ISLETS
2021, VOL. 13, NOS. 1–2, 24–31
https://doi.org/10.1080/19382014.2021.1890963

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

Protein kinase C-θ knockout decreases serum IL-10 levels and inhibits insulin secretion from islet β cells

Feng Hong[a], Yang Yang[a], Baiyi Chen[a], Peng Li[b], Guoguang Wang[a], and Yuxin Jiang[c]

[a]School of Preclinical Medicine, Wannan Medical College, Wuhu, China; [b]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan; [c]College of Medicine, Jiaxing University, Jiaxing, China

ABSTRACT

Various subtypes of protein kinase C (PKC) are expressed in islet β cells and regulate β cell proliferation and survival. PKC-θ is distributed in the immune system and promotes the secretion of IL-10, which manifests a critical role in the onset of diabetes, by the immune cells. However, the role of PKC-θ in islets has not been concerned. In the present study, we investigated the role of PKC-θ in the protection of islet β cells and insulin secretion. Fasting glucose and insulin measurement, glucose tolerant test, immunofluorescence, and ELISA were conducted to study the influence of PKC-θ knockout on islet β cell survival and function, and explore the mechanism underlying this regulation. PKC-θ knockout mice at 2 weeks manifested normal serum insulin levels, glucose tolerance, and β cell mass. Knockout mice at 8 weeks show decreased β cell mass, but manifested normal insulin levels and glucose tolerance. Knockout mice at 16 weeks manifested impaired glucose tolerance, β cell mass, and decreased glucose stimulated insulin secretion. Furthermore, knockout mice manifested decreased serum IL-10 level compared with normal mice since 2 weeks. IL-10 injection into knockout mice improved glucose tolerance, serum insulin level, and reduced β cell mass, and IL-10 administration into cultured pancreatic tissue increased glucose stimulated insulin secretion. PKC-θ knockout decreases the secretion of IL-10, reduces β cell mass and insulin secretion in pancreatic islets. The present study illuminates the critical role of PKC-θ in protecting the survival and function of islet β cells.

KEYWORDS

PKC-θ; gene knockout; islet β cells; insulin secretion; serum IL-10

Introduction

As a member of the serine/threonine protein kinase family, protein kinase C (PKC) is represented by more than 10 different functional isozymes.[1] Based on the structural differences, PKC can be classified into several categories: 1) classical, including α, β, and γ members; 2) novel, including δ, ε, and η; 3) atypical, including ζ and ι; and 4) PKC-related kinases (PRKs).[2] PKCs participate in many biological processes including cell proliferation, differentiation, and apoptosis.[3] Several PKC isoforms, such as PKC-α and PKC-ε, are present in the β cells of the pancreatic islets.[4] Glucose stimulates PKC-α synthesis and promotes its translocation from the cytosol to the membrane.[5,6] Inhibitors of both PKC-α and PKC-ε can decrease glucose-induced insulin secretion.[7,8] PKC-δ is also expressed in pancreatic islet β cells and is essential for pancreatic β cell replication during insulin resistance.[9]

PKC-θ mainly performs immunoregulatory functions by translocation to the synapses of immune cells after antigen-stimulation of T cells.[10,11] In the immune synapse, PKC-θ regulates the transmission of T-cell receptor signals and activates the downstream transcription factors to drive proliferation of T cells, B cells, and natural killer cells.[12,13] Studies have demonstrated that PKC-θ is required for IL-10 induction[14] and PKC-θ-knockout mice have manifested significantly decreased serum IL-10 levels. IL-10 has been considered to be a suppressive cytokine, which inhibits T helper (Th) 1 cell proliferation and cytokine production, and regulates proliferation and differentiation of immune cells, such as immune B cells and mast cells.[15] As an anti-inflammatory cytokine, IL-10 first increases and then decreases at the onset of diabetes in 14–16 week-old non-obese diabetic (NOD) mice.[16] Furthermore, a study reported by van Exel *et al.* demonstrated a decrease in serum IL-

CONTACT Yuxin Jiang jiangyx@zjxu.edu.cn School of Medicine, Jiaxing University, No.118 Jiahang Road, Jiaxing 341001, China.; Hong Feng hongfengshengli@163.com School of Preclinical Medicine, Wannan Medical College; No.22 Wenchang West Road, Wuhu 241002, China.

10 levels in diabetes patients.[17] In addition, IL-10 suppresses the progression of autoimmune pathogenesis associated with diabetes mellitus, protects β cells, and suppresses the onset of autoimmune diabetes in NOD mice,[18] which further indicate the possible protective effects of IL-10 on pancreatic islet cells.

In the present study, we aim to investigate the regulatory effects of PKC-θ knockout on the survival and function of islet β cells *in vivo* and *in vitro*, and then explore the potential mechanism underlying this regulation.

Materials and methods

Animal preparation

PKC-θ knockout mice were generated according to a previous report[19] and backcrossed with C57BL/6 mice for more than 15 generations. Male C57BL/6 wild-type (WT) mice, PKC-θ knockout mice, and IL-10 treatment knockout mice were housed and maintained under specific pathogen-free (SPF) conditions at Wannan Medical College (Wuhu, China). The body weight, food intake, and fecal number of each mouse were measured at 2, 8, and 16 weeks, respectively. The IL-10 treatment knockout mice were i.p. injected with 5 μg/kg recombinant mouse IL-10 (rIL-10) twice a week from 2 weeks to 16 weeks. All animal procedures were approved by the Animal Welfare Committee of Wannan Medical College.

Fasting glucose measurement

Subsequently, 14 PKC-θ knockout mice, 14 WT mice, and 10 IL-10 treatment knockout mice aged 2 weeks, 8 weeks, and 16 weeks each were fasted for 16 h from 5:00 pm to 9:00 am on the next day. The fasting blood glucose levels were measured in tail-snip samples using a handheld glucometer ACCU-CHEK (Roche, Mannheim, Germany).

Intraperitoneal glucose tolerance test (IPGTT)

After fasting for 16 h, each mouse was i.p. injected with glucose solution (1.5 g/kg). A drop of blood from the tail-snip was used to measure blood glucose levels using ACCU-CHEK (Roche, Mannheim, Germany) at 0, 15, 30, 60, and 90 min. In particular, blood from the tail tip of a 2-week-old mouse should be more carefully withdrawn according to the animal welfare guidelines. Blood samples were collected at 30 min for evaluating the serum insulin levels. The blood samples were coagulated for 30 min at room temperature and centrifuged for 10 min at 3,000 rpm at 4°C to collect the serum, and the serum was stored at −80° C for the measurement of insulin levels.

Glucose stimulated insulin secretion assay

The mice were sacrificed by decapitation, and pancreatic tissue was quickly removed from the mice. The pancreases were separately placed into an incubation tank equipped with a gas mixer containing 4.5 ml low-glucose (2 mM glucose) Krebs solution. IL-10 (20 ng/ml) was added to the solution in the IL-10 treatment group (n = 10). After incubation for 30 min at 37°C, the samples were collected for the measurement of basal insulin secretion. Subsequently, 20 mM glucose was added and samples of the solution were collected after 1 h for measuring glucose-induced insulin secretion. The insulin secretion index was calculated as the glucose stimulated insulin secretion divided by the basal insulin secretion.

Insulin measurement

Insulin was measured via radioimmunoassay using Millipore RI-3 K kits (Billerica, MA, USA) as reported previously.[20]

Immunofluorescence (IF)

Pancreatic sections (6 μm thickness) were prepared and washed with PBST (PBS containing 0.3% Triton X-100, pH = 7.2 ~ 7.4). After blocking in 5% horse serum (Gibco, USA), the sections were incubated with primary antibodies (anti-insulin, anti-caspase-3, Table 1) and appropriate secondary antibodies (Alexa Fluor® 488 donkey anti-goat IgG, Alexa Fluor® 594 donkey anti-rabbit IgG, Table 2), and the nuclei were stained with DAPI (Sigma, Mannheim, USA). The images were analyzed using a laser confocal TCSSP8 microscope (Leica, Germany). The β cell mass was calculated as described previously.[21]

Table 1. Primary antibodies.

Primary antibody	Code	Company	Host species	Dilution
Monoclonal Anti-Insulin antibody produced in mouse	I2018-2ML	Sigma	Mouse	1:1000
Insulin antibody	4590	Cell signaling	Rabbit	1:100
CASP-3 antibody	ab13847	Abcam	Rabbit	1:200

Table 2. Secondary antibodies.

Secondary antibody	Code	Company	Dilution
Alexa Fluor® 488 donkey anti-goat IgG (H + L)	A11055	Invitrogen	1:1000
Alexa Fluor® 594 donkey anti-rabbit IgG (H + L)	A21207	Invitrogen	1:1000

Morphometric analysis

To quantify fractional β cells, six islets per section were randomly selected and at least three sections per pancreatic section were randomly selected and examined from five WT mice, five PKC-θ knockout mice, and five IL-10 treatment knockout mice. The number of immunoreactive cells was counted by DAPI staining. The proportion of islet β cell was calculated as the number of immunoreactive β cells divided by the total number of nuclei in the islets. β cell mass (BCM) was then calculated as the product of proportion of islet β cells, determined by immunofluorescence staining in each individual, and the estimated pancreatic weight.

Serum IL-10, IL-2, and TNF-α assay

Peripheral blood samples were collected from the tail vein of mice 2 h after each subcutaneous injection of 80 μg MBPAc1-9[4Y] at 2, 8, and 16 weeks. The blood was centrifuged at 3,000 rpm, and the serum was separated and frozen at −80°C for analysis. IL-10, IL-2, and TNF-α concentrations were then measured using multiplex immunoassay kit Th1/Th2/Th9/Th17/Th22/Treg Cytokine 17-Plex Mouse Procarta Plex Panel (Invitrogen, USA).

Statistical analysis

All data are presented as the means ± SEM and analyzed using Student's unpaired *t*-test or one-way ANOVA; n refers to the number of mice. The statistical analysis was conducted and graphs were generated using GraphPad Prism 6.0. $p < .05$ was considered significant.

Results

PKC-θ knockout mice manifest non-significant changes in body weight, fasting blood glucose, and insulin levels

We continuously monitored the growth of WT mice, PKC-θ knockout mice, and IL-10 treatment knockout mice and measured their body weight, food intake, and feces number at 2, 8, and 16 weeks. The results showed that there were no differences in body weight change among the WT, knockout, and IL-10 treatment knockout mice (Figure 1(a)). Similarly, the food intake of mice at 8 and 16 weeks was almost the same, which was slightly higher than that at 2 weeks, with no difference among WT, knockout, and IL-10 treatment knockout mice (Figure 1(b)). In addition, there was also no significant difference between the three groups in terms of fecal number (Figure 1(c)).

Fasting blood glucose in the knockout mice showed no differences compared with that in the WT or IL-10 treatment knockout mice at 2, 8 and 16 weeks (Figure 1(d)). Furthermore, there was also no significant difference in serum insulin levels at 2, 8, and 16 weeks in all mice (Figure 1(e)).

PKC-θ knockout mice manifested significant differences in glucose tolerance

To explore the influence of PKC-θ knockout on pancreatic islet endocrine function and blood glucose level, glucose tolerance test (GTT) was conducted in mice. At 2 weeks, there were no significant differences in the GTT curves between WT and knockout mice (Figure 2(a)). Furthermore, there was no difference in blood insulin levels at 30 min after glucose administration (Figure 2(d)). At 8 weeks, the GTT curve of knockout mice was

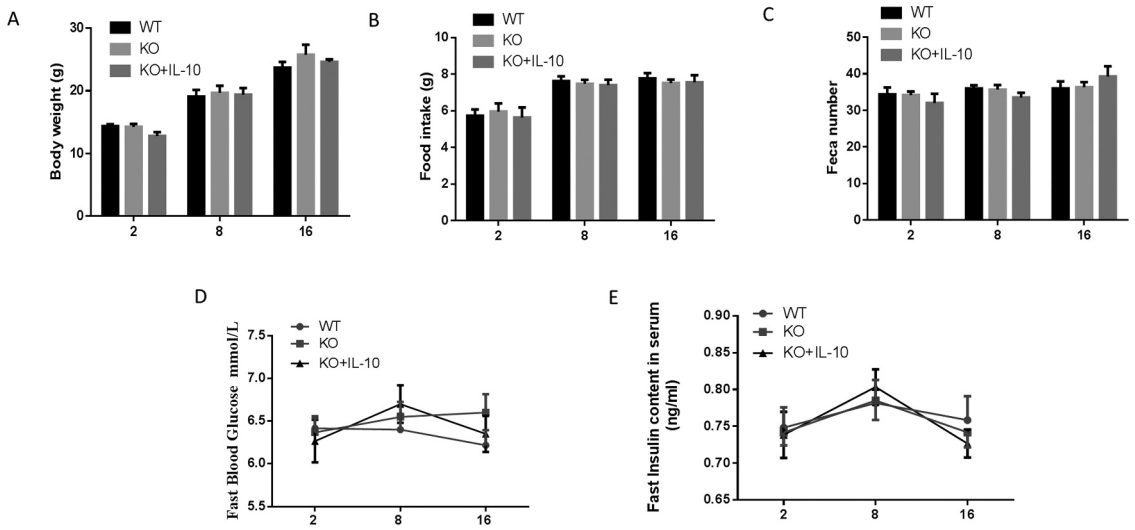

Figure 1. Body weight of WT mice, PKC-θ KO mice, and IL-10 treatment KO mice at 2, 8, and 16 weeks (a). Food intake of WT mice, PKC-θ knockout mice, and IL-10 treatment KO mice at 2, 8, and 16 weeks (b). Fecal number of WT mice, PKC-θ knockout mice, and IL-10 treatment KO mice at 2, 8, and 16 weeks (c). Fasting blood glucose of WT mice, PKC-θ knockout mice, and IL-10 treatment KO mice at 2, 8, and 16 weeks (d). Fasting serum insulin levels of WT mice, PKC-θ knockout mice, and IL-10 treatment KO mice at 2, 8, and 16 weeks (e) (n = 10–14). WT, wild-type; KO, knockout.

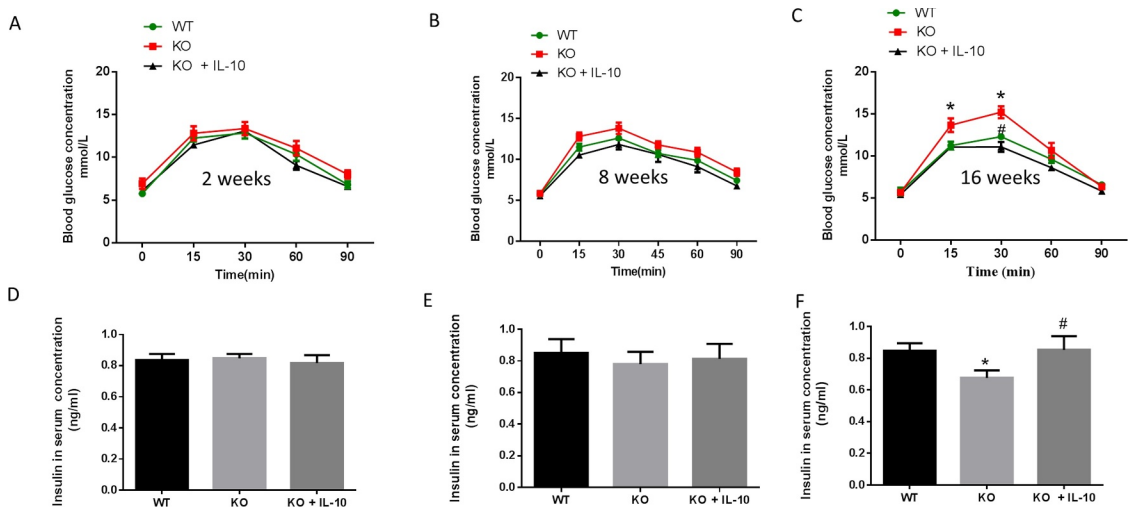

Figure 2. The IPGTT results in WT mice, PKC-θ KO mice, and IL-10 treatment KO mice at 2 weeks (a), 8 weeks (b), and 16 weeks (c). The serum insulin levels at 30 min after glucose injection (d, e, f) (#P< .05; n = 10–14). * P< .05, compared with WT mice, # P< .05, compared with PKC-θ KO mice. IPGTT, intraperitoneal glucose tolerant test; WT, wild-type; KO, knockout.

higher (Figure 2(b)) and the blood insulin levels was lower (Figure 2(e)) than that of the WT mice, but the difference was not significant. At 16 weeks, the GTT curve was elevated and the blood glucose levels at 15 and 30 min after GTT were significantly increased in the knockout mice compared with the WT mice (p < .05) (Figure 2(c)), and the insulin levels induced by glucose injection in the serum were significantly decreased at 30 min in the knockout mice (Figure 2(f)). All above mentioned results indicated that PKC-θ knockout decreased glucose-induced insulin secretion and induced glucose intolerance in mice.

PKC-θ knockout mice manifested decreased islet β cell mass

Sections of pancreatic tissue obtained from WT, PKC-θ knockout, and IL-10 treatment knockout mice were evaluated by immunofluorescence staining to evaluate insulin-secreting islet β cells. The results clearly showed that insulin-positive cells

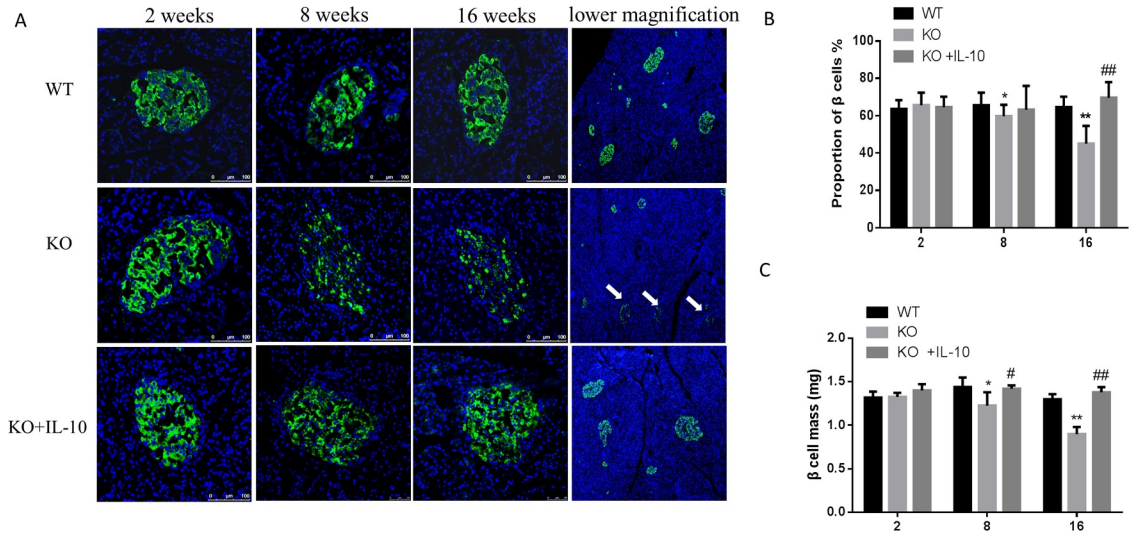

Figure 3. Islet structure as shown by insulin immunoreactive β cells; scale bar = 100 μm. The lower magnification showing the loss of β cell mass at 16 weeks, in which the arrow shows the location of the islets in KO mice (a). The proportion of β cells contained in islets of WT mice, PKC-θ KO mice, and IL-10 treatment KO mice at 16 weeks (b). The β cell mass in WT mice, PKC-θ KO mice, and IL-10 treatment KO mice at 16 weeks (c). ** *P*< .01, compared with WT mice, ## *P*< .01, compared with PKC-θ KO mice. WT, wild-type; KO, knockout.

were widely distributed in the central part of the islets in the WT group at 2, 8, and 16 weeks, but the number of the positive cells significantly decreased at 16 weeks (Figure 3(a)). At 2 weeks, the knockout mice showed no difference in the distribution and proportion of β cells (Figure 3(b)). The β cell mass was found to range from 1.3 to 1.4 mg, with no changes observed between knockout and WT mice (Figure 3(c)). At 8 weeks, the proportion of insulin-positive β cells (Figure 3(b)) and β cell mass were slightly decreased in the knockout group ($p < .05$) compared with the WT group (Figure 3(c)). At 16 weeks, the proportion of insulin-positive β cells (Figure 3(b)) and β cell mass (Figure 3(c)) were significantly lower in the knockout group than in the WT group ($p < .01$). These results further demonstrate that PKC-θ knockout influences the survival of mice islet β cells.

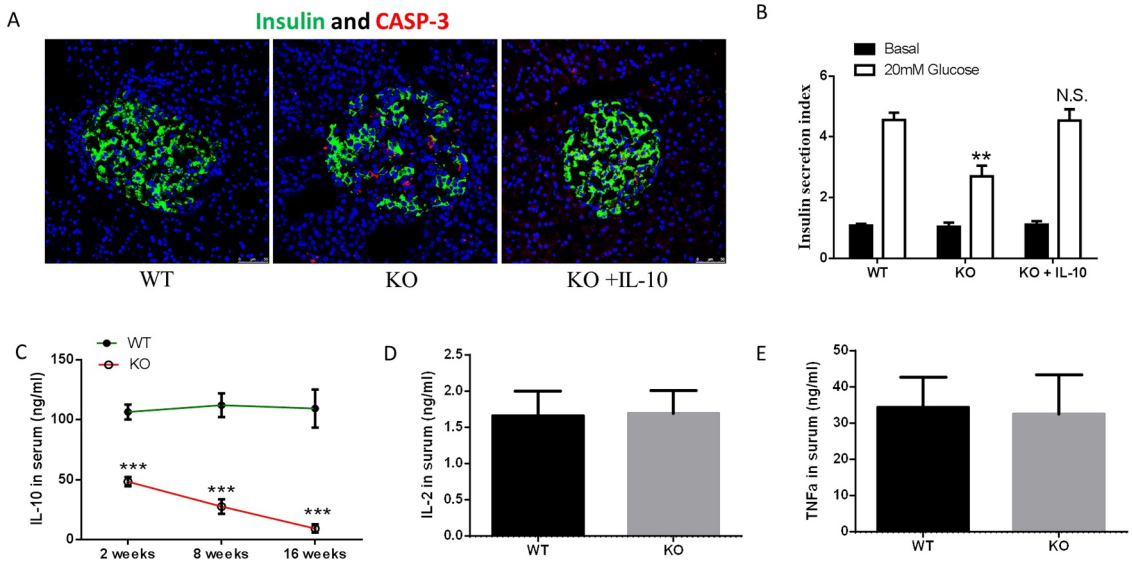

Figure 4. CASP-3 expression in pancreatic islets of WT mice, PKC-θ KO mice, and IL-10 treatment KO mice (a). Insulin secretion index of pancreatic tissue in WT mice, PKC-θ KO mice, and IL-10 treatment KO mice *in vitro* (b). Serum levels of IL-10, IL-2, and TNFα in WT mice, PKC-θ knockout mice, and IL-10 treatment mice after stimulation with 80 μg MBPAc1-9 (c). ** $p < .01$.*** $p < .001$, N.S. no significant change compared with WT mice. CASP-3, caspase-3; WT, wild-type; KO, knockout.

PKC-θ knockout mice manifested caspase-3 expression, decreased insulin secretion, and serum IL-10 levels

We further confirmed the effect of PKC-θ knockout on the number and function of islet β cells by glucose stimulation of the pancreatic tissue. A large number of cells staining positive for the apoptotic factor caspase-3 were observed in the islets of the knockout mice at 16 weeks (Figure 4(a)), which further confirmed that PKC-θ knockout leads to islet β cell apoptosis in mice with time. The secretion of glucose stimulated insulin in the knockout group was significantly decreased compared with that in the WT group (Figure 4(b)). These results indicated that the islet β cells were damaged by PKC-θ knockout, leading to decreased glucose stimulated insulin secretion, whereas the basal secretion was not affected.

Mechanism underlying the decreased islet β cell mass and insulin secretion in PKC-θ knockout mice

To investigate the mechanism of how PKC-θ knockout affects islet β cell survival and endocrine function, we detected the changes in the immune system. Serum levels of IL-10, IL-2, and TNFα after MBPAc1-9 stimulation were measured in 16-week-old mice. The results demonstrated that the levels of IL-2 and TNF-α in PKC-θ-knockout mice were similar to those in WT mice, whereas IL-10 content was significantly reduced in knockout mice compared with that in WT mice ($p < .001$) (Figure 4(c)). Indeed, the decrease of IL-10 in knockout mice was detected since 2 weeks. The IL-10 levels in WT mice did not change with the age of the mice, whereas the IL-10 levels in knockout mice showed a decreasing tendency with age (Figure 4(c)). These results suggested that PKC-θ knockout reduces IL-10 secretion, which then affects the structure and function of islet β cells. For further demonstrating the role of IL-10 in the decrease of islet β cell number and function, we tested the effect of IL-10 on islets of knockout mice *in vivo* and *in vitro*. IL-10 i.p. injected into knockout mice twice a week significantly decreased the high levels of blood glucose in GTT (Figure 2(c)) and increased the insulin levels in the serum (Figure 2(f)) at 16 weeks. In addition, the expression of insulin-positive cells (Figure 3(a)), the proportion of islet β cells (Figure 3(b)), and β cell mass (Figure 3(c)) at 16 weeks in IL-10 treatment knockout mice were all recovered to levels observed in WT mice. The expression of caspase-3 was significantly decreased in IL-10 treatment knockout mice, which demonstrated the protective effects of IL-10 on the impaired islet survival and function seen in PKC-θ knockout mice. Thus, administration of IL-10 into cultured pancreatic tissue significantly improved the glucose stimulated insulin secretion ability of pancreas in knockout mice (Figure 4(b)).

Discussion

The present study for the first time demonstrates that PKC-θ knockout decreases the secretion of IL-10, which is then leads to the destruction of islet β cells and in turn inhibits insulin secretion. Compared with WT mice, PKC-θ knockout mice showed significantly reduced proportions of β cells and glucose stimulated insulin secretion.

The PKC protein family is widely distributed throughout the body and participates in the regulation of many important physiological functions.[1] Studies have demonstrated that several PKC isoforms, such as PKC-α and PKC-ε, are expressed in islet β cells and regulate islet cell proliferation and insulin secretion.[7,8] PKC-θ is expressed mainly in immune cells and participates in the regulation of immune function.[10] In the present study, we explored the influence of PKC-θ on islet β cell survival and insulin secretion.

There were no significant differences in body weight, food intake, fasting insulin secretion, and blood glucose levels among the WT, knockout, and IL-10 treatment knockout mice at 2 weeks. However, the islet structure gradually destroyed, with a significant reduction in the proportion of β cells from 8 weeks onward. Furthermore, the levels of serum insulin secretion stimulated by glucose were greatly reduced, and glucose intolerance was observed in knockout mice at 16 weeks. In general, our data demonstrate that PKC-θ knockout contributes to the impairment of pancreatic islet structure and function.

Since PKC-θ is expressed by immune cells and not directly expressed in islet β cells, we needed to verify whether PKC-θ knockout causes changes in certain

immune factors which then affect the islet β cell structure and function. PKC-θ knockout leads to a decrease in IL-10 secretion, which indicates that PKC-θ is also an essential factor for promoting IL-10 secretion.[14] Given that IL-10 has been reported to manifest a therapeutic role in diabetes and the administration of IL-10 to adult NOD mice has been reported to decrease the incidence and delay the onset of autoimmune diabetes,[18] we here explored the possible role of IL-10 in impaired insulin secretion in knockout mice. Our results demonstrated that serum IL-10 levels in WT mice manifested no change depending on the age of the mice at 2, 8, and 16 weeks, whereas serum IL-10 levels in the knockout mice greatly reduced from 2 week onward and decreased with age. These results lead to the conclusion that PKC-θ knockout is associated with islet destruction and also a decrease in IL-10 levels. Fortunately, we then detected that IL-10 administration *in vivo* and *in vitro* significantly protected islet β cells and improved glucose tolerance in PKC-θ knockout mice. Indeed, restoring IL-10 production from T cells in PKC-θ knockout mice by adoptive cell transfer could further demonstrate the critical role of IL-10 in PKC-θ knockout-induced islet β cell decrease, which need more researches to demonstrate.

In summary, the present study for the first time demonstrates that PKC-θ, which is mainly expressed in immune cells, can indirectly protect islet β cell and promote insulin secretion by regulating IL-10 secretion.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by National Natural Science Foundation of China (81172790, 81671586, and 91671586) and Anhui Natural Science Foundation (1708085QH206), and the Academic and Technical Leader Project of Wannan Medical College (010202041703).

ORCID

Baiyi Chen http://orcid.org/0000-0002-2462-1472
Guoguang Wang http://orcid.org/0000-0002-8532-5730

References

1. Stross C, Keitel V, Winands E, Häussinger D, Kubitz R. Expression and localization of atypical PKC isoforms in liver parenchymal cells. Biol Chem. 2009;390(3):235–244. doi:10.1515/BC.2009.031.
2. Mellor H, Parker PJ. The extended protein kinase C superfamily. Biochem J. 1998;332(Pt 2):281–292. doi:10.1042/bj3320281.
3. Zeng L, Webster SV, Newton PM. The biology of protein kinase C. Adv Exp Med Biol. 2012;740:639–661.
4. Tian YM, Urquidi V, Ashcroft SJ. Protein kinase C in beta-cells: expression of multiple isoforms and involvement in cholinergic stimulation of insulin secretion. Mol Cell Endocrinol. 1996;119(2):185–193. doi:10.1016/0303-7207(96)03811-7.
5. Deeney JT, Cunningham BA, Chheda S, Bokvist K, Juntti-Berggren L, Lam K, Korchak HM, Corkey BE, Berggren PO. Reversible Ca2+-dependent translocation of protein kinase C and glucose-induced insulin release. J Biol Chem. 1996;271(30):18154–18160. doi:10.1074/jbc.271.30.18154.
6. Trexler AJ, Taraska JW. Regulation of insulin exocytosis by calcium-dependent protein kinase C in beta cells. Cell Calcium. 2017;67:1–10. doi:10.1016/j.ceca.2017.07.008.
7. Yedovitzky M, Mochly-Rosen D, Johnson JA, Gray MO, Ron D, Abramovitch E, Cerasi E, Nesher R. Translocation inhibitors define specificity of protein kinase C isoenzymes in pancreatic beta-cells. J Biol Chem. 1997;272(3):1417–1420. doi:10.1074/jbc.272.3.1417.
8. Nesher R, Anteby E, Yedovizky M, Warwar N, Kaiser N, Cerasi E. Beta-cell protein kinases and the dynamics of the insulin response to glucose. Diabetes. 2002;51(Suppl 1):S68–73. doi:10.2337/diabetes.51.2007.S68.
9. Lakshmipathi J, Alvarez-Perez JC, Rosselot C, Casinelli GP, Stamateris RE, Rausell-Palamos F, O'Donnell CP, Vasavada RC, Scott DK, Alonso LC, et al. PKCzeta is essential for pancreatic beta-cell replication during insulin resistance by regulating mTOR and Cyclin-D2. Diabetes. 2016;65(5):1283–1296. doi:10.2337/db15-1398.
10. Thebault S, Ochoa-Garay J. Characterization of TCR-induced phosphorylation of PKCtheta in primary murine lymphocytes. Mol Immunol. 2004;40(13):931–942. doi:10.1016/j.molimm.2003.10.014.
11. Villalba M, Altman A. Protein kinase C-theta (PKCtheta), a potential drug target for therapeutic intervention with human T cell leukemias. Curr Cancer Drug Targets. 2002;2(2):125–137. doi:10.2174/1568009023333908.
12. Lin HF, Shao JZ, Xiang LX, Wang HJ. Molecular cloning, characterization and expression analysis of grass carp (Ctenopharyngodon idellus) NF45 (ILF2) cDNA, a subunit of the nuclear factor of activated T-cells (NF-AT). Fish Shellfish Immunol. 2006;21(4):385–392. doi:10.1016/j.fsi.2006.01.003.

13. Liu Y, Witte S, Liu YC, Doyle M, Elly C, Altman A. Regulation of protein kinase Ctheta function during T cell activation by Lck-mediated tyrosine phosphorylation. J Biol Chem. 2000;275(5):3603–3609. doi:10.1074/jbc.275.5.3603.
14. Britton GJ, Mitchell RE, Burton BR, Wraith DC. Protein kinase C theta is required for efficient induction of IL-10-secreting T cells. PLoS One. 2017;12(2): e0171547. doi:10.1371/journal.pone.0171547.
15. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol. 1993;11:165–190. doi:10.1146/annurev.iy.11.040193.001121.
16. Schloot NC, Hanifi-Moghaddam P, Goebel C, Shatavi SV, Flohé S, Kolb H, Rothe H. Serum IFN-gamma and IL-10 levels are associated with disease progression in non-obese diabetic mice. Diabetes Metab Res Rev. 2002;18(1):64–70. doi:10.1002/dmrr.256.
17. van Exel E, Gussekloo J, de Craen AJ, Frölich M, Bootsma-Van Der Wiel A, Westendorp RG. Leiden 85 Plus Study. Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study. Diabetes. 2002;51 (4):1088–1092. doi:10.2337/diabetes.51.4.1088.
18. Pennline KJ, Roque-Gaffney E, Monahan M. Recombinant human IL-10 prevents the onset of diabetes in the non-obese diabetic mouse. Clin Immunol Immunopathol. 1994;71(2):169–175. doi:10.1006/clin.1994.1068.
19. Manicassamy S, Yin D, Zhang Z, Molinero LL, Alegre ML, Sun Z. A critical role for protein kinase C-theta-mediated T cell survival in cardiac allograft rejection. J Immunol. 2008;181(1):513–520. doi:10.4049/jimmunol.181.1.513.
20. Zhang Q, Chibalina MV, Bengtsson M, Groschner LN, Ramracheya R, Rorsman NJ, Leiss V, Nassar MA, Welling A, Gribble FM, et al. Na+ current properties in islet alpha- and beta-cells reflect cell-specific Scn3a and Scn9a expression. J Physiol. 2014;592 (21):4677–4696. doi:10.1113/jphysiol.2014.274209.
21. Mendes MC, Bonfleur ML, Ribeiro RA, Lubaczeuski C, AFJ F, Vargas R, Carneiro EM, Boschero AC, Araujo ACF, Balbo SL. Duodeno-jejunal bypass restores beta-cell hypersecretion and islet hypertrophy in western diet obese rats. Endocrine. 2018;60(3):407–414. doi:10.1007/s12020-018-1578-4.