Transplantation of Sertoli-Islet Cell Aggregates Formed by Microgravity: Prolonged Survival in Diabetic Rats

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Transplantation of pancreatic islets is a potentially attractive treatment for type I diabetes. We generated the transplantable, tissue-like aggregates composed of Sertoli cells and islets in rotating wall vessel bioreactors, SICA (Sertoli-islet cell aggregates), to improve their biological function *in vitro* and *in vivo*. The isolated islet equivalent and Sertoli cells were purified from Wistar rats and cocultured for 5 days in bioreactor to generate SICA. The SICA, islets aggregates, and fresh isolated islets were transplanted under the kidney capsule of diabetic Sprague-Dawley (SD) rats, respectively. The functions of different grafts were ascertained by blood glucose level measurements and an *in vivo* glucose tolerance test. In response to elevated glucose, insulin secretion from SICA was 1.4-fold higher ($P < 0.05$, $n = 5$) than islet aggregates cultured alone. Of the rats that received SICA, 90% (9/10) remained normoglycemic at 60 days post-transplantation, and the survival significantly increased compared with recipients bearing homotypic islets aggregates or freshly isolated islets. The former responded similarly with healthy rats to the glucose tolerance test. Our results support the usefulness of SICA for the treatment of type 1 diabetes without any immunosuppressive agents. Exp Biol Med 234:595–603, 2009

Key words: Sertoli-islet cell aggregates (SICA); rotating wall vessel (RWV) bioreactor; local immunoprotection; diabetes; islets transplantation

Introduction

The possibility of using xenogeneic islets for transplantation in insulin-dependent diabetes mellitus (IDDM) necessitates characterization of their potential for growth and functional differentiation (1). Allogeneic islet transplants have been successfully used in combination with chronic immunosuppressive agents to prevent the rejection of transplanted cells in diabetic recipients (2–4).While this approach is a breakthrough in islet transplantation, the limitations in tissue availability and the need for continuous systemic immunosuppression restrict the widespread clinical use of allotransplants (5).

For decades the immunoprivileged status of the testis has been appreciated for a variety of transplanted tissues, such as human breast sarcoma, skin, parathyroid fragment, pancreatic islets, and insulinoma cells, all of which survived to various degrees following grafting into the testis (6–9). When relocated into the abdominal cavity, the testis provides an extraordinarily immunoprotective environment for the extended survival of islet grafts (10, 11). More recent studies suggest that the local immunosuppressive and trophic supportive properties, exhibited by the abdominally placed testis, are provided by Sertoli cells. In the past, Sertoli cells have been co-transplanted with islets to provide local immunoprotection for the islet grafts in animals (12–14) and humans (15). However, there are some inherent

This work was supported by the National High Technology Research and Development Program of China (2003AA744051), the Tissue Engineering Special Project of Beijing (H020920040220), Natural Science Foundation of China (30570498), and NASA (NAG9-651, NCC9-130, and NNJ04HC81G).

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Received December 10, 2008.
Accepted February 10, 2009.

DOI: 10.3181/0812-RM-359
1535-3702/09/2345-0595$15.00

problems limiting the usefulness of the facilitation protocol, including difficulties in utilizing primary isolates and the inability to insure and maintain the necessary close proximity of Sertoli cells and islets following transplantation.

In order to overcome these limitations, Cameron et al (16, 17) utilized the NASA-developed rotating wall vessel (RWV) bioreactor system to generate a transplantable, tissue-like aggregate composed of Sertoli cells from SD rats and islets from neonatal pigs. They termed it as Sertoli-islets cell aggregates, or SICA. The different cell types remained viable and retained their differentiated functions, that is, normal insulin secretion by the beta-cells of the islets and immunoprotective and trophic support by the Sertoli cells. RWV bioreactors can provide a continuous low-shear rotation of fluid medium inside the culture vessel. Cells that are suspended in this rotating cell culture system are in continuous free fall at a terminal velocity. Under optimized conditions, the solid-body-like motion of the cells through the medium provides low hydrodynamic shear stress and high efficiency of nutrient transfer (18, 19). The resulting spatial co-location of the cells facilitates formation of macroscopic, viable aggregates and enhances their differentiation into tissue-like constructions (20–22). Rutzky et al (23) transplanted the fresh mouse islets or mouse islets cultured in stationary dishes or microgravity bioreactors to streptozotocin (STZ)-induced diabetic mouse recipients. They found that microgravity condition decreased immunogenicity and significantly improved the function of secretory cells.

Up to now, it is not clear whether the function of SICA generated in RWV bioreactor *in vivo* is better than that of islet aggregates cultured alone in a bioreactor or a mixture of islets and Sertoli cells without coculture in a bioreactor. In this paper we demonstrated that islets and Sertoli cells in SICA were organized together, and the islets retained their insulin-secreting competence. After transplantation into STZ-induced diabetic rats, the viability and function of the islets in the SICA grafts was significantly improved. Indeed, the animals have remained euglycemic for the entire duration of our *in vivo* studies, and the SICA did not elicit obvious allogeneic transplantation rejection, thus alleviating the need for immunosuppressive agents.

Materials and Methods

Animals. Prepubertal male Wistar rats (7–10 days) were used for the isolation of Sertoli cells. Pancreatic islets were harvested from adult male Wistar rats (42–49 days). The recipients were adult age-matched SD rats (42–49 days). Both donor and recipient animals were provided by the Experimental Animal Center of the Beijing Institute of Basic Medical Sciences and kept in the controlled environment with free access to food and water. All experiments were approved by the Animal Experimental Committee of the Beijing Institute of Basic Medical Sciences.

Media. All media used in this study were freshly prepared immediately before use. Sertoli cells were cultured in Medium 199 (GIBCO, USA) supplemented with 2.7g/l sodium bicarbonate, 10% FCS (Hyclone, USA), 100 U/ml penicillin, and 100 mg/l streptomycin. Islets were maintained in RPMI 1640 (GIBCO) supplemented with 2 mmol/l L-glutamine, 10 mmol/l nicotinamide, 2.28 g/l sodium bicarbonate, 100 mg/l penicillin, 100 U/ml streptomycin, and 10% FCS.

Sertoli Cells Isolation and Culture. Testes from prepubertal Wistar rats were collected in Hank's balanced salt solution (HBSS). Sertoli cells were prepared according to Cameron et al (16). Briefly, after removal of the tunica vaginalis propria, tissue from 10 testes was minced into 1-mm pieces with fine scissors and digested for 5 min at 37°C with 10 ml 0.1% type V collagenase (Sigma, USA) in freshly prepared Hank's buffer. The cell-enzyme slurry was added to an equal volume of complete medium (M199 supplemented with 10% heat-inactivated FCS) to terminate enzymatic digestion and then filtered through a 75-μm stainless sieve. The total mixture was centrifuged for 5 min at 87 × *g* (Sigma 3K18). The isolated cells were treated with sterile 0.5 mmol/l Tris-HCl buffer pH7.5 for 1 min to hypotonically remove contaminating germ and red blood cells followed by restoration of the tonicity by adding 1/10 volume of 10× PBS. Following two additional washes with complete M199, the cells were resuspended in fresh complete M199 in T75 flasks and placed for 2 days into the incubator at 37°C in 5% CO_2/95% air.

Islet Isolation and Purification. Pancreases of adult Wistar rats were surgically removed, washed in HBSS, and then carefully minced with scissors to yield tissue fragments <1mm in diameter prior to incubation in 10 ml 0.1% collagenase (Sigma, type V) for 17 min. The digested tissue was filtered through a metal mesh (pore size, 300 μm) and washed with 10% FCS in Hank's solution. The filtrate was centrifuged at 87 × *g* for 5 min, and the supernatant was discarded. The pellet was thoroughly resuspended in 4 ml 32% Dextran 40 (Amersham, USA) and placed in a centrifuge tube. Equal volumes of 27%, 22%, and 11% Dextran 40 were then carefully added sequentially. The samples were centrifuged at 4°C first at 87 × *g* and then at 146 × *g* for 10 min each. The cells at the interfaces between the 11%, 22%, and 22%/27% layers were collected and washed with Hank's solution. The islets were counted and their purity evaluated after staining with Dithizone (DTZ) (24). The final islet yield was assessed by counting two 10-μl aliquots of purified islets two times each and evaluated by converting islet numbers to islet equivalent (IEQ) with an average diameter of 150 μm (25). The viability of the purified islet was determined by acridine orange–propidium iodide (AO-PI) double staining. Under a fluorescent microscope, live cells were in green, whereas dead cells were in red.

Preparation of SICA. Fresh 2000 IEQ islets from a pancreas were cocultured with 1×10^7 Sertoli cells from

prepubertal Wistar rats in a 10-ml RWV bioreactor in the incubator at 37°C and 5% CO_2 for 5 days. The incipient rotational speed of the bioreactor was 15–20 r/min and raised gradually with the increase of the aggregates size. Then fresh 2000 IEQ islets from a pancreas were cultured in a RWV as control.

Glucose Challenge and Radioimmunoassay. After having been cultured in the RWV bioreactor for 5 days, homotypic islets aggregates formed by 2000 IEQ islets and heterotypic aggregates (SICA) formed by islet and Sertoli cells were collected and assayed for basal and glucose-stimulated insulin release. The *in vitro* function was assayed by three consecutive incubations of 45 min each in KRB supplemented with 3.3 mmol/l, 16.7 mmol/l, and 3.3 mmol/l glucose, respectively. Each experiment was performed three times. The supernatants were collected and frozen (−20°C) for subsequent insulin assay. Insulin secretion in the supernatant was assayed using a commercial RIA kit (Sensitive Rat Insulin RIA Kit, LINCO Research, Missouri, USA) as previously described (26). Stimulation index (SI) was calculated by dividing the mean insulin release during high glucose stimulation (16.7 mmol/l) by the mean insulin release during lower glucose stimulation (3.3 mmol/l). Data are expressed as mean ± SEM of at least three independent experiments.

Electron Microscopic Observation. SICA were routinely processed for ultrastructural analysis and either stained with uranyl acetate/lead citrate for transmission electron microscopy or dried with hexamethyldislazane for scanning electron microscopy.

Histology and Immunohistochemistry. Histological analysis was performed according regular H&E procedure. Immunohistochemical analysis was performed using primarily cultured Sertoli cells or cryosections (10 μm) of SICA. FasL antibody was used to identify Sertoli cells (27), and insulin was used to identify islet beta-cells using a commercial streptavidin-biotin kit (SABC, Boster, WuHan, China). Briefly, the sections were treated with 3% H_2O_2 for 10 min to block endogenous peroxidase and 5% BSA for 20 min to block non-specific staining. The samples were reacted with primary antibodies against FasL (1:100, rabbit polyclonal antibody, sc-834, Santa Cruz, USA) or insulin (1:100, mouse monoclonal antibody, ZhongShan, Beijing, China) overnight at 4°C. After washing with PBS (three times for 15 min each), biotin-conjugated secondary antibody was added and incubated for 30 min at 37°C. After three washes (as above), the samples were incubated with streptavidin-horseradish peroxidase for 30 min at 37°C. The reaction products were detected using DAB as substrate. All immunostained slides were examined under an Olympus BX51 microscope (Olympus, Japan).

***In Vivo* Transplantation.** The SD rats were rendered diabetic by injecting STZ (50 $mg \cdot kg^{-1}$, Sigma) 7–10 days prior to transplantation. Rats with glucose levels greater than 22.2 mmol/l in two consecutive measurements were considered to have developed diabetes. There were three experimental groups. Group 1: diabetic SD rats receiving 2000 IEQ and 1×10^7 Sertoli cells aggregates (SICA) cocultured in the bioreactor for 5 days ($n = 10$); Group 2: diabetic SD rats receiving 2000 IEQ islets cultured alone in the bioreactor for 5 days ($n = 12$); Group 3: diabetic SD rats receiving 2000 IEQ fresh isolated islets ($n = 11$). The grafts were transplanted under the left kidney capsule of the recipients. Glucose levels were measured in blood collected from a tail vein three times per week using a One Touch Basic Plus blood glucose meter (Lifescan, Milpitas, USA). Successful cure of diabetes was defined as lowering glucose levels to less than 7.0 mmol/l in two consecutive measurements. A blood glucose level of more than 11.4 mmol/l was defined as a reappearance of diabetes. Nephrectomy of the graft-bearing kidney and reappearance of hyperglycemia was used to ascertain the function of the grafts.

Glucose Tolerance Test. Euglycemic SICA recipient rats ($n = 9$) that had normal glucose levels for 30 days postimplantation were fasted for 4 hr and injected intraperitoneally with 2.0 $g \cdot kg^{-1}$ of glucose. Blood glucose levels were analyzed at 0, 15, 30, 45, 60, 75, 90, 105, and 120 min postinjection. As a control, glucose tolerance tests were also performed in untreated healthy rats ($n = 9$), STZ-treated diabetic rats ($n = 5$), and healthy rats receiving SICA grafts ($n = 9$). The results were presented as mean blood glucose level (mmol/l) ± standard deviation (MBS ± SD).

Cell Composition of SICA. In this study, we examined the cell compositions of SICA grafts by immunohistochemistry at 8 h posttransplantation (day 1), at 12–18 days posttransplantation in rats in which diabetes recurred (within the previous 24 h), and at 12–18 and 30 days posttransplantation in rats that were still normoglycemic.

Grafted SICA were also immunostained with antibodies against insulin, CD45, FasL, and TGF-β1 according to the procedures described above when recipients remained euglycemic for 30 days.

Graft Cell Preparations. SICA grafts were removed from the renal subcapsular spaces, placed in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (Life Technologies), and kept on ice; they were then transferred into Eppendorf tubes containing 50-μl Ca2+/Mg2+–free phosphate-buffered saline (PBS) with 0.2 mg/ml EDTA (cell dissociation buffer) (Life Technologies). Grafts were processed separately from each rat. While on ice, the grafts were cut into small pieces with fine scissors, disrupted mechanically by syringe injection through progressively narrower gauge needles, and dissociated into single cells by incubation in the cell dissociation buffer at 37°C for 20 min.

Immunohistochemical Studies. Cells isolated from SICA grafts were identified by immunohistochemistry. Cell preparations were stained in duplicate with each test or control antibody, and 1,000 cells were scored blindly by two independent observers who each scanned 60 different microscopic fields (oil immersion, 100×). The quantity of

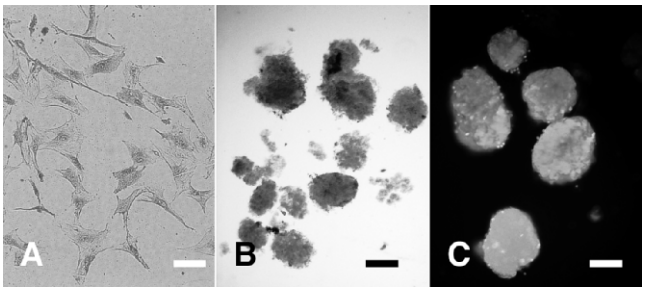

Figure 1. Identification of Sertoli cells and islets isolated from Wistar rats. A: FasL immuno-staining of primary Sertoli cells isolated from the testes. Bar = 30 μm. B: Dithizone staining showed islets were in crimson red. Bar = 90 μm. C: AO-PI double staining showed the viability of purified islets. Live cells were stained in green, and dead cells were in red under a fluorescent microscope. Bar = 90 μm.

positive cells in the first day was defined as 100%, the baseline that other time points were compared with. Significance was determined by analysis of variance followed by Tukey-Kramer's test.

Results

Identification and Viability of Testicular Sertoli Cells and Pancreatic Islets. We isolated purified Sertoli cell populations from the testes of prepubertal Wistar rats with an average purity of 95%. Most of the primary isolates of Sertoli cells were immunopositive for antibody against FasL (Fig. 1A). The purified cells or cell clusters (average purity over 90%) were stained crimson red by DTZ (Fig. 1B), while acinar and ductal tissue failed to incorporate the stain. The typical results confirmed the existence of pancreatic beta-cells in our preparation. The viability of the isolated islets was 95% ± 2%, as assessed by AO-PI double staining in which live islets stained green and the dead stained red (Fig. 1C). From each Wistar rat, the yield of islets was on the average 950 ± 24 ($n = 8$) IEQ.

Morphology and Immunohistochemical Analysis of SICA. The aggregates became larger and more compact by day 3 (Fig. 2A). By day 5, the aggregates had reorganized into pycnotic and homogeneous spherical structures (Fig. 2B). Since the size and spherical shape of the aggregates appeared to stabilize by day 5, collection, morphology, and assay of the formed aggregates were performed on those harvested following 5 days of incubation.

The HE staining (Fig. 3A, 3B) and scanning electron microscopy analysis staining indicated that the SICA were generally spherical in shape (Fig. 3E) In SICA, Sertoli cells were characterized by one or more indentations along the course of the membrane and a filamentous zone just inside and associated with the inner surface of the nuclear membrane (Fig. 3C). The islets had many insulin secretion granules inside the SICA (Fig. 3D). The most significant observation in SICA was a tight junction complex (Fig. 3F, 3G, 3H).

The immunohistochemical staining of SICA revealed

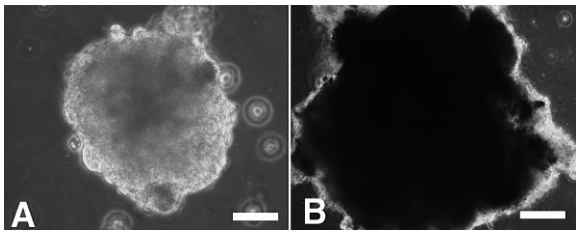

Figure 2. Phase contrast microscopic observation of SICA. A: SICA cultured in RCCS for 3 days. Bar = 500 μm. B: SICA cultured in RCCS for 5 days. Bar = 250 μm.

cells that stained positively for insulin and FasL (Fig. 3C), indicating the presence of immunoreactive beta-cells and Sertoli cells, respectively.

***In Vitro* Glucose Challenge Test.** Samples of SICA and homotypic islets cell aggregates were collected and assayed for insulin, following a glucose challenge. Insulin secretion was expressed in ng/ml, and the SI was calculated by dividing insulin output during high glucose (16.7 mmol/l) stimulation by insulin output during basal glucose (3.3 mmol/l) incubation. The SI of SICA was 1.45 ± 0.2 above basal control and higher than that of homotypic islets cell aggregates ($P < 0.05$, $n = 5$) (Fig. 4). Upon return to basal glucose medium conditions, the SI in both homotypic and heterotypic returned close to control values. No insulin was detected in Sertoli cell aggregates under basal conditions or upon exposure to elevated glucose (data not shown).

***In Vivo* Function of Different Grafts.** Homotypic islets aggregates formed by 2000 IEQ, 2000 IEQ fresh isolated islets, and SICA formed by 2000 IEQ and 1×10^7 Sertoli cells were transplanted under the left kidney capsule of diabetic SD rats, respectively. The rats that received SICA grafts, 90% (9 of 10) remained normoglycemic at 60 days posttransplantation (Fig. 5). All diabetic animals receiving homotypic islet aggregates allografts alone, 83% (10 of 12) achieved euglycemia within 2–7 days, but returned to a diabetic state (blood glucose levels returned to >11.4 mmol/l) by 20–30 days posttransplantation and then died. The diabetic rats bearing fresh islets kept euglycemia for 8–10 days.

Insulin Immunostaining. Kidneys bearing SICA grafts of diabetic SD rats were removed 30 days posttransplantation and evaluated by HE staining and immunostaining for insulin. As seen in Figure 6A, there were obvious grafts in kidneys at 30 days posttransplantation. There was also evidence for cells staining positive for insulin in kidneys bearing SICA (Fig. 6B).

***In Vivo* Glucose Tolerance Test.** Glucose tolerance tests performed 30 days posttransplantation showed significant functionality of the grafted SICA (Fig. 7). As expected, no evident change in blood glucose level was seen in the diabetic SD rats without treatment. STZ-treated animals bearing SICA responded appropriately to the glucose tolerance test. Their blood glucose levels were

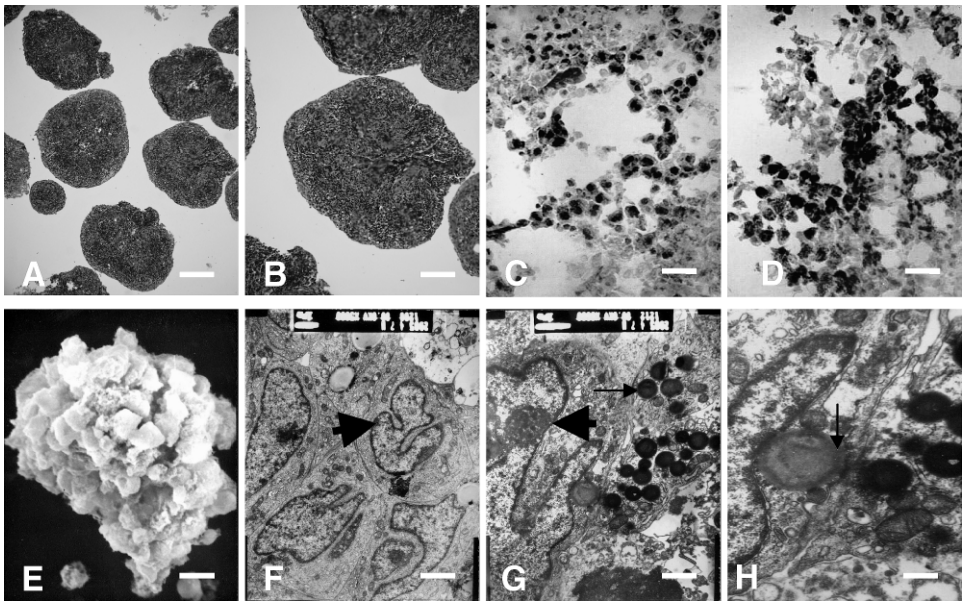

Figure 3. Histological and immunohistochemical analysis and ultrastructure of SICA. A and B: HE staining of SICA. C: SICA stained by FasL. D: Insulin staining of SICA. E: Scanning electron microscopic observation of the surface of SICA. F: Transmission electron microscopic observation of the ultrastructure of SICA. Sertoli cells (▶) were characterized by one or more indentations along the course of the membrane and a filamentous zone just inside and associated with the inner surface of the nuclear membrane. G: Ultrastructure of SICA. Cells in islets had many secretion granules (→), and Sertoli cells showed one or more indentations along the course of the membrane (▶). H: Tight junction (→arrow) between Sertoli cells and islet cells. Bars: 200 μm (A), 100 μm (B), 60 μm (C, D), 10 μm (E), 2 μm (F), 0. 5 μm (G), 0.2 μm (H).

transiently elevated from 5 mmol/l to 12 mmol/l and returned to normal 2 hours postinjection, which was similar to healthy rats without treatment.

Cell Compositions of Islet and Sertoli Cell Grafts. Cellular compositions of SICA grafts that prevented diabetes recurrence were compared with compositions of grafts that failed to maintain normoglycemia. At 12–18 days posttransplantation, insulin-positive cells were significantly decreased, and leukocytes were significantly increased in islet grafts of diabetic rats compared with values in islet grafts initially transplanted. In contrast, insulin-positive cell numbers were maintained in high levels, and leukocytes were significantly less numerous in SICA grafts of normoglycemic rats than diabetic rats at 12–18 days and at 30 days posttransplantation. Notably, FasL expression by Sertoli cells decreased significantly from 98 ± 5% at the time of transplantation to 40 ± 3% in diabetic rats and 35 ± 3% in normoglycemic rats at 12–18 days posttransplantation. At 30 days posttransplantation, FasL expression was very low in normoglycemic rats (29 ± 2%). In contrast to FasL, TGF-β1 expression by Sertoli cells remained high (>60%) after transplantation in all groups of rats (Fig. 8).

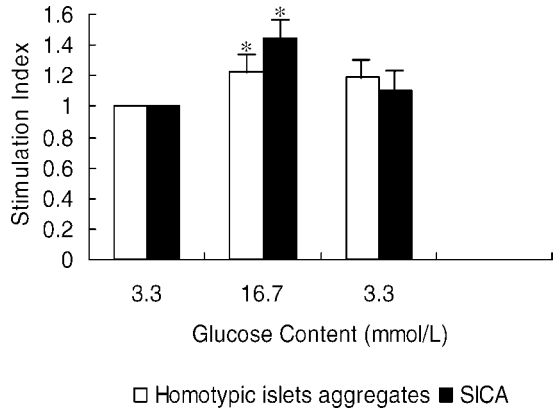

Figure 4. Secretory response of homotypic islets aggregates and SICA to glucose stimulation. Stimulation Indices (SI) were calculated by dividing the amount of insulin released at 16.7 mmol/L glucose by that released at 3.3 mmol/L glucose. The SI of SICA is 1.45 ± 0.2 and higher than that of homotypic islets aggregates at 16.7 mmol/L glucose (*$P < 0.05$, $n = 5$).

Discussion

The failure of pancreatic islet allotransplants in all clinical trials to date is caused by various factors such as poor initial islet function, graft rejection, and side effects of immunosuppressive agents. To address these problems we implanted functional heterotypic 3-D tissue-like constructs, SICA, in a rodent model. SICA were generated *in vitro* by coculturing prepubertal rat Sertoli cells and adult rat islets for 5 days in RWV bioreactors. The ultrastructure of SICA, assessed by TEM and immunostaining, showed tissue-like constructs, co-mingled Sertoli cells, and islets. Upon transplantation of SICA constructs, the recipient diabetic rats remained euglycemic throughout the duration of our study (>60 days to date). We transplanted the same amount of cells, viz, 2000 islets and/or 1×10^7 Sertoli cells, into recipients as did Korbutt et al (13). The major difference

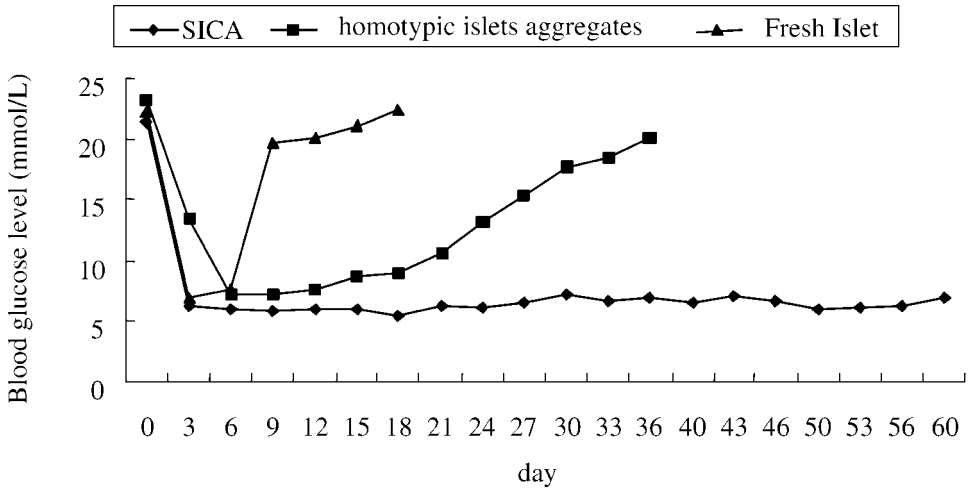

Figure 5. Blood glucose levels of recipients at different time points after transplantation of SICA, homotypic islets aggregates, or fresh islets. ♦: Diabetic SD rats treated by SICA ($n = 10$); ■: Diabetic SD rats treated by homotypic islets aggregates ($n = 12$); ▲: Diabetic SD rats treated by fresh islets ($n = 11$).

was that we cultured or cocultured the grafts in the RWV bioreactors for 5 days before transplantation.

In line with several prior studies that demonstrated that Sertoli cells can create immunoprivileged sites outside the testis (28), we cocultured the islets with Sertoli cells in the RWV bioreactor to provide local immunoprotection for the graft. Dufour and colleagues results showed Sertoli cells were able to prolong the survival of islet xenografts when combined with anti-mouse lymphocytes (29). Luca et al (30) strongly suggest that neonatal porcine cell clusters that are considered for human islet substitutes pretreatment in simulated microgravity with Sertoli cells may enhance the transplantation success of neonatal porcine cell clusters in the diabetic recipient. Selawry and Cameron (12) cografted rat Sertoli cells with isolated islets beneath the rat kidney capsule. However, they used a short course of an immunosuppressant, cyclosporin A, to fully exploit the immunoprotective traits of Sertoli cells. Dufour et al (31) examined the difference between successful and rejected islet/SC cografts in order to further improve this procedure for optimal extension of islet allograft survival, and their results showed a consequence of long-term graft acceptance is the formation of SC tubule structures, which may be an additional requirement for optimal protection of islet allografts. Sertoli cells provided two key functions in transplantation protocol: local immunosuppression and nutritional support. Although the precise mechanisms are not yet clear, it is evident that Sertoli cells secrete numerous bioactive proteins, some of which are known to have immunosuppressive capabilities and the others are well-known trophic factors that promote cell growth and differentiation (32). Several mechanisms have been suggested. For example, the expression of FasL may down-regulate the activated T-lymphocyte population by FasL-Fas–induced apoptosis, thereby protecting the graft from the cytotoxic effects of FasL-positive invading immune cells (33). Additionally, Sertoli cells may secrete one or more protein(s) that suppress the proliferation of activated T-lymphocytes by competitive binding of IL-2 receptors on the lymphocytes and/or suppress lymphocytic IL-2 secretion. This latter possibility for immunoprotection is reminiscent of the mechanism of action of cyclosporin A, which also suppressed the production of IL-2 (34, 35). Because of their unique immunosuppressive/trophic properties, Sertoli cells have also been used in both neuronal allografts and xenografts into the rat brain. For example, Othberg (36) found that Sertoli cells provided trophic support of rat and human ventral mesencephalic cells and hNT neurons in vitro. Furthermore, Willing et al (28, 37) demonstrated that Sertoli cells could decrease microglial response and increase engraftment of human hNT neurons in the hemiparkinsonian rat striatum.

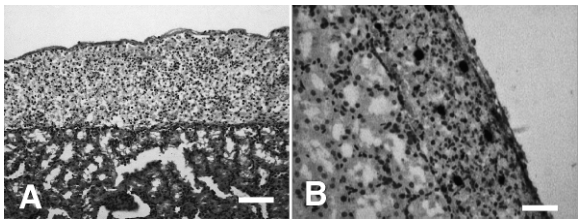

Figure 6. Histological and immunohistochemical analysis of kidney bearing SICA grafts. A: H&E. staining of diabetic rat kidney capsule bearing SICA graft. B: Insulin immunohistochemical staining of the diabetic kidney capsule bearing SICA graft showed insulin-positive islets. Bar = 30 μm.

However, there are some inherent problems limiting the usefulness of the facilitation protocol, such as the difficulty in utilizing primary isolates and the inability to ensure and maintain the necessary close proximity of Sertoli cells and islets following transplantation.

Rutzky et al (23) found that culturing islets in RWV bioreactors could a) decrease their immunogenicity by

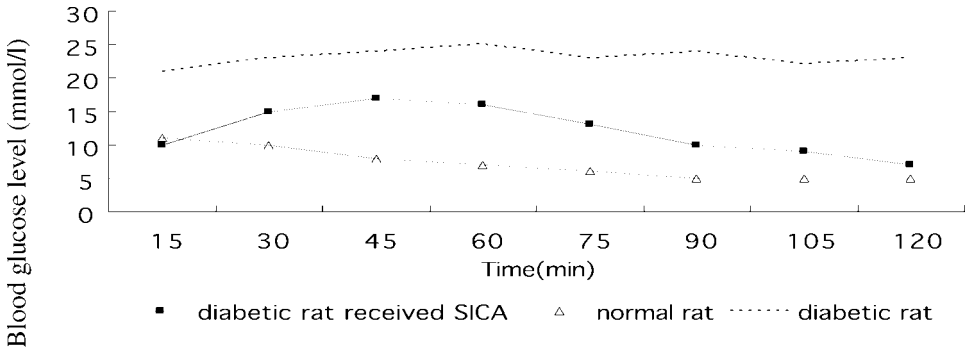

Figure 7. Glucose tolerance tests. Glucose tolerance tests were performed in normal SD rats, diabetic SD rats, diabetic, and normal SD rats with SICA grafts. The rats were challenged by intraperitoneal injection of 2.0 $g \cdot kg^{-1}$ glucose. Blood glucose levels were analyzed every 15 min for 2 hours.

eliminating the class II MHC-expressing dendritic cells, b) improve the secretion function by forming multiple nutritional channels between individual islets, and c) fewer bioreactor-cultured islets were necessary to produce euglycemia. Cameron et al (16) cocultured isolated pig islets and rat Sertoli cells in the RWV bioreactors for 5 days to form SICA. They found that SICA retained the ability to secrete insulin when exposed to elevated glucose *in vitro*. But there is no experimental evidence to prove that the SICA can work well *in vivo*.

To achieve successful islet transplantation without any immunosuppressive agent and prolonged survival time, we made two significant changes in the experimental approach pioneered by Selawry and Cameron, Rutzky, and Korbutt. First, they merely implanted the mixture of Sertoli cells and islets, whereas we implanted the tissue-like Sertoli-islet cell aggregates cocultured under microgravity in the RWV bioreactor. In the aggregates, the Sertoli cells contacted with islets tightly, which may be helpful for their interaction. Second, they used immunosuppressive agents, but we did

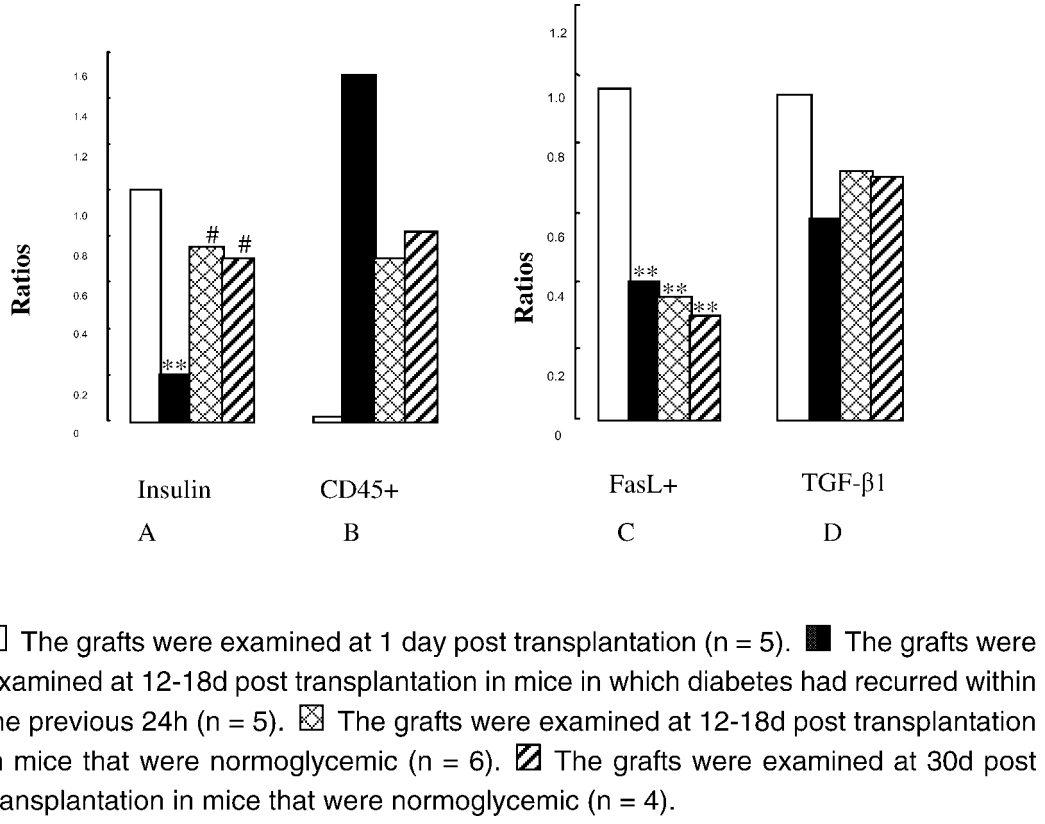

Figure 8. Cell composition of SICA grafts. A: The ratios of the number of insulin-positive cells in the whole. B: The ratios of the number of CD45-positive cells in the whole. C: The ratios of the number of FasL-positive cells in the whole. D: The ratios of the number of TGF-β1–positive cells in the whole. Values are means ± SE. $^{**}P < 0.01$ vs. day 1 posttransplantation; $\#P < 0.01$ vs. days 12–18, diabetic.

not. From the above differences, we can summarize that the novelty of this work is that we cocultured the Sertoli cells and islets under microgravity environment before transplantation and hence attenuated the immunogenicity of the islets. In the aggregates, the diverse cell types retained their differentiated functions, namely normal insulin secretion by pancreatic beta-cells and immunoprotective and trophic support by the testicular Sertoli cells.

In the SICA, the Sertoli cells spread mainly around the outside of islets, while some also went to the inside of islet. The aggregates have a tissue-like constructs. Sertoli cells outside the SICA play an important role of immunologic barrier, and Sertoli cells inside the SICA not only improved the connection of the Sertoli cells and islet, but also helped to supply nutrition to the inner islets. Although this impaction may change the natural structure of islets, the two properties resulted from the specific structures could be more helpful to improve the function and viability of islets. Taken together, our approach decreased the rejection of the grafts and improved the function of islets. In addition, the coculture in RWV bioreactors appears to optimize the immunoprotective/trophic effects of Sertoli cells as evidenced by the prolonged survival and function of the transplanted tissue-like constructs.

We also investigated two immunoregulatory products of Sertoli cells, FasL and TGF-β1, as possible mediators of the protective effects of these cells against autoimmune destruction of islet β-cells. Our findings indicate that FasL may not qualify as a mediator of the protective effects of Sertoli cells against autoimmune β-cell destruction. Thus, immunohistochemical analysis of SICA grafts revealed that FasL was highly expressed by Sertoli cells initially implanted in diabetic NOD rats; however, this decreased progressively after transplantation. At 30 days posttransplantation, FasL expression was very low (only 29 ± 2%). Nevertheless, despite this loss of FasL expression by the Sertoli cells, the rats were normoglycemic. In contrast, TGF-β1 cells were maintained at high levels at posttransplantation. Suarez-Pinzon et al (38) found that testicular Sertoli cells protect islet β-cells from autoimmune destruction in NOD rats by a TGF-β1–dependent mechanism. Our results suggested that TGF-β1 maybe play an important role in protecting islet β-cells from autoimmune destruction.

There is great potential to use xenogeneic islets for transplantation in treatment of type I diabetes. In previous studies, most researchers cocultured the islets and Sertoli cells *in vitro* and assessed the viability of the islets. Even some of them transplanted the mixture of the islets and Sertoli cells to the diabetes rats; however, it resulted in failure because of the immunological rejection. By utilizing the immunoprotective and trophic properties of Sertoli cells and the unique cell/tissue culture environment of RWV bioreactors, we have been able to significantly improve the viability of allogeneic islet grafts upon implantation and prevent their rejection without having to use any immunosuppressive agents. In conclusion, this study demonstrated feasibility toward overcoming the main limitations of islet transplantation therapy.

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