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Long-term inhibition of dipeptidyl-peptidase 4 reduces islet infiltration and downregulates IL-1β and IL-12 in NOD mice

Xinran He, Wangen Li, Yunliang Xie, Yunjuan Zhao*

Department of Endocrinology, The Second Affiliated Hospital of Guangzhou Medical University, The East Chang-Gang Road, Guangzhou, China

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ABSTRACT

Dipeptidyl-peptidase 4 (DPP-4) inhibitor (sitagliptin) is a novel anti-hyperglycemia drug in the treatment of type 2 diabetes. However, its potential in type 1 diabetes is still unclear. Recent studies show that increased infection, especially respiratory tract infection, is significantly associated with DPP-4 inhibitors. In this study, we aimed to explore the effects of long-term inhibition of DPP- 4 on innate immunity in type 1 diabetes. Forty mice were randomly divided into 4 groups (n = 10 in each group): control group, lipopolysaccharide (LPS) group, sitagliptin group and sitagliptin + LPS group. The concentrations of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ were measured with Mesco Scale Discovery multiplexed-assay kit. Immunohistochemistry staining of pancreases was performed and insulitis scores for each islet were determined. The results showed that DPP-4 inhibition has no effect on incident rate of diabetes and metabolic parameters in NOD mice. Long-term inhibition of DPP-4 reduced CD4 + T cells to infiltrate into islets and ameliorated insulitis in NOD mice. DPP-4 inhibition downregulated serum interleukin IL-1β and IL-12 in NOD mice. However, it had no significant effect on LPS-induced IL-1β, IL-6, IL-10, IL-12, tumor necrosis factor (TNF)-α and interferon (IFN)-γ in NOD mice. In conclusion, Long-term inhibition of DPP-4 exists anti-inflammatory effect in type 1 diabetes probably by reducing CD4 + T cells to infiltrate into islets and downregulating L-1β and IL-12 in serum.

1. Introduction

Type 1 diabetes is an organ-specific autoimmune disease characterized by the selective destruction of islet β-cell destruction and dysfunction of insulin secretion [1]. Over the past two decades, studies about the pathogenesis of type 1 diabetes focus on adaptive immunity. Wen L et found that MyD88 deficiency prevents the onset of type 1 diabetes in nonobese diabetic (NOD) mice (an automatic autoimmune diabetes mouse model) [2]. Their further study shows that deficiency of the nucleotidebinding oligomerization domain, leucine-rich containing family and pyrin domaincontaing protein 3 (NLRP3) prevents type 1 diabetes by inhibiting pathogenic T cell migration to the islets in NOD mice [3]. These studies suggest a critical role of innate immunity in the pathogenesis of type 1 diabetes.

Toll-like receptors (TLRs) are pattern recognition receptors which plays a key role in innate immune responses [4]. TLR4 is considered the core molecular directly modulating innate immunity with subsequent activation of adaptive immunity [4]. TLR4 is localized on the cell surface and functions as a specific receptor for lipopolysaccharide (LPS) [5]. LPS binds to TLR4, which causes activation of MyD88 and Trif adaptor molecules, leading to secretion of inflammatory cytokines IL-1β, TNF-α and IL-6 [4]. TLRs primarily regulates both pro-and anti-diabetic signals which are triggered by microbiota in type 1 diabetes [6]. TLR4 selectively damages β cells and involves initiation of type 1 diabetes in NOD mice [7]. TLR-4 knockout downregulates MyD88 levels, NF-κB activity and reduced macrophage to secrete IL-6, IL-1β, TNF-α, and IFN-β in streptozotocin-induced diabetic mouse [8]. TLR4-Ab reserves type 1 diabetes, alleviates insulitis and preserves pancreatic islets in NOD mice [9].

Dipeptidyl peptidase-4 (DPP-4) inhibitor is a kind of anti-diabetic drug for the treatment of type 2 diabetes. In recent years, many studies have tried to use DPP-4 inhibitors in type 1 diabetes. DPP-4 inhibitor and quercetin co-administration alleviates insulitis and improves glucose metabolism in type 1 diabetic rats [10]. Our previous study shows that sitagliptin preserves β cell function in patients with latent-autoimmune diabetes in adults [11]. Combination therapy with anti-CD3

Abbreviations: **DPP-4**, Dipeptidyl-peptidase 4; **IFN**, interferon; **IPGTT**, intraperitoneal glucose tolerance test; **LPS**, lipopolysaccharide; **NOD**, nonobese diabetic; **NLRP3**, nucleotidebinding oligomerization domain, leucine-rich containing family and pyrin domaincontaing protein 3; **TNF**, tumor necrosis factor; **TLRs**, Toll-like receptors; **WHO**, World Health Organization

* Corresponding author.
E-mail address: yougukedream@163.com (Y. Zhao).

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and MK626 increases CD4 + CD25 + Foxp3 + T cells in pancreatic draining lymph nodes, alleviates insulitis and preserves islet β -cell function in recent-onset diabetic NOD mice [12]. To investigate the immunoregulatory effect of DPP-4 inhibitor, Pinheiro MM et al cultured peripheral blood mononuclear cells of healthy volunteers with sitagliptin, and they found that sitagliptin suppresses lymphocytes proliferation and inhibits the differentiation of Th1, Th17 and Th2 cells [13]. These studies suggest DPP-4 inhibitor is a promising drug for type 1 diabetes, probably involving its immunoregulatory effect. However, there are very few studies about the effect of DPP-4 inhibitor on innate immunity in type 1 diabetes [14].

A nested case-control study based on adverse drug reaction database of World Health Organization (WHO) shows that increased infection (especially respiratory tract infection) is significantly associated with DPP-4 inhibitors, which suggests DPP-4 inhibitors may play a potential role in innate immunity [15]. In this study, we aimed to explore the effect of long-term dipeptidyl-peptidase 4 inhibition on innate immunity in type 1 diabetes.

2. Materials and methods

2.1. Ethics statement

All experiments complied with institutional guidelines and was approved by Institutional Animal Ethics Committees of Guangzhou Medical University.

2.2. Experimental groups and animal treatment

Female NOD mice (6–8-wk-old) (Nanjing Biomedical Research Institute of Nanjing University) were used in the experiment. All mice were housed in an SPF environment and had free access to water and food. 5 mice were maintained in each cage and received light for 12 h every day. All animal procedures were approved by the animal ethics committee of Guangzhou Medical University. Mice (n = 40) were randomly divided into 4 groups (n = 10 per group): control group, LPS group, sitagliptin group and sitagliptin + LPS group. Among the 4 groups, mice in sitagliptin and sitagliptin + LPS group received intragastric administration with sitagliptin (30 mg/kg) (Januvia ®, Merck Pharmaceuticals) every day. Mice in control group and LPS group received intragastric administration with an equal volume of 0.9% saline. The mice were treated for 33 weeks. The information random blood glucose, weight, food intake and water intake were collected each week. The mice in LPS group and sitagliptin + LPS group received intraperitoneal injection of LPS (5 mg/kg) (lipopolysaccharide, sigma, USA) for 3 h before sacrificing.

2.3. Metabolic parameters and blood glucose monitoring

Bodyweight, food intake and water intake were measured once a week. Blood samples were collected from the tail vein of the mice for the measurement of blood glucose with a glucometer (Accu-Chek Performa). Random capillary blood glucose was determined once a week. If two consecutive glucose levels ≥16.7 mmol/L, the mouse was diagnosed with diabetes.

2.4. Intraperitoneal glucose tolerance test

The intraperitoneal glucose tolerance test (IPGTT) was performed on NOD mice at week 32 (224 days after treatment with sitagliptin). For the IPGTT, mice were fasted for 12 h and blood glucose were measured at 0, 30, 60 and 120 min after intraperitoneal injections of glucose (2 g/kg, 20% glucose solution).

2.5. Cytokine assays

Blood samples were collected before sacrificing the mice. Serum was separated by centrifugation and stored at −80℃ until assayed. Serum levels of pro-inflammatory and inflammatory cytokines were determined with Mesco Scale Discovery multiplexed-assay kit (Meso Scale Discovery, Gaithersburg, MD, USA). Serum levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ were measured according to the references [1617].

2.6. Histology, immunohistochemistry and insulitis score

2.6.1. H&E staining

Pancreases were harvested at 33 weeks after treatment with sitagliptin. After isolation, pancreas tissue was fixed with 4% paraformaldehyde and embedded in paraffin. H&E staining was performed. Histology slides were observed using a microscope (BX41-32P02-SD0, OLYMPUS) and photomicrographs were captured using Canon Utilities software. Insulitis scores for each islet were determined as follows: 0, no mononuclear cells infiltrate in islet; 1, mononuclear cells infiltrate around the islet but not in islet; 2, mononuclear cells infiltrate in and around the islet, but the infiltrated area is less than one-third of the islet; 3, intra-islet infiltration of mononuclear cells is 1/3 to 1/2 of the islet area; 4, intra-islet infiltration of mononuclear cells is more than 1/2 of the islet area [18].

2.6.2. Immunohistochemistry

Immunohistochemistry was done according to the reference [19] . Primary specific antibodies for CD4 (GB13064-2, 1:300 dilution, Servicebio, Wuhan, China), CD8(GB13429, 1:400 dilution, Servicebio, Wuhan, China), CD11b (GB11058, 1:500 dilution, Servicebio, Wuhan, China), CD11c(GB11059, 1:300 dilution, Servicebio, Wuhan, China), Second antibody: HRP-labeled goat anti-rabbit IgG(GB23303, 1:200 dilution, Servicebio, Wuhan, China), IL-1β(GB11113, 1:100 dilution, Servicebio, Wuhan, China), IL-12(PA82345ML, 1:100 dilution, Servicebio, Wuhan, China). The positive express was shown by diaminobenzidine (DAB, G1211, brown color, Servicebio, Wuhan, China) after second antibody incubation.

2.6.3. An immunohistochemical evaluation

An immunohistochemical evaluation was conducted by two pathologists blindly. We randomly take three areas at least for each sample to take photograph at a magnification of 200 and 400 (Figs. 4 and 5). The mean of values obtained in these areas was used for data analysis.

2.7. Statistical analysis

All these data were presented as mean ± standard error of the mean. Data were analyzed by using the SPSS 20.0 software (IBM SPSS Statistics). Diabetes incidence was compared between different groups using Chi-square test. ANOVA or non-parametric tests were used to compare differences between group, depending on the distribution and characteristics of data. P value < 0.05 was considered statistically significant.

3. Results

3.1. DPP-4 inhibitor has no effect on incident rate of diabetes in NOD mice

NOD mice were diagnosed with diabetes after two sequential glucose levels ≥ 16.7 mmol/L. In the end of the experiment, there are 5 mice alive in control group, 5 mice alive in LPS group, 6 mice alive in sitagliptin group and 6 mice alive in sitagliptin + LPS group. By 33 weeks, the incident rate of diabetes is 40% in control group, 40% in LPS group, 67% in sitagliptin group and 33% in sitagliptin + LPS

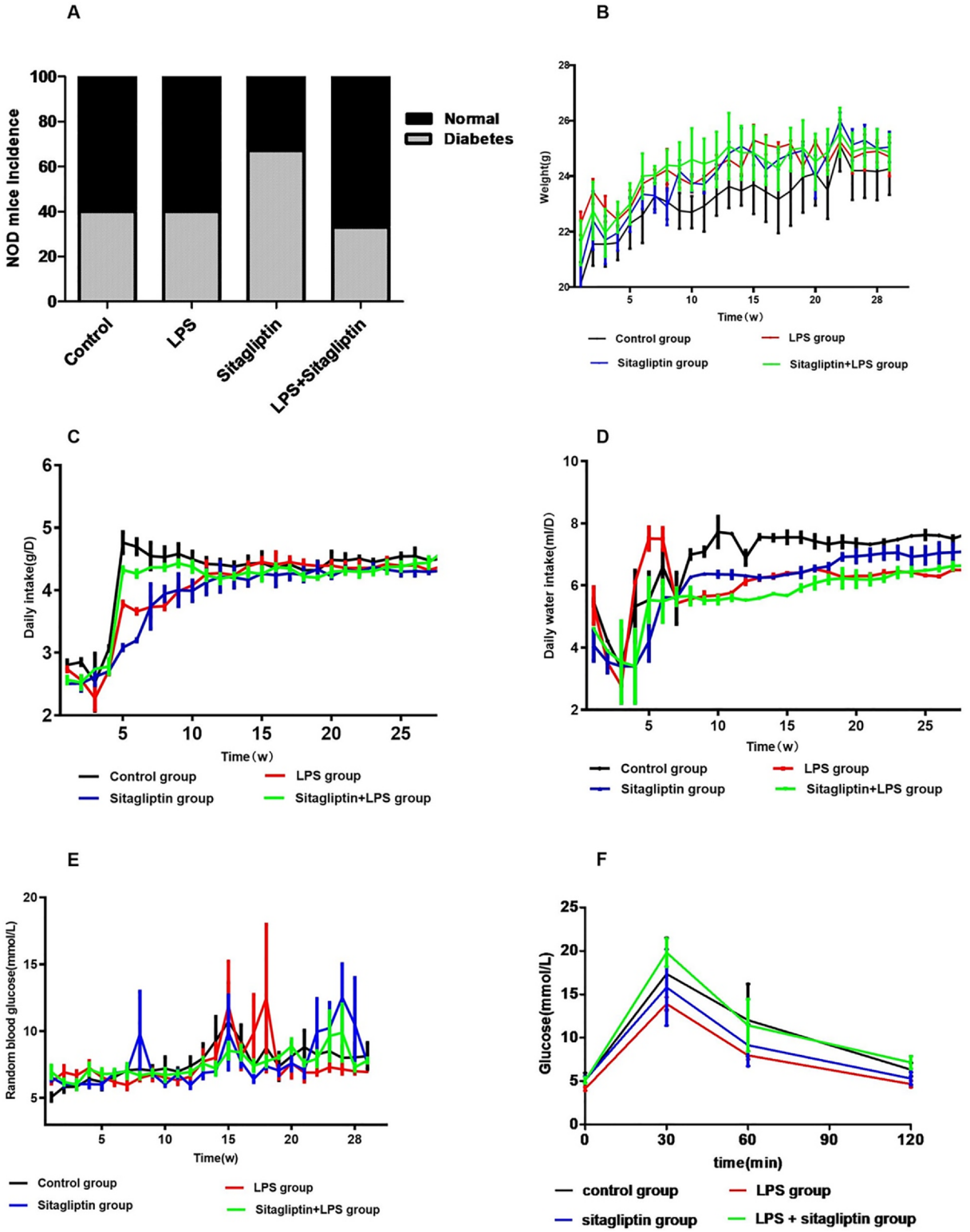


Fig. 1. A, Sitagliptin has no effect on incident rate of diabetes in NOD mice. Fig. 1B–E, Sitagliptin has no effect on metabolic parameters in NOD mice. We measured body weight, food intake, water intake, and random blood glucose in NOD mice. Sitagliptin has no significant difference on body weight, food intake, water intake, and random blood glucose. Fig. 1F, sitagliptin/sitagliptin + LPS has no effect on glucose tolerance in NOD mice. IPGTTs were performed with glucose (2 g/kg) in 40-week-old non-diabetic or diabetic NOD mice in control group (n = 5), sitagliptin group (n = 6), LPS group (n = 5) and sitagliptin + LPS group (n = 6). The glucose tolerance has no significant difference between the 4 groups ($P > 0.05$).

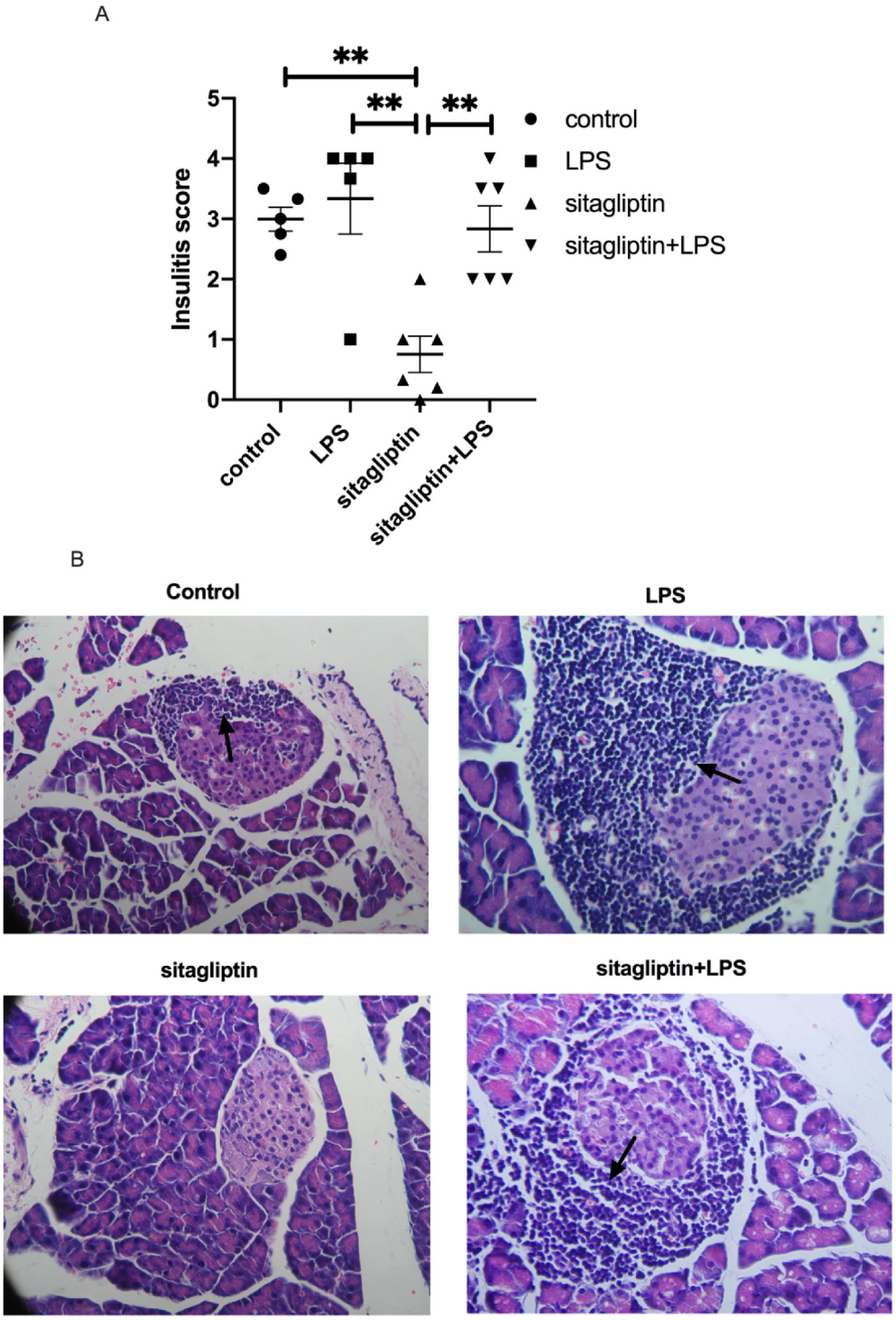


Fig. 2. Long-term inhibition of sitagliptin ameliorates insulitis in NOD mice. A Insulitis scores were calculated as follows: 0, no mononuclear cells infiltrate in islet; 1, mononuclear cells infiltrate around the islet but not in islet; 2, mononuclear cells infiltrate in and around the islet, but the infiltrated area is less than one-third of the islet; 3, intra-islet infiltration of mononuclear cells is 1/3 to 1/2 of the islet area; 4, intra-islet infiltration of mononuclear cells is more than 1/2 of the islet area. B. Representative images of pancreases were stained with H&E. The arrow points to the islets infiltrated with mononuclear cells. Magnification of images: ×400.

group, which has no significant difference between the 4 groups (Fig. 1A).

3.2. DPP-4 inhibitor has no effect on metabolic parameters in NOD mice

We measured body weight, food intake, water intake, and random blood glucose in NOD mice. DPP-4 inhibitor has no effect on body weight, food intake, water intake, and random blood glucose (Fig. 1 B, C, D and E). IPGTT was performed at 32 weeks after sitagliptin treatment. There was no significant difference in blood glucose level during an IPGTT between the 4 groups (Fig. 1 F).

3.3. Long-term DPP-4 inhibition ameliorates insulitis in NOD mice

Pancreases of NOD mice were separated and stained with H&E. Islets of the mice in control group, LPS and sitagliptin + LPS group

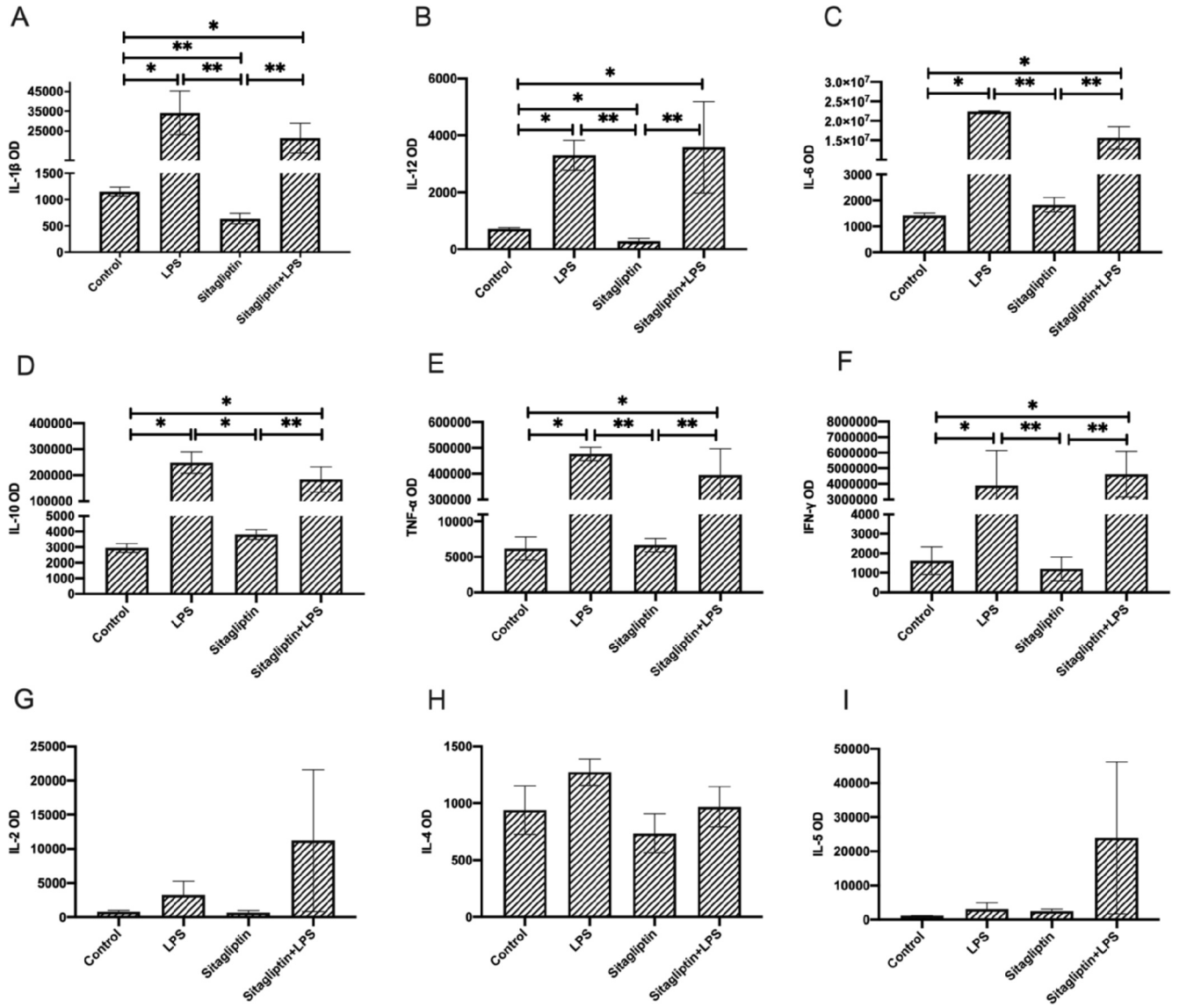


Fig. 3. A and B, Sitagliptin downregulates serum IL-1β and IL-12 in NOD mice. Sitagliptin significantly downregulates serum IL-1β and IL-12 in NOD mice in sitagliptin group compared with control group, LPS group and sitagliptin + LPS group ($p < 0.05$). Fig. 3C–I, Pretreatment with sitagliptin has no effect on LPS-induced IL-1β, IL-6, IL-10, IL-12, TNF-α and IFN-γ in NOD mice ($P > 0.05$).

showed widespread infiltration of mononuclear cells. Mice receiving sitagliptin showed significantly less infiltration of mononuclear cells, compared with control group (Fig. 2A and B). These findings were furthered confirmed by insulitis scoring, which suggested that long-term DPP-4 inhibition ameliorates insulitis in NOD mice.

3.4. DPP-4 inhibition downregulates serum IL-1β and IL-12 in NOD mice

To explore the effect of sitagliptin on the pro-inflammatory and inflammatory cytokines in NOD mice, we determined the secretion of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ in the serum of NOD mice. Sitagliptin significantly downregulates serum IL-1β and IL-12 in NOD mice in sitagliptin group compared with control group, LPS group and sitagliptin + LPS group ($p < 0.05$) (Fig. 3A and B).

In order to explore the effect of long-term inhibition of DPP-4 on the expression of IL-1β and IL-12 in pancreases, we determined IL-1β and IL-12 levels by immunohistochemistry (Fig. 4C). Unfortunately, there was no significantly difference in sitagliptin, compared with control group, LPS group and sitagliptin + LPS group ($P>0.05$) (Fig. 4A and B).

3.5. Pretreatment with DPP-4 inhibitor has no effect on LPS-induced IL-1β, IL-6, IL-10, IL-12, TNF-α and IFN-γ in NOD mice

To identify the effect of pretreatment with sitagliptin on LPS-induced inflammation, we analyzed the levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ in the serum of NOD mice between sitagliptin and sitagliptin + LPS group. There was no difference of the levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ between sitagliptin and sitagliptin + LPS group (Fig. 3C, D, E, F, G, H and I). It suggests that pretreatment with sitagliptin has no effect on the levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ in the serum of NOD mice.

3.6. Long-term inhibition of DPP-4 significantly reduced CD4 + T cells to infiltrate into islets

The immune cells infiltrated into islets and led insulitis in the characterization of type 1 diabetes. In the present study, long-term inhibition of DPP-4 reduced CD4 + T cells to infiltrate into islets in NOD mice ($P<0.01$) (Fig. 5A and E). LPS injection induces CD11b + cells to

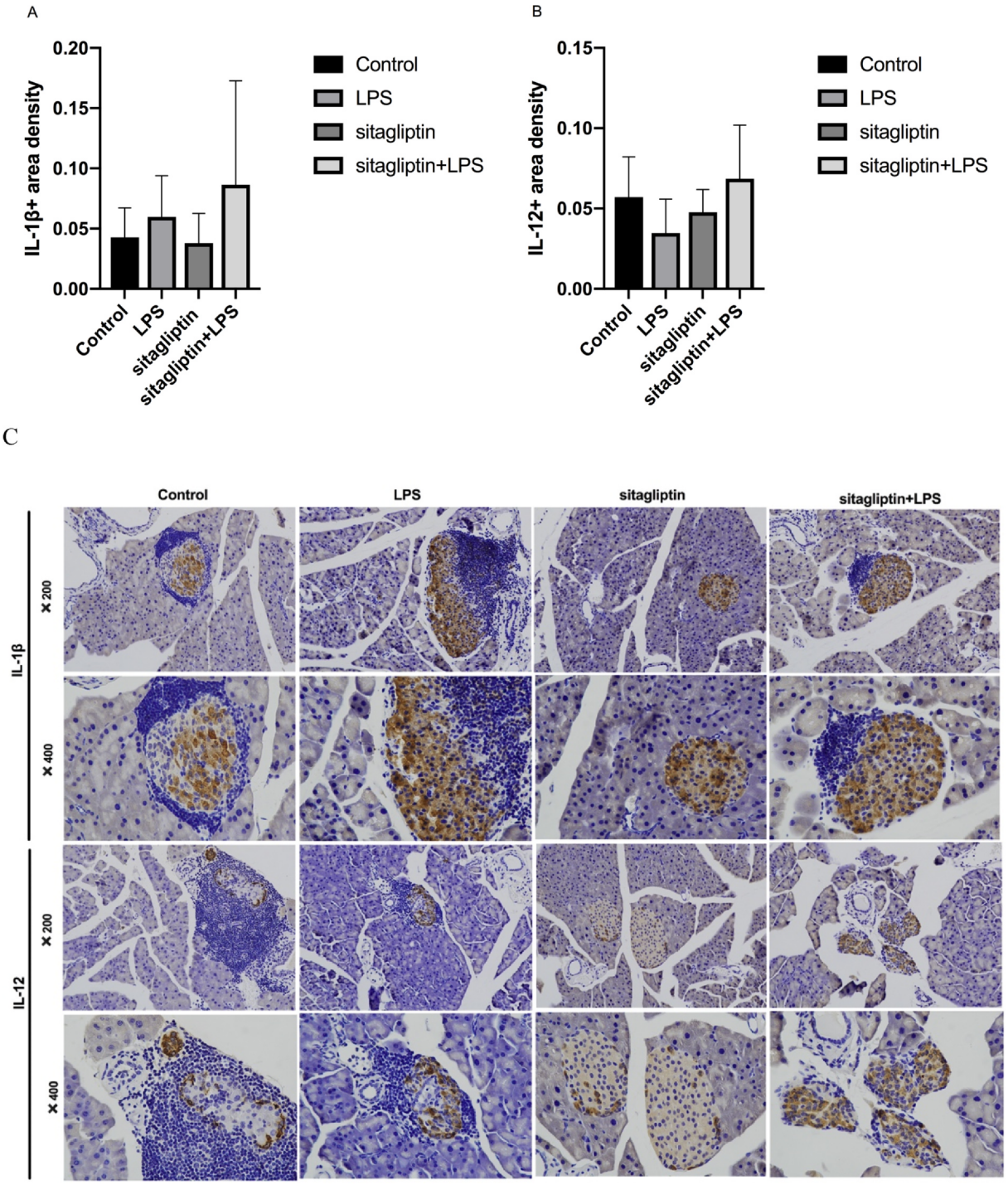
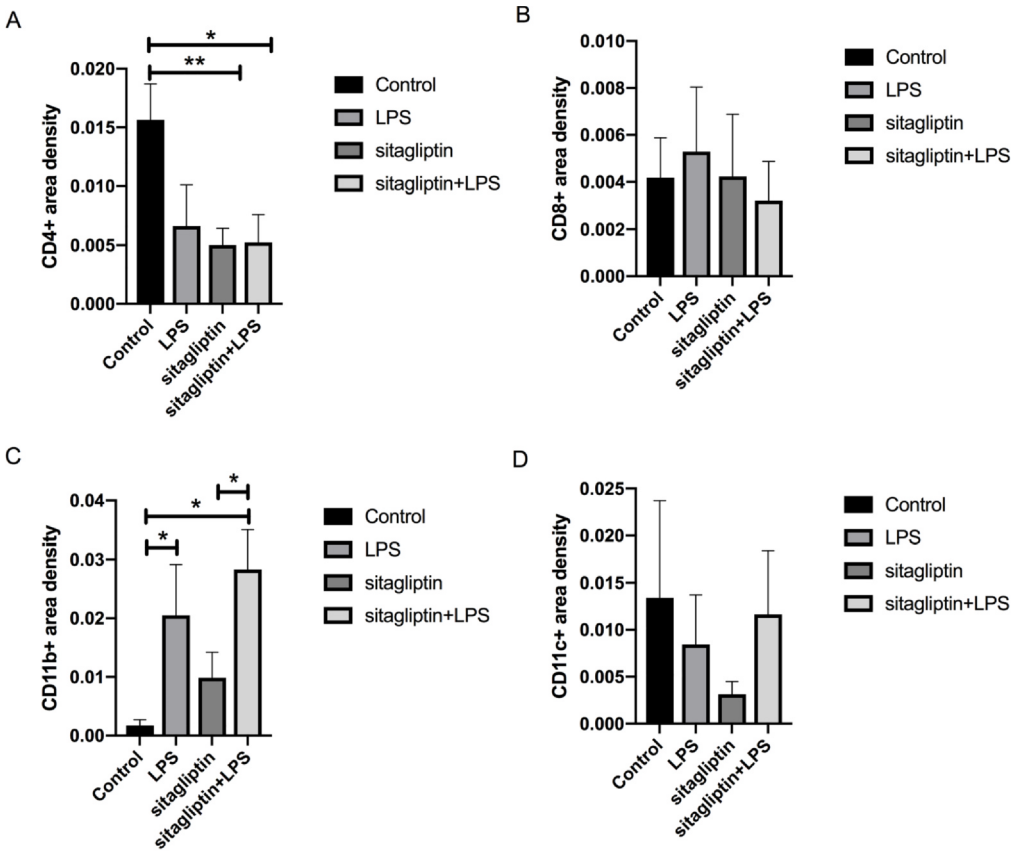


Fig. 4. Long-term inhibition of DPP-4 has no effect on IL-1β and IL-12 expression in islets in NOD mice. A. Long-term inhibition of DPP-4 has no effect on IL-1β expression in islets in NOD mice (P>0.05). B. Long-term inhibition of DPP-4 has no effect on IL-12 expression in islets in NOD mice (P>0.05). C. Protein expression was assessed by immunohistochemistry staining. Brown color was considered positive. (n = 4 in control group, n = 5 in LPS group, n = 6 in sitagliptin group, n = 5 in sitagliptin + LPS group).

infiltrate into islets in NOD mice (Fig. 5C and E). long-term inhibition of DPP-4 had no effect on CD8 + T and CD11c + cells infiltration in islets in NOD mice ((Fig. 5B, D and E) (P>0.05).

4. Discussion

In the present study, long-term inhibition of DPP-4 significantly reduces CD4+T cells to infiltrate into islets, ameliorates insulitis and downregulates serum levels of IL-1β and IL-12 in NOD mice. Long-term treatment of sitagliptin, is more effective than insulin in β cell preservation for at least 4 years in type 1 diabetes, probably due to the immunoregulatory effect of sitagliptin [20]. However, there are few studies about the immunoregulatory effect of DPP-4 inhibitors on innate immunity in type 1 diabetes. Recently, Davanso MR et al. found that treatment of DPP-4 inhibitor for 90 days had no effect on CD3 + CD4+T cells and CD3 + CD8 + T cells in the spleen in streptozotocin-induced type 1 diabetic mouse [21]. However, in the present study, we found that long-term inhibition of DPP-4 (more than



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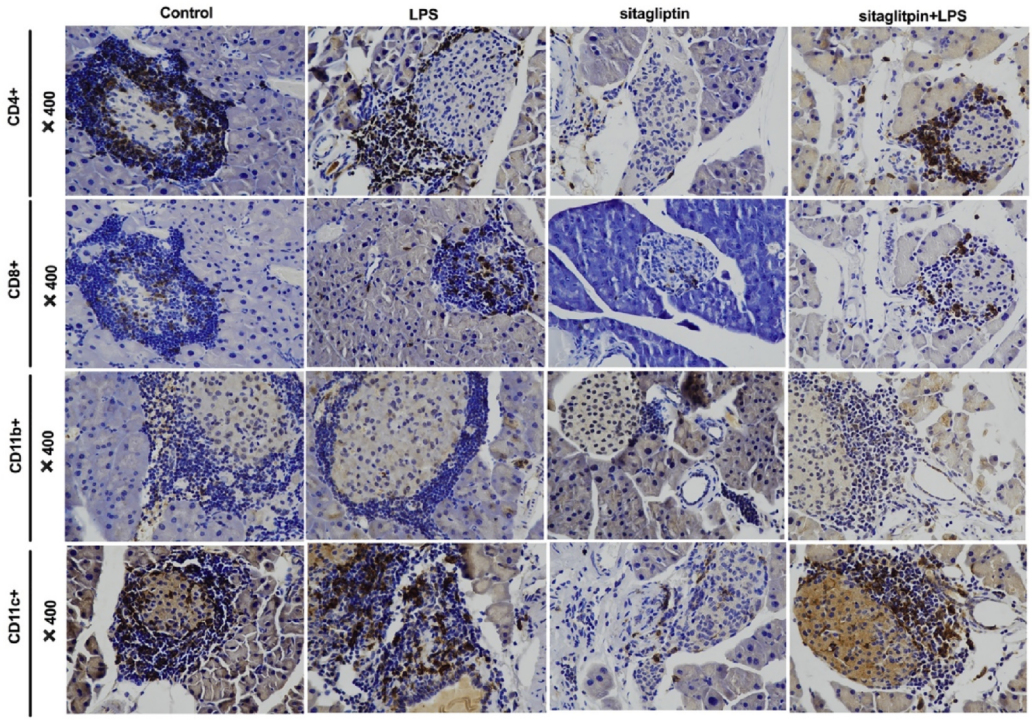

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Fig. 5. Long-term inhibition of DPP-4 significantly reduced CD4 + T cells to infiltrate into islets in NOD mice. A. Compared with control group, sitagliptin treatment significantly reduced CD4 + T cells to infiltrate into islets in NOD mice ($P<0.01$). Administration of LPS following with sitagliptin treatment also reduced CD4 + T cells to infiltrate into islets in NOD mice ($P<0.05$). B. Sitagliptin treatment has no effect on CD8 + T cells infiltration in islets ($P>0.05$). C. Administration of LPS following with sitagliptin treatment increased CD11b + cells to infiltrate into islets in NOD mice, compared with control group and sitagliptin treatment alone ($P<0.05$). Administration of LPS increased CD11b + cells to infiltrate into islets in NOD mice, compared with control group ($P<0.05$). D. Sitagliptin treatment has no effect on CD11c + cells infiltration in islets ($P>0.05$). E, Protein expression assessed by immunohistochemistry staining of the immune cells infiltrating into pancreatic islets, for CD4 + T cells, CD8 + T cells, CD11b + cells as well as CD11c + cells. Brown color was considered positive. CD4 + T cells, CD8 + T cells and CD11c + cells were the dominant immune cell types infiltrating into the islet. Normal islet structure was destroyed when immune cells infiltrated into the islets. (n = 4 in control group, n = 5 in LPS group, n = 6 in sitagliptin group, n = 5 in sitagliptin + LPS group).

8 months) significantly reduces CD4 + T cells to infiltrate into islets and ameliorates insulitis. Many immunoregulatory drugs were effective in early stage of type 1 diabetes, however, very few drugs were still effective in the late stage of disease. In the present study, we used sitagliptin to treat NOD mice for 33 weeks (more than 8 months), which showed to reduce CD4 + T cells infiltration. It is a highlight of our study. long-term inhibition of DPP-4 had no effect on CD8 + T and CD11c + cells infiltration in islets in NOD mice. LPS injection induces CD11b + cells to infiltrate into islets in NOD mice, which suggested LPS induced inflammation in type 1 diabetes.

IL-1 gene may involve in the pathogenesis of type 1 diabetes [22,23]. Administration of high dose IL-1β significantly increases the onset of type 1 diabetes in the Biobreeding rat [24]. IL-1 signaling plays an important role in β cell dysfunction probably via the mitogen activated protein kinase (MAPK) and NF-κB pathway [25]. IL-1 block in the early stage of type 1 diabetes prevents virus-triggered insulitis in Kilham rat virus (KRV)-induced diabetes [26]. In the present study, we found the OD value of serum IL-1β by MSD in sitagliptin group is significantly decreased compared with control group, which suggests sitagliptin downregulates serum IL-1βlevel. Meanwhile, we found sitagliptin protects βcell function in NOD mice. The decrease of IL-1βpossibly contributes to the protection of βcell function in NOD mice. IL-12 is mainly produced by phagocytes (macrophages, monocytes and neutrophils) and dendritic cells [27]. It is a key proinflammatory cytokine which promotes naïve $CD4^+$T cells to differentiate into T helper1 (Th1) cells [28]. It is considered as a link between innate and adaptive immunity [27]. In the present study, we found sitagliptin downregulates serum IL-12 level, which might play some role in innate and adaptive immunity in NOD mice. IL-12 is positively associated with risk of type 1 diabetes [29,30,31]. IL-12 mRNA expression of mononuclear leukocytes isolated from islets is correlated with β cell destruction in NOD mice [32]. Administration of IL-12 induces severe lymphocyte infiltration in the pancreatic islets and accelerates type 1 diabetes in NOD mice [33]. In the present study, we found sitagliptin downregulates IL-1β and IL-12 in the serum, but not in the pancreases, which might own to the different distribution of these cytokines in the body. Down regulation of IL-1β and IL-12 was probably related with amelioration of insulitis by an indirect effect in type 1 diabetes.

In the present study, we determined serum levels of IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α and IFN-γ, which shows no significant difference between sitagliptin and control group. There were few studies about the effect of DPP-4 inhibitor on cytokine profile in autoimmune diseases. DPP-4 inhibitor (PKF275-055) does not affect IL-2 in kidney and serum IL-6 in Sprague-Dawley (SD) rats of type 1 diabetes [34]. Sitagliptin treatment for 90 days decreased TNF-α and IL-10 in pancreatic homogenate in streptozotocin-induced type 1 diabetic mice [21], which was different with our result. We considered it was owing to: (1) the concentrations of TNF-α and IL-10 were higher in pancreas than in serum in type 1 diabetes; (2) there were different effects of cytokines in different stages of disease.

Marjolein J et al. found that increased infection (especially respiratory infection) is significantly associated with DPP-4 inhibitors [15]. Corona virus infectious disease (COVID-19) is a new epidemic disease with high mortality. Diabetes is one of the most common comorbidities among the patients with COVID-19 [35]. The patients suffered from severe COVID-19 with diabetes had higher mortality than patients without diabetes [36]. ICU patients infected with COVID-19 had higher inflammatory cytokines such as IL-2, IL-7, IL-10 and TNF-α, compared with non-ICU patients [37]. DPP-4 has been identified as a functional receptor for human coronavirus-Erasmus Medical Center [38], which is essential for coronavirus infection [38]. It means there is intense contact between DPP-4 and coronavirus infection. Mice expressing human DPP-4 were susceptible to Middle East respiratory syndrome coronavirus (MERS-Cov) [39]. Human DPP-4 transgenic diabetic mice exhibit prolonged severe disease and immune dysregulation following MERS-Cov infection [40]. However, Rinkoo Dalan presents that DPP-4 inhibitors might not be beneficial to COVID-19 infection for the following reasons: (1) DPP-4i may not effectively reduce infection transmission; (2) DPP-4i may be a disadvantage for suppressing T cell immunity; (3) DPP-4i may increase fibrotic lesion in the lung; (4) DPP-4i recruits regenerative stem cells; (5) DPP-4i is related to a prothrombotic state [41]. To date there is no direct consequences between COVID-19 infection and DPP-4 inhibitors. A case control study shows that treatment with DPP-4 inhibitors in T2D patients with COVID-19 has no influence to disease outcome, compared with those without DPP-4 inhibitors treatment [42]. Because the intense contact of DPP-4 and coronavirus infection, further research between DPP-4i and coronavirus in diabetes is warranted.

Stervbo et al found that gravitation stress decreased serum levels of inflammatory mediators and they provides promising design to assess the effect of cytokines with or without LPS stimulation ex vivo [43]. In this study, we injected intraperitoneally the mice in LPS group and sitagliptin + LPS group with LPS (5 mg/kg) 3 h before sacrificing these mice to initiate innate immune response. After LPS injection, the serum concentration of IL-1β, IL-6, IL-10, IL-12, TNF-α and IFN-γ is significantly increased. However, pretreatment with sitagliptin for 33 weeks does not decrease the production of pro-inflammatory cytokines, probably owing to the following reasons: first, LPS is strong activator of serious inflammatory reaction, which cann't be reversed by pretreatment of sitagliptin; second, treatment with sitagliptin is before LPS injection, which acts like effects of pretreatment.

5. Conclusions

Long-term inhibition of DPP-4 exists anti-inflammatory effect in type 1 diabetes probably by reducing CD4 + T cells to infiltrate into islets and downregulating L-1β and IL-12 in serum.

CRediT authorship contribution statement

Xinran He: Investigation, Methodology, Software, Formal analysis. **Wangen Li:** Validation. **Yunliang Xie:** Writing - review & editing, Supervision. **Yunjuan Zhao:** Conceptualization, Resources, Writing - original draft, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Compliance with ethical standards

All experiments were complied with institutional guidelines and approved by Institutional Animal Ethics Committees of Guangzhou Medical University.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.106945.

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