Facilitated Engraftment of Isolated Islets Coated With Expanded Vascular Endothelial Cells for Islet Transplantation

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ABSTRACT

Background. Diabetes is complex disease, which involves primary metabolic changes followed by immunological and vascular pathophysiological adjustments. However, it is mostly characterized by an unbalanced decreased number of the β-cells unable to maintain the metabolic requirements and failure to further regenerate newly functional pancreatic islets. The objective of this study was to analyze the properties of the endothelial cells to facilitate the islet cells engraftment after islet transplantation.

Methods. We devised a co-cultured engineer system to coat isolated islets with vascular endothelial cells. To assess the cell integration of cell-engineered islets, we stained them for endothelial marker CD31 and nuclei counterstained with DAPI dye. We comparatively performed islet transplantations into streptozotocin-induced diabetic mice and recovered the islet grafts for morphometric analyses on days 3, 7, 10, and 30. Blood glucose levels were measured continuously after islet transplantation to monitor the functional engraftment and capacity to achieve metabolic control.

Results. Cell-engineered islets showed a well-defined rounded shape after co-culture when compared with native isolated islets. Furthermore, the number of CD31-positive cells layered on the islet surface showed a direct proportion with engraftment capacities and less TUNEL-positive cells on days 3 and 7 after transplantation.

Conclusions. We observed that vascular endothelial cells could be functional integrated into isolated islets. We also found that islets that are coated with vascular endothelial cells increased their capacity to engraft. These findings indicate that islets coated with endothelial cells have a greater capacity of engraftment and thus establish a definitely vascular network to support the metabolic requirements.

OVER the past 15 years, islet transplantation has been transformed from an experimental therapy to an elective therapeutic choice for diabetes mellitus definite treatment. An update of nearly 10 years after islet transplantation with Edmonton protocol reported up to 73% to be c-peptide–positive and 15% insulin-independent, respectively [1]. The long-term results have shown that patients with partial graft function still benefit as c-peptide positive remains within detectable levels. Since then, transplantation significantly contributes to revert the progression of chronic diabetic complications and thus improve the quality of life [1,2]. The objective of our study was to analyze

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the properties of vascular endothelial cells (VECs) to facilitate islet cell engraftment after islet transplantation.

METHODS

Islets and VEC Isolation

All procedures performed on animals were approved by the Institutional Animal Care and Use Committee and were thus within the guidelines for humane care of laboratory animals.

Balb/C mice, weighing 20 g and 10 to 12 weeks old, were used as pancreas donors. Islet isolation was performed with Hank's balanced salt solution (Gibco BRL, Grand Island, New York) containing 2 mg/mL type-V collagenase (Sigma-Aldrich, St Louis, Missouri), 2 mg/mL soybean trypsin inhibitor (Sigma-Aldrich), and 0.2% bovine serum albumin (Sigma-Aldrich), using a modified Gotoh's method with Histopaque 1077–RPMI-1640 medium gradient (Sigma-Aldrich). Islets were hand-picked under a microscope and cultured with RPMI-1640 (Gibco BRL) at 37°C and 5% CO_2 for in vitro analyses. Freshly isolated islets were used immediately for transplantation experiments. Islet viability was evaluated with the use of a Live & Dead detection kit (Molecular Probes, Eugene, Oregon), in accordance with the manufacturer's instructions. Dithizone staining assessed purity of islets, and islet equivalents' yield was determined with the use of a phase-contrast microscopy with a squared calibrated grid. One islet equivalent (IEQ) was equal to a spherical islet of 150 μm in diameter [3].

Moreover, the tissues suspended from the middle density Histopaque 1077 layer were further washed and cultured with RPMI-1640 medium. The resultant adherent cells were gently recovered with Accutase at room temperature for 10 minutes. Samples of these cells were immunostained with FITC-conjugated anti-mouse CD31 antibody (eBiosciences) to verify their phenotype as vascular endothelial cells (VECs) with purity >90% [4].

Integration of VEC Coating the Isolated Islets

The VECs were gently mixed and then incubated, floating 2.5×10^4 cells with 450 IEQ to induce coating of islets in 500 μL of RPMI-1640 culture medium at 37°C for 2 hours. After incubation, the islets coated with VECs were cultured overnight to allow complete integration for further analyses.

Morphological Evaluation and Integration of Islets Coated With VECs

The islets coated with VECs, namely cell-engineered islets, were morphologically evaluated by use of phase contrast light and immunofluorescent microscopy for CD31 with FITC-conjugated and DAPI for counterstaining.

Animal Experiments

Female inbred Balb/C mice, weighing 20 g and 10 weeks old, received a single intraperitoneal injection of 220 mg streptozotocin per kg of body weight. Mice with blood glucose levels ≥360 mg/dL on a minimum of 2 consecutive measurements were selected as recipients. For transplantation, either isolated islets or cell-engineered islets were suspended in 10 μL RMPI-1640–S medium and then transplanted under the kidney capsule of diabetic mice [3,5].

Transplantation Experiments

Balb/c mice recipients of age 8 to 10 weeks were randomly divided into following experimental groups:

1. (G1) (n = 12) diabetic mice marginal receptor of 150 IEQ (native isolated islets)
2. (G2) (n = 12) diabetic mice marginal receptor of 150 IEQ (cell-engineered islets)
3. (G3) (n = 3) diabetic mice treated with saline solution as sham controls
4. (G4) (n = 3) healthy control mice

We performed a comparative morphometric analyses within the recovered grafts on days 3, 7, 10, and 30 after islet transplantation. The analyses were based both on TUNEL and CD31 staining assessments for the recovered grafts tissues. Blood glucose levels were measured in parallel continuously, using a portable glucose meter after islet transplantation to monitor the capacity of metabolic control.

Statistical Analysis

The analyses of variance and the Mann-Whitney *U*-tests were used to calculate the significance between mean values. A *P* value of <.05 was considered to be statistically significant.

RESULTS

Endothelial Cells on the Surface of Islets Facilitate Successful Engraftment

To investigate the capacity of endothelial cells to facilitate engraftment of islets, we comparatively assessed specific markers within the grafts recovered. The follow-up for a successful engraftment was evaluated by the total amount of positive cells immunostained for CD31. On the other hand, the amounts of TUNEL-positive cells were counted to monitor the occurrence of apoptosis.

The number of CD31-positive cells layered on the islet surface showed a direct proportion with engraftment capacities and fewer TUNEL-positive cells from day 3 to day 10 after islet transplantation (Fig 1).

Naturally, the number of cells stained CD31-positive was increased in cell-engineered islets because, when coated with VECs in vitro, they kept rising with CD31-positive cells from 113.33 ± 12.58 on day 0, 147.67 ± 12.64 on day 3, 165.67 ± 13.95 on day 7, 182.33 ± 11.50 on day 10, and 174.67 ± 15.28 on day 30 after islet transplantation Particularly, a few TUNEL-positive cells were detected from 26.58 ± 8.14 on day 3 and 10.89 ± 5.25 on 7, 6.33 ± 3.83 on day 10 and, then reached comparable levels on day 30 4.67 ± 3.79 (G2). In contrast, when (G1) native isolated islets were transplanted, a few endothelial cells were detected initially and greater amounts of TUNEL-positive cells were seen (from 80.25 ± 13.4 on day 3, 45.00 ± 10.25 on day 7, 27.67 ± 9.61 on day 10, and 5.33 ± 3.51 on day 30; Fig 2A).

A Marginal Graft Could Support the Metabolic Demands to Ameliorate Experimental Streptozocin-Induced Diabetes

To assess function of the islet grafts after transplantation, we followed blood glucose levels in animals. The blood glucose levels rapidly decreased after transplantation in both groups (G1 and G2); however, only the islet graft that was engrafted in a timely manner could achieve the

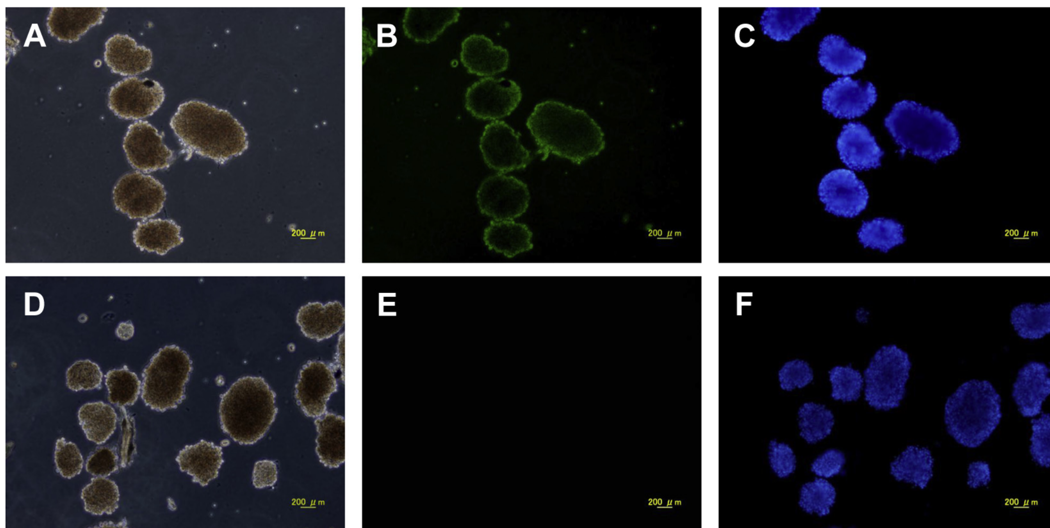

Fig 1. Morphological evaluation and integration of islets coated with VECs. Cell-engineered islets: (**A**) Phase contrast, (**B**) FITC-CD31 immunostaining-positive cells coating the surface of the islets, and (**C**) DAPI nuclei counterstaining. Native isolated islets: (**D**) Phase contrast, (**E**) FITC-CD31 immunostaining, which shows no positive cells on the islets, and (**F**) DAPI nuclei counterstaining.

normoglycemia within acceptable ranges to control diabetes, once revascularization had been completed.

Overall, animals that received isolated islets only showed (G1) showed poor metabolic control with abnormally persistent glucose levels. On the other hand, animals (G2) showed an initial partial function from 198.33 ± 33.52 on day 3 to 134.92 ± 26.32 mg/dL on day 7. After that, glycemic measurements were found within normal parameters, with 117.83 ± 17.37 on day 10 to 115.08 ± 15.05 mg/dL on day 30 for G2 ($P < .01$; Fig 2B). This finding suggests that complete revascularization initiated within 1 week can dramatically affect the outcome from partial control to tight glycemic control within <126 mg/dL (Fig 3).

DISCUSSION

We have previously reported the use of islet-derived fibroblasts as a co-culture system to maintain islet functions [6]. The mechanism underlying its benefits appears to be through secretion of fibroblast growth factor-2 (FGF-2). On these bases, we also used FGF-2 and found beneficial effects on both islets cells and vessels. The blood vessels have a tremendous influence, particularly on the pancreatic islets, because the pancreatic islets have the highest metabolic rate of the entire gland. The natural environment of the islets provides them with a rich vascular supply [5,7]. The vascular network supplies them with oxygen, nutrient delivery, and signals from neighboring and distant tissues [7]. It is well known that during islet isolation, both vascular and surrounding tissues are extensibility disrupted. Therefore, to properly engraft islets and provide function, islets must re-establish an appropriate vascular supply [3,8]. The natural process of revascularization after islet transplantation starts with the residual endothelial cells and progenitors, and it is completed within 2 weeks [9,10]. We first thought that providing them a suitable cell source of endothelial cells it

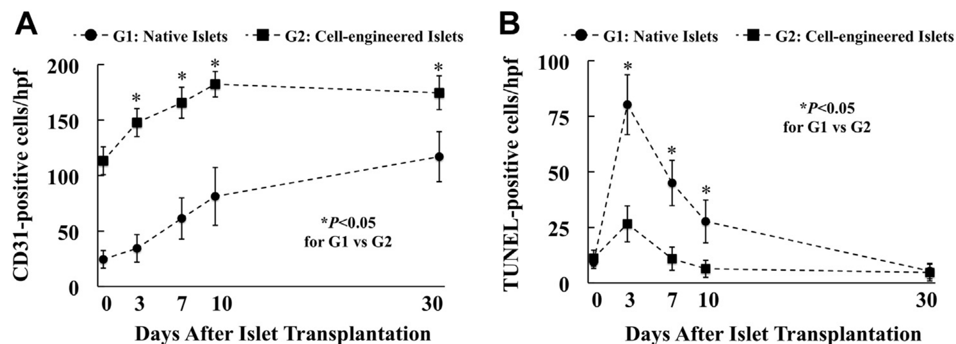

Fig 2. Graphical representation of the amount of biomarkers detected within the recovered grafts. (**A**) Amount of cells that were stained and detected with CD31 antibody from days 3, 7, 10, and 30 after islet transplantation for both G1, native isolated islets, and G2, cell-engineered islets. (**B**) Amount of cells detected in apoptosis with TUNEL labeling TMR from days 3, 7, 10, and 30 after islet transplantation for both G1, native isolated islets, and G2, cell-engineered islets.

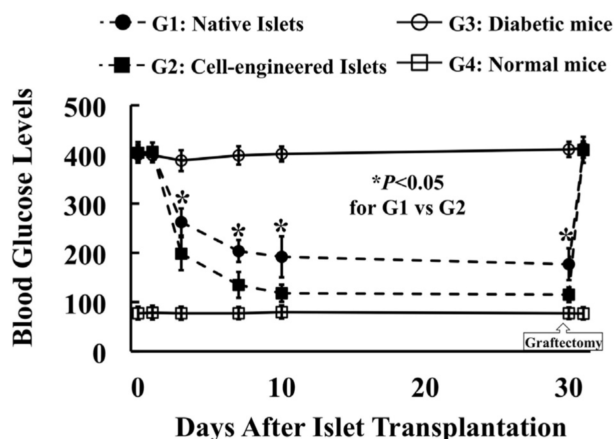

Fig 3. Blood glucose levels after islet transplantation: Glycemic follow-up to monitor the capacity of grafts to maintain the metabolic requirements after islet transplantation.

might speed up the process; however, we also found that coating islets with endothelial cells allows us to facilitate both cellular integration of the islets and vascular engraftment. Our ongoing experiments will be focused on the use of this strategy with an experimental model of intraportal islet transplantation.

In conclusion, we have demonstrated that VECs could be functionally integrated into isolated islets. We also found that once the islets were coated with expanded VECs, their capacity to engraft after islet transplantation increased. These findings indicate that cell-engineered islets have a greater capacity of engraftment and thus establish a definitely vascular network to support the metabolic requirements.

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