cell line CRL-10052TM, which produces sCR1, was obtained from the ATCC (Manassas, VA, USA). sCR1 was purified from the culture medium of CHO cells using a heparin column, followed by centrifugal filtration as described previously [18,19], and was stored at 4 °C until use.

2.3. Layer-by-layer immobilization of sCR1 and heparin on islets

sCR1 and heparin were immobilized on the islet surface as shown in Scheme 1 [16]. Briefly, thiol groups were introduced to sCR1 by treatment with Traut's reagent, and the thiol-carrying sCR1 (sCR1-SH) was purified by Sephadex G25 Column.

* Corresponding author. Tel./fax: +81 75 751 4119.
E-mail address: iwata@frontier.kyoto-u.ac.jp (H. Iwata).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.03.041



Scheme 1. Immobilization of sCR1 and heparin on the islet cell surface.

To detect heparin on islets, heparin-carrying FITC (Dojindo Laboratories Co., Ltd., Japan) was used in the modification procedure. FITC-heparin was prepared as described previously [16]. We added 50 μL islet suspension to 2 mL Mal-PEG-DPPE solution (500 μg/mL in PBS), and this mixture was incubated at room temperature for 20 min. After washing three times with MEM, islets were hand-picked and transferred to 2 mL sCR1-SH solution (1 mg/mL in PBS), and this islet suspension was incubated for 40 min at room temperature. The islets (sCR1-islets) were then washed thoroughly with MEM, and were alternatively treated with heparin (1 mg/mL in MEM) and sCR1 (500 μg/mL in MEM) for 10 min and 30 min, respectively. To confirm the presence of sCR1 on the islet surface, modified islets were immunostained using fluorescein isothiocyanate (FITC)-conjugated anti-sCR1 antibody (FITC-J3D3, Dako). Islets immobilized with two double layers of sCR1 and heparin (with heparin as the outermost layer) were used for transplantation experiments.

2.4. Preparation of diabetic mice

Diabetes was induced in eight-week-old male C57BL6/c mice (Shimizu Co., Shizuoka, Japan) by a single injection of streptozotocin (STZ; Nacalai Tesque, Kyoto, Japan). The STZ solution in citrate buffer (13 mg/mL, pH 4.5) was given at a dose of 130 mg/kg after 14–20 h of fasting. Mice with blood glucose exceeding 450 mg/dL on two consecutive days were considered diabetic and used as recipients for transplantation.

2.5. Islet transplantation

Transplantation was performed between syngeneic islet donors and diabetic recipients (all C57BL6/c mice). Two-day cultured islets were randomly divided into two groups. In the experimental group, islets were immobilized with two double layers of sCR1 and heparin (sCR1-heparin islets), while untreated islets were used in the control group. Diabetic mice were anesthetized by inhalation of isofurane using a specialized instrument (400 Anesthesia Unit; Univentor, Malta). Then, 100, 125, or 250 islets were transplanted into the livers of diabetic mice through the portal vein. To evaluate the outcome of the transplantation, blood glucose levels of all recipients were determined every one or two days using the DIAmeter-α glucocard (Arkray, Kyoto, Japan). Blood was collected from the tail vein. Body weights of mice were also recorded.

2.6. Intraperitoneal glucose tolerance test (IPGTT)

At 30 days post-transplantation, the IPGTT was performed on mice transplanted with 100 sCR1-heparin islets to evaluate graft function. Non-diabetic mice were used as references. Mice were fasted for 14–20 h, followed by intraperitoneal injection of a glucose solution (2 g glucose per kg body weight). Blood was collected before glucose challenge and at 10, 20, 30, 45, 60, 90, and 120 min after glucose challenge. Glucose levels were determined using the DIAmeter-α glucocard.

2.7. Analysis of plasma insulin

To evaluate islet damage during early time of post-intraportal infusion of islets, 250 islets with/without sCR1-heparin modification were intraportally transplanted into the livers of diabetic mice. One hour after transplantation, blood was collected from recipient mice. Blood samples from normal and untreated diabetic mice were used as references. All blood samples were kept on ice, then centrifuged at 2500 rpm at 4 °C for 10 min. Plasma was collected and stored at −30 °C until assayed for insulin concentration by ELISA (Shibayagi, Gunma, Japan).

2.8. Immunohistochemical staining

Livers and pancreases of recipient mice were retrieved at 1 h, 50 days, and 200 days after islet transplantation. They were immediately fixed with 10% neutral buffered formalin (Wako), and thin tissue sections (4 μm in thickness) were prepared. Insulin, fibrin, and von Willebrand Factor (vWF) were stained using standard immunohistochemical procedures [20]. Hematoxylin and eosin (H&E) staining was also performed as a counter staining.

3. Results

3.1. Layer-by-layer immobilization of sCR1 and heparin on islets

Two double layers of sCR1 and heparin were immobilized onto the islet surface, as shown in Scheme 1. Fig. 1A shows a confocal microscopic image of immunostaining of sCR1 on islets; clear fluorescence was observed at the islet periphery, indicating sCR1 presence on the islet surface. To visualize heparin adsorption onto sCR1-islets, FITC-heparin was used in the immobilization procedure; green fluorescence was clearly visible after two layers of FITC-heparin were formed, indicating immobilization of heparin on islet surfaces through its interaction with sCR1 (Fig. 1B).

3.2. Syngeneic transplantation of sCR1-heparin islets

To determine the minimal number of islets required to cure chemically induced diabetic mice in a syngeneic transplantation model, 100 or 125 non-treated islets were intraportally transplanted into the livers of diabetic mice. No recipient demonstrated

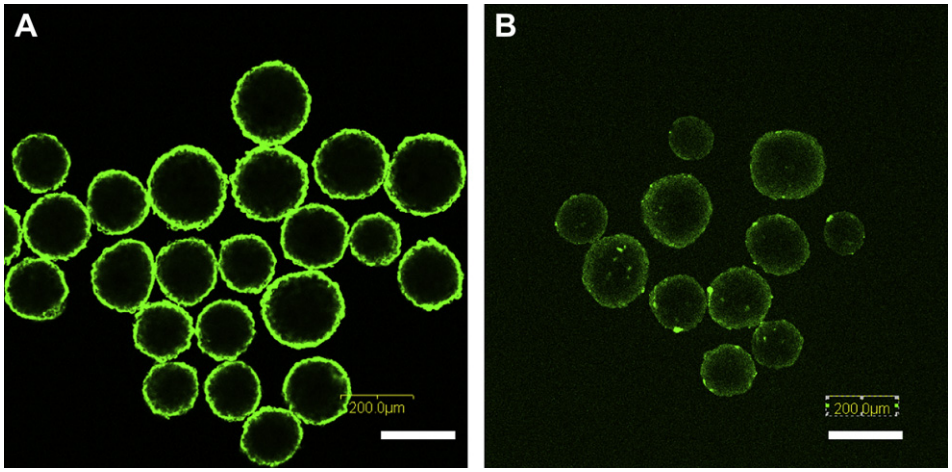


Fig. 1. Layer-by-layer immobilization of sCR1 and heparin on islets. (A) sCR1 on islets. The islets with two double layers of sCR1 and heparin were subjected to immunostaining for sCR1 using fluorescent-labeled anti-CR1 antibodies (FITC-J3D3, Dako). (B) Heparin on islets. FITC-heparin was used to cover islets with two double layers of sCR1 and heparin. The islets were observed under a confocal laser-scanning microscope (Fluoview, FV500, Olympus Optical Co. Ltd., Tokyo, Japan). Scale bar = 200 μm.