

Regulatory T Cells Promote Pancreatic Islet Function and Viability via TGF- β 1 *in vitro* and *in vivo*

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조절 T 세포 유래 TGF- β 1에 의한 췌장섬세포의 기능 및 활성 증가

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Regulatory T cells (Treg), known as immune-suppressors, may help modulate the immune response. In this study, we investigated the effect of Treg-derived TGF- β 1 on pancreatic islet cell function *in vitro* and *in vivo*. One hundred eighty IEQ (islet equivalents) of pancreatic islets, the marginal amount to regulate blood glucose level after syngeneic islet transplantation in mouse type 1 diabetes (T1D) model, were co-cultured with 4×10^6 Treg cells for 48 hours. The changes in TGF- β 1, interleukin-6 (IL-6), and insulin secretion levels were measured and analyzed among the Treg-only group, the islet-only group, and the Treg/islet co-cultured group. In the Treg/islet co-cultured group, IL-6 and insulin secretion levels were increased ($P < 0.0005$, $P < 0.005$) and islet viability was improved ($P < 0.005$) compared with the islet-only group. Furthermore, after transplantation, the co-cultured islets regulated blood glucose levels efficiently in the T1D mouse model. These data suggest that Treg could improve islet functions and viability via the TGF- β 1 secretion pathway ($P < 0.05 \sim 0.005$), thus the use of Treg in islet transplantation should be explored further.

Key words: Interleukin 6, Islet, Regulatory T cell, TGF-beta, Transplantation

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INTRODUCTION

As a multifunctional cytokine, transforming growth factor β 1 (TGF- β 1) plays various roles in many cellular differentiations and in immune regulation [1]. TGF- β 1 regulates inflammatory responses through the control of activation or chemotaxis, and survival of various immune cells such as lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes [2, 3].

TGF- β 1 molecules are also expressed in the leukocytes [2]. And early studies showed that activated T cells produce TGF- β 1 [4]. Some later studies of oral tolerance models revealed that a subpopulation of CD4⁺ T cells, named Th3 cells, express TGF- β 1 as an effector cytokine [5]. TGF- β 1 produced by Th3 cells has been proposed to inhibit Th1 and Th2 cell differentiation and helps B cells to produce IgA [6]. The regulatory T cells (Tregs) express surface TGF- β 1, which mediates their suppressor function [7-9].

However, the role of TGF- β 1 in Treg function is still controversial. Because TGF- β 1^{-/-} Treg cells still inhibit proliferation of responder T cells *in vitro*, and this result indicates that TGF- β 1 synthesis by Tregs is not required for suppression under these culture conditions [10]. The protective activity of Treg cells against inflammatory bowel disease induced by transfer of CD4⁺/CD45RB^{high} T cells into severe combined immunodeficiency (SCID) mice is reversed by the treatment of the recipients with anti-TGF- β 1 antibody [11]. However, in an autoimmune gastritis model, Treg-mediated protection is not affected by the same treatment [10]. Even in the same colitis model, TGF- β 1^{-/-} Treg cells were shown to be protective or inactive depending on experimental systems [12-14].

Some researchers even reported that TGF- β 1 is also related to the conversion and induction of Treg from naïve T cells [15, 16]. And it is also reported that TGF- β 1 regulates the development and function on type 1 diabetes (T1D) mice of natural Treg (nTreg) *in vivo* [17]. However, these investigations are opened to be disputed, because recent other report suggested that TGF- β 1 mediated Treg induction is just available for Treg phenotypes, not functioned [18]. On the other hands, it is the present progress that these reports suggest that TGF- β 1 may also promotes pancreatic islet function by paracrine, autocrine, or both effects, thus we focus on the contribution of TGF- β 1 produced from Treg to pancreatic islet cell survival and function.

In our experiments, more TGF- β 1 condition is required for the better function and viability of islet *in vitro* and *in vivo*. When islet cells were not exposed to Treg, insulin secretion and islet cell viability level was lower, while the addition of TGF- β 1 during Treg co-culture improved the expression of interleukin-6 (IL-6), insulin and cell viability levels *in vitro*. Moreover, Treg co-cultured islet regulated the blood glucose levels of T1D mice more efficiently. Those might be resulted from the data that TGF- β /Smad3 signaling regulates insulin gene transcription and pancreatic islet beta cell function [19]. TGF- β signaling plays as a promoter in pancreatic development although the role of this pathway in the adult pancreas is obscure. Smad3

deficiency results in improved glucose tolerance and enhanced glucose-stimulated insulin secretion *in vivo* [19]. It was also reported that neutralization of TGF- β 1 and Treg characters (CTLA-4, GITR, or CD25) had no effect on inflammation reduction in diabetic islets [20], islet graft can prolong via a TGF- β 1 and Treg-dependent mechanisms in allograft survival [21]. On the other hand, TGF- β /Smad4 signaling was reported that E-dnSmad4-transgenic mice show an age-dependent increase in the size of islets, in parallel, an expanded population of replicating cells expressing the E-dn Smad4 transgene is found in the stroma between the enlarged islets and pancreatic ducts [22]. Despite the marked enlargement, E-dnSmad4 islets contain normal ratios and spatial organization of endocrine cell subtypes and have normal glucose homeostasis [22]. Replication of cells derived from primary duct cultures of wild-type mice, but not E-dnSmad4 mice, was inhibited by the addition of TGF-beta family proteins, demonstrating a cell-autonomous effect of the transgene. These data show that, in the adult pancreas, TGF- β family signaling plays a role in islet size by regulating the growth of a pluripotent progenitor cell residing in the periductal stroma of the pancreas. These reports suggest that TGF- β 1 may also influence pancreatic islet functions. Thus we focus on the contribution of TGF- β 1 produced from Treg to pancreatic islet cell survival and function.

The induction of immune tolerance by suppressive effect of Treg is well known. Here, we suggest that Treg cells may also promote pancreatic islet viability and function by TGF- β 1 secretion. It is different scheme from other reports, and proposes the effective value of Treg in clinical syngeneic transplantation.

MATERIALS AND METHODS

1. Mice

Inbred C57BL/6 mice, aged 6 to 10 weeks, were purchased from Charles River Laboratories, Inc. (Wilmington, MA, USA) and fed standard rodent chow (PMI Nutrition International, St. Louis, MO, USA) in sterile cages in a

barrier animal facility under a 12 h light/dark cycle. Selected C57BL/6 mice (6–8 week-old) were sacrificed, and their spleens were harvested to isolate Treg cell population. And other C57BL/6 mice (8~10 week-old) were sacrificed *in vivo* islet transplantation test. All animal handling procedures adhered to regulations specified by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) in attendance of veterinary doctors.

2. Isolation of CD4⁺/CD25⁺ Treg cells & confirmation of the purity

Mouse spleens were ground on a 40 μ m-scale nylon mesh (Becton Dickinson, Franklin Lakes, NJ, USA), and incubated in hemolysis buffer (Sigma-Aldrich, St. Louis, MO, USA). Splenocytes without erythrocyte were stained with an FITC conjugated anti-CD4 (eBioscience, San Diego, CA, USA) and an PE conjugated anti-CD25 (eBioscience), double-stained cells were sorted by flow cytometry (FACS) Aria (Becton Dickinson). Sorted cells were analyzed by FACS Aria or Vantage (Becton Dickinson) to confirm the purity of isolated Treg population. Then, mostly isolated Treg population was sorted by MACS Treg isolation kit again to eliminate the intervention of foreign cells. CD4⁺ T cells were negatively selected, and CD4⁺/CD25⁺ cells were positively selected with MACS kit for Treg cell isolation (Miltenyi Biotec, Bergisch Gladbach, Germany).

3. Pancreatic islet isolation

Pancreatic islets were isolated from male syngeneic C57BL/6 mice, aged 8 to 10 weeks by digesting pancreatic tissues with collagenase P (Roche, Mannheim, Germany) and purified by ficoll (Biochrom AG, Berlin, Germany) gradient purification and cultured free floating in Medium 199 (M199) with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). Each islet was handpicked under an inverted microscope under sterile conditions and the purity was assessed by diphenylthiocarbazone (Sigma) staining.

4. Co-culture of Treg cells and pancreatic islet cells

Isolated CD4⁺/CD25⁺ Treg cells were cultured in 24-well culture dishes at a density of 4×10^6 cells/mL in RPMI-1640 (Hyclone, Waltham, MA, USA) containing T cell culture supplements [17]. Cultures were maintained at 37°C with 5% CO₂. TCR (T cell receptor) stimulation was performed by coating the bottom of the culture plate with cross-linked anti-mouse CD3 antibody (5 μ g/mL, eBioscience) and by adding a soluble anti-mouse CD28 antibody (5 μ g/mL, eBioscience) for 48 h, before co-culture with islet cells following previous report [7]. Interleukin-2 was not added for Treg culture to exclude intervention on results. Finally, isolated islets were seeded as 180 IEQ (islet equivalents) in a trans-well insert dish (Millipore, Billerica, MA, USA), then co-cultured with Treg for 48 hours. At this time, culture medium was concentrated to M199 (Hyclone).

5. Detection of protein levels (TGF- β 1, IL-6, Insulin) and cell death by ELISA

Cultures were prepared as three groups as islet culture, Treg/islet co-culture or Treg culture then secreting soluble cytokines were measured from each culture supernatant. Islet concentration was reached to 180 IEQ in a trans-well insert dish. These islets were washed with PBS, medium was changed to M199 with 10% FBS. After 48 hours incubation, cultured medium was harvested. And then, to evaluate the change of soluble factors in medium of “islet only” or “Treg co-cultured islet”, and also “Treg only” were investigated. All of collected medium were filtered with PES (polyethersulfone) membrane 0.22 μ m pore size. After collecting the supernatants, the soluble cytokines [transforming growth factor- β 1 (TGF- β 1), interleukin-6 (IL-6), insulin], and cell death index (fragmented and solubilized nucleosomal DNA) levels were determined using an enzyme-linked immunosorbent assay (ELISA) kits for TGF- β 1, IL-6, and insulin (R&D Systems, Minneapolis, MN, USA), and for cell death (Roche Applied Science, Indianapolis, IN, USA). Absorbance was measured at 450 nm using a microplate reader. Each sample was tested in quintuple.

6. Glucose-stimulated insulin secretion (GSIS) in vitro

After overnight culture in M199 with 10% FBS, aliquots of islets were incubated in M199 medium containing 1.7 mM glucose at 37 °C for 30 min. Five hand-picked islets were incubated in M199 medium containing either 1.7 or 16.7 mM glucose at 37°C for 1 hr in replicates of five. After incubation, supernatants were collected for immunoreactive insulin measurement by enzyme immunoassay (Linco, St. Charles, MO, USA). The mean amount of released insulin in response to the high concentration of glucose (16.7 mM) was divided by the mean amount released by the low concentration of glucose (1.7 mM) to yield the glucose-stimulated insulin secretion (GSIS) index.

7. Islet transplantation beneath the kidney capsule of type 1 diabetic mouse in vivo

To identify the function and survival of cultured islets in vivo, our study was conducted at Semyung University animal research facility following an Institutional Animal Care and Use Committee (IACUC)-approved protocol. Male inbred syngeneic C57BL/6 mice, aged 8 to 10 weeks, were used as donors and transplantation recipients. Diabetes (as defined as a blood glucose >16.65 mmol/L) and was produced by a single intraperitoneal (IP) injection of streptozotocin (STZ) (200 mg/kg) (Sigma) 2 days before transplantation. And recipient mice were classified into two groups, namely, islet transplantation (IT) without any intervention (control, N=6), and Treg co-cultured islet transplantation (TCIT, N=6). 180 IEQ of islets were implanted beneath the renal capsule of the left kidney in a syngeneic recipient C57BL/6. Daily monitoring of body weight was performed to assess animal condition and activity, and blood glucose levels were used to assess islet graft function. Graft failure was defined as a reversal of hyperglycemia (>11.1 mmol/L) by three consecutive measurements. This experiment repeated three times.

8. Intraperitoneal glucose tolerance test (IPGTT)

To investigate the glucose regulation ability in vivo, IPGTT was performed on graft recipients from all two

treatment groups at 21 days after transplantation. Briefly, after overnight fasting, unanesthetized mice were injected with 20% glucose solution (1 mg/kg body weight) by IP. Plasma glucose concentrations were then measured at 0, 15, 30, 60, 90, 120, and 180 minutes after glucose injection.

RESULTS

1. Purity of the isolated natural Treg

We used FACS ARIA to sort into the mouse endogenous nTreg cells and analyze their phenotype. Ninety four percent of the cells showed typical nTreg cell phenotype, CD4⁺/CD25⁺ among the sorted cells (Figure 1). To eliminate 6% of contaminating population (CD4⁺/CD25⁻, CD4⁻/CD25⁺, and CD4⁻/CD25⁻) completely, we additionally performed MACS isolation. Thus, the purity of nTreg in this study was about 98% averagely (data not shown).

2. TGF-β1 levels in Treg, islet and Treg/islet co-culture groups

It was needed to classify the groups into three for *in vitro* cytokine analysis: Treg group (Treg single culture),

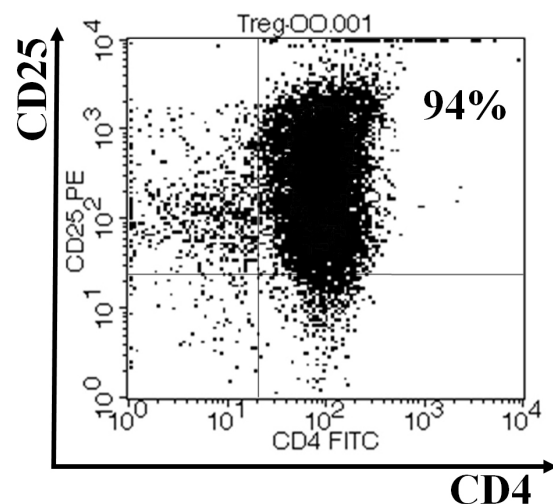


Figure 1. Purity of the isolated natural Treg. FACS analysis revealed that 94 % of the isolated cells displayed the CD4⁺/CD25⁺ phenotype (Figure 1; upper right), while a “contaminated” other cell accounted for 6 % of the FACS ARIA sorted cells. We then additionally performed MACS isolation again to remove the contaminating populations completely.

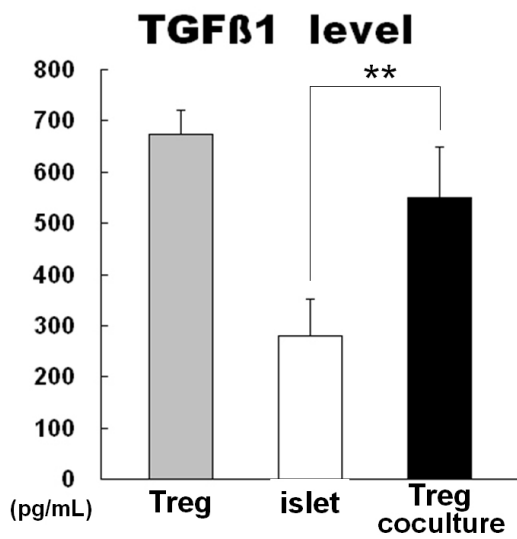


Figure 2. TGF- β 1 levels in Treg, islet, and Treg/islet co-culture group. It was needed to classify the groups into three for *in vitro* analysis: Treg group (nTreg culture control), Islet group (islet culture only), and Treg/islet co-culture group (islet cultured with nTreg). The differences and changes of TGF- β 1 secretion levels were compared among three groups *in vitro*. In Treg group, data showed the largest amount of TGF- β 1 among three groups, secreted TGF- β 1 by nTreg was 648.29 pg/mL on the average. In Treg coculture group, 526.55 pg/mL of TGF- β 1 was secreted, it was diminished as 18.80 %. Islet group showed 256.02 pg/mL of TGF- β 1 secretion as the smallest amount in our conditions. This experiment was repeated 5 times, the numbers of samples in each batch were over quintuple (** $P < 0.005$).

Islet group (islet single culture), and Treg/islet co-cultured group (islet culture with Treg). The TGF- β 1 secretion levels were compared among these three groups. In Treg group, 648.29 pg/mL of TGF- β 1 was measured and it was similar to previous study [4] (Figure 2). The TGF- β 1 level was different between Islet and Treg/islet co-culture group. It was 256.02 pg/mL and 526.55 pg/mL by group, respectively (Figure 2).

3. Measurement of IL-6 level and islet cell viability

The secretion level of IL-6 was measured by ELISA. The amount of IL-6 was 43.59 pg/mL in Islet group, while it was 153.68 pg/mL in Treg/islet co-culture group (Figure 3A). In Treg/islet co-culture group, IL-6 was secreted 3.53 fold more. These results might be related to islet cell viability. The detection of fragmented DNA contents was measured as 0.064 and 0.024 (OD value) in Islet group and Treg/islet co-culture group, respectively (Figure 3C). The Treg co-cultured islet showed less fragmented DNA contents (cell death index) than islet only as 2.67 fold.

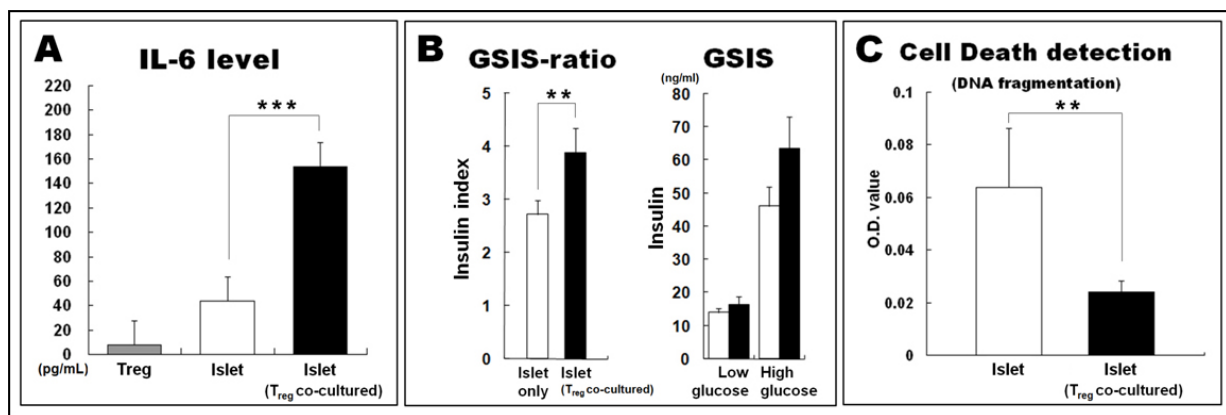


Figure 3. Measurement of IL-6, insulin levels and cell viability. ELISA analysis to measure the cytokines secretion levels of the IL-6, insulin from islet and nTreg-cocultured islet changed by exposing to Treg-derived TGF- β 1 or not. (A) The amount of IL-6 secretion was 43.59 pg/mL in Islet group and 153.68 pg/mL in Treg/islet co-culture group. Compared islet group with Treg/islet co-culture group, the difference of TGF- β 1 level was 3.53 fold. ***This experiment was repeated 3 times, the numbers of samples in each batch were quintuple ($P < 0.0005$), (B) Exposed to TGF- β 1 by Treg/islet co-culture, the insulin secretion level was also increased. When low-dose glucose (60 mg/dL) was added to culture, 2.43 ng/mL of more insulin was measured in Treg/islet co-culture group than in Islet group (Figure 3B). When high glucose (300 mg/dL) was added, 17.43 ng/mL of more insulin was secreted in co-cultured group than in islet group. The converted insulin secretion ratio with these data showed that the islet cells which were co-cultured with Treg and were exposed to TGF- β 1 secreted more insulin as 1.43 fold. **This experiment was repeated 5 times, the numbers of samples in each batch were quintuple ($P < 0.005$), (C) On the average index, Islet group and Treg/islet co-culture group showed 0.064 and 0.024 each in optical density value (OD value). The Treg/islet co-culture group showed less fragmented DNA contents (cell death index) than Islet group as 2.67 fold. **This experiment was repeated 5 times, the numbers of samples in each batch were quintuple ($P < 0.005$).

4. Measurement of insulin secretion level by GSIS test

When the islet cells were co-cultured with Treg, the insulin level was also increased in GSIS test (Figure 3B). When low-dose glucose (60 mg/dL) was added to culture, 2.43 ng/mL of more insulin was measured in Treg/islet co-culture group than in Islet group (Figure 3B). When high glucose (300 mg/dL) was added, 17.43 ng/mL of more insulin was secreted in co-cultured group than in islet group. The converted insulin secretion ratio with these data showed that the islet cells which were co-cultured with Treg and were exposed to TGF- β 1 secreted more insulin as 1.43 fold (Figure 3B, right graphs).

5. Comparison the transplanted islet cell function in T1D mice model

Considering the effect of TGF- β 1 on islet cells which were exposed to Treg before transplantation, we designed to transplant the minimal IEQ (180) of syngeneic islet under the kidney capsule of T1D C57BL/6 mice. It was aimed for an efficient comparison the blood glucose regulation with IT (Islet Transplantation) and TCIT (Treg-co-cultured islet transplantation) group. After same IEQ of islet transplantation in IT or TCIT group, the blood glucose level of each group was measured for 22 days (Figure 4A). It showed much more efficient down-regulation of blood glucose when the islets which were previously exposed to Treg-derived TGF- β 1 were transplanted. IT group showed the regulation range of 269~310.75 mg/dL, while TCIT group showed within the 145~156.60 mg/dL stably comparing the IT and TCIT group between the day 10 to 22 after transplantation (Figure 4A).

To investigate the functional responses of transplanted islets *in vivo*, we performed IPGTT test at 21 days after transplantation (Figure 4B). Fasted overnight mice were injected with 20% glucose solution (1 mg/kg body weight) intraperitoneally. In IPGTT test, plasma glucose concentrations were then measured at 0, 15, 30, 60, 90, 120, and 180 minutes after glucose injection. Two groups showed similar high-regulation aspects from 0 to 30 minutes. Data showed, at 30 minutes, the glucose levels of 324.75 or 325

mg/dL in IT or TCIT, respectively. However, TCIT group regulated the plasma glucose much more efficiently than IT group after 60 minutes. The blood glucose levels of

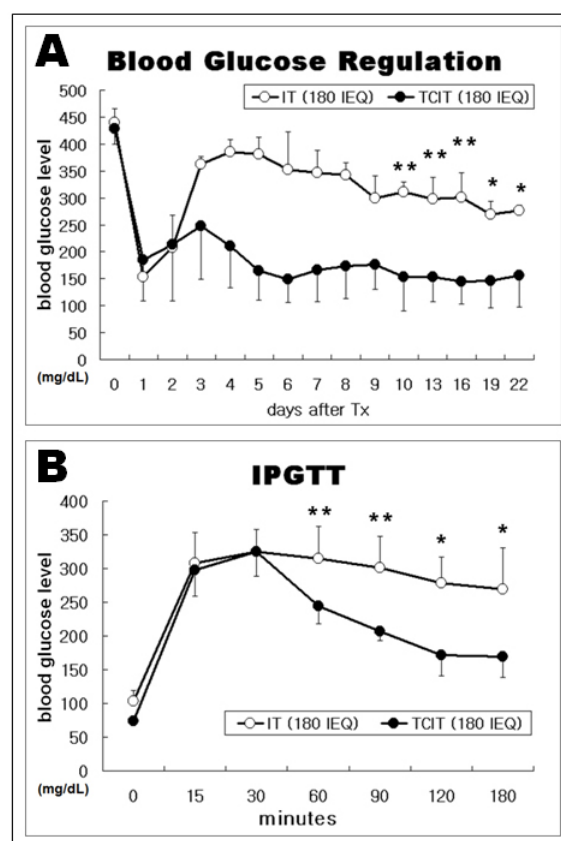


Figure 4. Measurement of transplanted islet function with or without Treg co-culture. (A) We determined the minimal IEQ of islet *in vivo* experiment to compare the blood glucose level regulation ability between Islet and Treg/islet co-cultured islet. Then we transplanted 180 IEQ of islet cells or same IEQ of Treg-co-cultured islet cells in T1D mice. The groups were divided as Islet Transplantation group (IT) and Treg-co-cultured islet transplantation group (TCIT). The blood glucose levels of the IT group were distributed in the range of 269~310.75 mg/dL, while TCIT group regulated the blood glucose levels in a normal range within 145~156.6 mg/dL stably. This experiment was repeated three times, the numbers of samples in each batch were quintuple (* P <0.05, ** P <0.005). (B) To investigate the functional responses of transplanted islets *in vivo* we achieved IPGTT test at 21 days after transplantation. Plasma glucose concentrations were then measured at 0, 15, 30, 60, 90, 120, and 180 minutes after glucose injection. TCIT group commenced with plasma glucose regulation more efficiently than IT group after 60 min. Data showed similar phases, 324.75 and 325 mg/dL in IT and TCIT respectively at 30 min. The blood glucose levels were diminished as 3.08, 4.29, 7.47, 3.32 % at 60, 90, 120, 180 minutes respectively in IT group, while 24.92, 15.76, 17.15, 1.02 % at same period respectively in TCIT group. Because the plasma glucose level of TCIT was already attained in normal blood glucose range at that time point, the blood glucose regulation efficiency of TCIT was less than IT at 180 min. This experiment was repeated three times, the numbers of samples in each batch were quintuple (* P <0.05, ** P <0.005).

TCIT group were reached to normal plasma glucose level after 120 minutes (Figure 4B).

DISCUSSION

In this study, it was suggested that nTreg might be helpful to improve the function and viability of pancreatic islet cells *in vitro* and *in vivo* study. First, we investigated whether islet cells could increase the secreting insulin level when they were exposed to nTreg-derived TGF- β 1 in our Treg/islet co-culture system. In GSIS result, Treg/islet co-culture group secreted significantly more insulin than Islet group (Figure 3B). Several reports support these results. C-peptide (a precursor of insulin) expression was increased by the expression of TGF- β 1 and the viability and function of islet were also improved [23] and TGF- β 1 also stimulates insulin gene [28]. In order to evaluate the effect of Treg-derived TGF- β 1 on islet cell function *in vivo* transplantation, 180 IEQ of syngeneic islet cells (IT group) or 180 IEQ islet cells which were co-cultured with Treg (TCIT group; using the trans-well culture system, islet and Treg were not contacted directly) were transplanted under the kidney capsule of T1D mice. In TCIT group, the co-cultured islet cells broke into regulate the blood glucose much more efficiently after day 3. The stable regulation of blood glucose in TCIT group was maintained until the end of observation (Figure 4A). IPGTT data also demonstrated that the co-cultured islet functioned more effectively than the islet without co-culture after transplantation. These results clearly demonstrated that the previously co-cultured islet cells with Treg improved the function of islets even *in vivo* transplantation.

The islet cell death was also decreased through Treg co-culture (Figure 3C). These data showed that two main factors that are closely related to the function of islet were improved through the co-culture with Treg. The improvement of viability and function of islet should be associated with the increase of IL-6 secretion through Treg/islet co-culture (Figure 3A). The IL-6 is a pleiotropic cytokine that influences metabolic regulation through

both normal physiology and disease [26]. It has been reported that the viability and function of islet were improved when it was co-cultured with IL-6 secreting umbilical mesenchymal stem cell [24]. And also, it has been demonstrated that IL-6 protects pancreatic islet beta cells from pro-inflammatory cytokines-induced cell death and functional impairment *in vitro* and *in vivo* [25]. Moreover, IL-6 knock-out mice displayed the glucose intolerance due to incomplete β -cell functions, which is caused by the abnormal expansion of α -cell in the absence of IL-6 [26]. A basal level of IL-6 was observed in islet only condition, meanwhile, the level of IL-6 secretion was clearly increased in Treg co-culture condition. In figure 3A, basal level of IL-6 in Treg only group just shows 7.73 pg/mL, while in Treg co-culture group shows 153.58 pg/mL.

Altogether Treg-derived TGF- β 1 would improve directly the viability and function of islet and indirectly through the secretion of IL-6, this increase of IL-6 (Figure 3A) might be mediated by TGF- β 1 which is secreted by Treg (Figure 1). It has been reported that X-linked inhibitor of apoptosis protein functions as a cofactor in transforming growth factor- β signaling [27] and IL-6 protects pancreatic islet beta cells from pro-inflammatory cytokines-induced cell death and functional impairment *in vitro* and *in vivo* [25].

In conclusion, we demonstrate that Treg-produced TGF- β 1 up-regulate IL-6 and insulin secretion levels from islet, while concomitantly inhibit islet cell death *in vitro*. The *in vivo* results show that islet under the TGF- β 1 condition down-regulates the blood glucose levels of T1D mice *in vivo* more efficiently. We propose that Treg cell could be used as an assistant-cell for the improvement of islet transplantation efficiency.

요약

본 연구에서는 면역 억제 역할을 하는 것으로 알려져 있는 조절 T 세포 (regulatory T cell, Treg)의 새로운 생리학적 기능 대하여 확인해보고자 하였다. 시험관내나 동물실험에서 조절 T 세포가 분비하는 transforming growth factor β 1 (TGF- β 1)에

의하여 이식 직전까지 췌장섬세포의 생존률을 향상시키면서 동시에 혈당조절 기능이 향상될 수 있을 것이라는 가설이다. 이를 증명하기 위하여 마우스를 이용한 1형 당뇨병 모델을 제작한 뒤, 180 IEQ (islet equivalents)의 췌장섬세포를 동종간 이식하였다. 췌장섬세포는 이식 수술 시행 전까지 48시간 동안 4×10^6 의 Treg 세포와 함께 배양하여 Treg 유래 TGF- β 1에 충분히 노출시킨 뒤 사용하였다. Treg 단독군, 췌장섬세포 단독군 및 Treg/islet 동시 배양군에서 각각 TGF- β 1, IL-6 및 인슐린 분비 수준의 변화를 측정하였다. Treg/islet 동시 배양군에서 IL-6와 인슐린 분비는 증가하였고 ($P < 0.0005$, $P < 0.005$), 췌장섬세포 단독군과 비교하여 생존율이 향상되었다($P < 0.005$). 또한, 이식 후, 동시 배양된 췌장섬세포는 1형 당뇨병 마우스 모델에서 혈당 수치를 보다 효율적으로 조절하였다. 이러한 결과는 Treg 세포가 TGF- β 1 분비를 통하여 췌장섬세포의 기능과 생존력을 향상시킬 수 있음을 시사한다.

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