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ICOS/ICOSL upregulation mediates inflammatory response and endothelial dysfunction in type 2 diabetes mellitus

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Abstract. – OBJECTIVE: ICOS/ICOSL plays a crucial part in various disease-mediated immune responses. However, the exact role of ICOS/ICOSL in type 2 diabetes mellitus (T2DM) development remains unexplored. This study aims to investigate the role of ICOS/ICOSL in the pathogenesis of T2DM.

MATERIALS AND METHODS: Human peripheral blood T-lymphocytes (CD3) and umbilical vein endothelial cells (HUVECs) were treated with high-glucose (HG) or advanced glycation end products (AGEs). A portion of CD3 cells was co-cultured with HUVECs and treated with different mediums or anti-ICOS mAbs. The ICOS/ICOSL and caspase-3 protein expression was measured by Western blotting. ELISA (enzyme-linked immunosorbent assay), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and NOx production assays were respectively used to detect cytokines level, cell viability and the production of NOx.

RESULTS: HG and AGEs significantly upregulated ICOS/ICOSL expressions in T cells and HUVECs. T cell contact with HUVECs secreted more IFN-γ, IL-4, and IL-10 compared to non-contact cells, while cytokines from IL-6-, IL-1β-, and CM- (the conditioned medium) treated cells did not differ from the control. A significant increase of IL-8 and IL-6 was found in HUVECs under both contact and non-contact conditions *vs.* control cells. Similar results were also observed in the comparison between CM1- (T cell condition medium) or CM2- (co-culture condition medium) treated cells and control cells. However, CM1 and CM2 treatment significantly inhibited cell viability and increased caspase-3 and NOx production; blocking ICOS/ICOSL remarkably decreased cytokines secretion, enhanced cell viability and reduced caspase-3 and NOx production.

CONCLUSIONS: HG and AGEs cause T cell inflammatory response and vascular endothelial dysfunction by upregulating ICOS/ICOSL, which may be one of the possible mechanisms of cardiovascular complications development in T2DM patients.

Key Words
Type II diabetes mellitus, ICOS/ICOSL, Inflammatory response, Endothelial dysfunction.

Introduction

Type 2 diabetes mellitus (T2DM), a major type of diabetes, has become a serious threat to public health worldwide with increasing clinical incidence rates[1]. T2DM development is generally accompanied by a series of adverse reactions such as excessive food intake and frequent urination along with clinical symptoms including cataracts and diabetic foot[2,3]. Patients suffering from T2DM are associated with a higher prevalence of cardiovascular events comparable to non-diabetic patients, especially diabetic heart diseases. Accordingly, the average expectancy of T2DM patients decreases by about 10 years and approximately 80% of T2DM patients die from cardiovascular complications[4]. Although important progress has been made regarding the therapy of T2DM, it remains an incurable disease over the world. It is urgent, therefore, to identify the pathogenesis mechanisms of T2DM at the gene level, which may provide a novel

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therapeutic target for T2DM treatment. Chronic hyperglycemia often occurs in the development of diabetes, which initiates a great deal of vascular complications[5]. Generally, hyperglycemia contributes to large production and accumulation of AGEs that are glycated in the diabetic vasculature stimulated by a hyperglycemic environment[6]. AGEs have been found to be involved in the development of several serious microvascular and macrovascular complications (which include nephropathy, neuropathy, retinopathy and angiopathy) by binding with their specific receptor (RAGE, receptor for advanced glycation end-products) and damaging the biological membranes and the endothelium[7]. Diabetes, therefore, is generally associated with serious inflammatory reactions and endothelial dysfunction[8,9]. ICOS (CD278), an inducible co-stimulator, is an important member of CD28 superfamily receptors and is mainly expressed on memory CD4 and CD8 T cells[10]. ICOS signaling is activated upon the participation by its unique ligand, ICOSL (CD275, B7-H2, B7h, B7RP-1) that is normally expressed on B cells, macrophages and dendritic cells[11,12]. In ICOS knockout mice, the immune cells are at a highly activated state and the susceptibility of type I diabetes is significantly increased[13,14]. The important role of ICOS-ICOSL interacting with the immune regulation has been confirmed by a growing list of evidence and has the ability to promote the proliferation of regulatory T (Treg) cells[15-17]. Meanwhile, the activation of the ICOS/ICOSL pathway-driven generation of Tregs has been found in both periphery[18] and microenvironment of diversified tumor cells[19,20]. The above findings suggest that the ICOS/ICOSL pathway plays an important role in immune responses and is triggered by various diseases. So far, a few studies have paid attention in exploring the potential role of ICOS/ICOSL signaling in the pathogenesis of type 2 diabetes mellitus. In the present work, the main aim is to explore the ICOS/ICOSL expression in HG- and AGEs-treated human peripheral blood T-lymphocytes and umbilical vein endothelial cells, as well as uncover the potential role of ICOS/ICOSL in T2DM-mediated inflammatory response and endothelial dysfunction. The findings obtained from this study provide a valuable supplement for exploring the biological functions and molecular mechanisms of ICOS/ICOSL in T2DM development, which may lay the foundation of the gene therapy for diabetes mellitus.

Material and Methods

Cell Lines

Human peripheral blood T-lymphocytes (CD3) and umbilical vein endothelial cells (HUVECs) were obtained from 20 patients who were newly diagnosed with type-II diabetes at the First People's Hospital of Yunnan Province from August 2015 to June 2016, and 20 healthy controls. All participants provided the researchers of this work with written informed consent. The study protocol was approved by the Institute Research Ethics Committee of First People's Hospital of Yunnan Province.

Cell Treatment

All collected cells were cultured in humidified conditions with 95% air and 5% CO_2 at 37°C in Roswell Park Memorial Institute-1640 medium (RPMI-1640; Invitrogen, Carlsbad, CA, USA) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% fetal calf serum. Two days later, the cells were collected and transferred into an appropriate medium supplying with glucose (Sigma-Aldrich, St. Louis, MO, USA) (0, 5.5, 16.7, 33.3 mmol/L) or AGEs (generated in a non-enzymatic protein glycation process) (0, 50, 100, 200 μg/mL). At the time of 24, 48, and 72 hours after different stimulation, the cells were collected for Western blot assay.

Co-Culture of CD3 and HUVECs

In co-culture experiment, CD3 cells were co-cultured with unstimulated HUVECs or HUVECs pretreated with anti-ICOS in the presence of 2 mg/mL anti-ICOS mAbs (eBioscience, San Diego, CA, USA) for 24 hours. Transwell cell culture chambers (1 μM, Millipore, Billerica, MA, USA) were used for co-culture assay. T-lymphocytes were seeded at the upper chamber with HUVECs monolayer on the lower chamber and were allowed to direct attach overnight. After the incubation process, the cell suspension and supernatants were collected after centrifugation and stored for future experiments.

ELISA Assay

Cytokines including IFN-γ, IL-4, IL-6, IL-8, and IL-10 production secreted from T cells or HUVECs were quantified using enzyme-linked immunosorbent assay according to the manufacturers' instructions. For assays, the cell suspensions from different groups were centrifuged and the separated supernatants were harvested for analysis with the use of the respective capture

antibodies respectively against IFN-γ, IL-4, IL-6, IL-8, and IL-10 (8 μg/mL) from BioSource (Camarillo, CA, USA). The value of absorbance at 450 nm was recorded using a spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA).

MTT Assay

MTT assay was used to evaluate the viability of HUVECs. First, appropriate MTT (Genview, Houston, TX, USA) was dissolved in Phosphate-Buffered Saline (PBS; Sigma-Aldrich, St. Louis, MO, USA). After mixing thoroughly and sterilizing, MTT stock solution at the concentration of 5 mg/mL was obtained. Then, 10 μl of MTT solution was transferred into each well of 96-well dishes containing the culture medium. The mixture was then incubated at 37°C for 4 hours. The plates were centrifuged and the medium was discarded. 200 μL dimethyl sulfoxide (DMSO; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to each well to dissolve the dark blue crystals. The absorbance at a wavelength 490 nm was recorded using a SpectraMax M2/M2e microplate reader (Molecular Devices, Sunnyvale, CA, USA).

NOx Production Measurement

NOx (NO and its oxidative metabolic products, NO_2^- and NO_3^-) production in cell supernatants were detected using a NO assay kit (Beyotime Institute of Biotechnology, Shanghai, China) following the manufacturer's protocol. The amount of NOx was represented as nmol/10^5 cells for HUVECs.

Western Blot Analysis

Total proteins were extracted from the cells using a cytosol protein extraction kit (Beyotime Institute of Biotechnology, Shanghai, China). 20 μg of proteins were subjected to 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE; Bis-Tris, Novex, San Diego, CA, USA) and then transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). After being blocked with 5% non-fat dried milk (BD Biosciences, Franklin Lakes, NJ, USA), the membranes were incubated with the primary antibodies respectively against ICOS, ICOSL, and caspase-3 with appropriate dilution overnight at 4°C, followed by a horseradish peroxidase-linked secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 37°C for 60 minutes. The films were developed using an ECL detection kit (Millipore, Billerica, MA, USA). Antibodies against ICOS, ICOSL and caspase-3 were purchased from Cell Signaling Technology (Danvers, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as the internal control.

Statistical Analysis

All values were shown as a means ± SEM of at least triplicate detections. All statistical analyses were conducted using GraphPad Prism software version 5.0 (GraphPad Software, La Jolla, CA, USA). Statistical significance was evaluated using unpaired Student's *t*-test. $p<0.05$ was considered statistically significant.

Results

HG and AGEs Upregulates ICOS Expression in T Cells

To confirm the impacts of HG or AGEs on ICOS expression in human peripheral blood T-lymphocytes, CD3 cells derived from diabetic patients and healthy people were respectively treated with high-glucose at a concentration of 0, 5.5, 16.7, 33.3 mmol/L, or AGEs at a concentration of 50, 100, 200 μg/mL. The corresponding results are shown in Figure 1. After 24 hours following stimulation, no alteration of ICOS expression in CD3 cells was observed. However, the relative ICOS expression was significantly increased in a concentration-dependent manner at the time point of 48 and 72 h in both normal or diabetes group ($p<0.01$, Figure 1A), suggesting that the glucose at high concentration was able to induce an effective upregulation of ICOS in T cells. Not surprisingly, a similar phenomenon was also represented in AGEs-treated cells. We found that the ICOS expressed on CD3 cells was elevated gradually with the increase of AGEs dose ($p<0.05$, $p<0.01$). A well-marked enhancement of ICOS level was shown after treatment with 100, 200 μg/mL AGE for 48 and 72 hours ($p<0.01$, Figure 1B). These findings suggest that HG or AGEs treatment has the ability to upregulate the ICOS expression level in human peripheral blood T-lymphocytes.

HG and AGEs Upregulates ICOSL Expression in HUVECs

The researchers of this study further explored the effects of HG or AGEs on ICOSL expression in human umbilical vein endothelial cells. Clear-

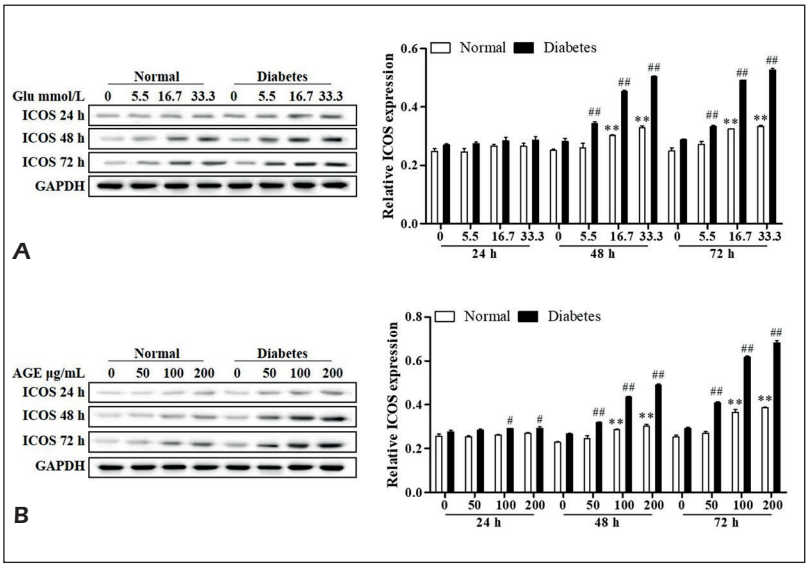

Figure 1. HG and AGEs upregulates ICOS expression in T cells. **A**, Western blot analysis showing the protein levels of ICOS in T cells after treatment with HG at the concentration of 0, 5.5, 16.7, 33.3 mmol/L for 24, 48 and 72 hours. **B**, Western blot analysis showing the protein levels of ICOS in CD3 cells after treatment with AGEs at the concentration of 50, 100, 200 μg/mL for 24, 48 and 72 hours. The results are reported as means ± standard deviation. #p<0.05, ##p<0.01 for the cells derived from diabetes patients; *p<0.05, **p<0.01 for cells derived from normal subjects.

ly, an important increase of ICOSL expression in HUVECs at the time point of 72 h post treatment was observed in the presence of 200 μg/mL AGEs comparing with the cells treated for 24 or 48 hours (p<0.01, Figure 2A), revealing that a long-term treatment with AGEs is more likely to induct the high expression of ICOSL than short-term treatment. Besides, the ICOSL expression in HUVECs post stimulation with AGEs for 72 hours was gradually improved with AGEs dose increasing (p<0.01, Figure 2A), which supported that a high concentration of AGEs is critical to upregulate ICOSL expression. Similarly, the administration of HG also led to an enormous enhancement of ICOSL protein level in a concentration and time-dependent manner (p<0.05, p<0.01, Figure 2B). We conclude that HG or AGEs treatment can promote ICOSL expression in human umbilical vein endothelial cells.

Cell-Cell Contact is Necessary for HG and AGEs Inducing T Cells Inflammatory Response

It is well-recognized that ICOS/ICOSL signaling plays an important role in organism immune system[16,17]. Considering our prior observation

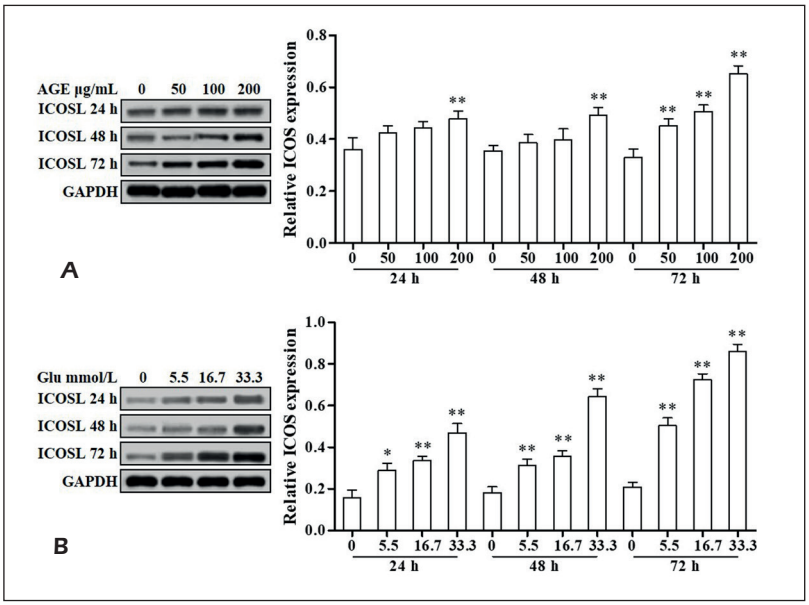

Figure 2. HG and AGEs upregulates ICOSL expression in HUVECs. **A**, Western blot analysis showing the protein levels of ICOSL in HUVECs after treatment with HG at the concentration of 0, 5.5, 16.7, 33.3 mmol/L for 24, 48 and 72 hours. **B**, Western blot analysis showing the protein levels of ICOSL in HUVECs after treatment with AGEs at the concentration of 50, 100, 200 μg/mL for 24, 48 and 72 hours. The results are reported as means ± standard deviation. *p<0.05, **p<0.01.

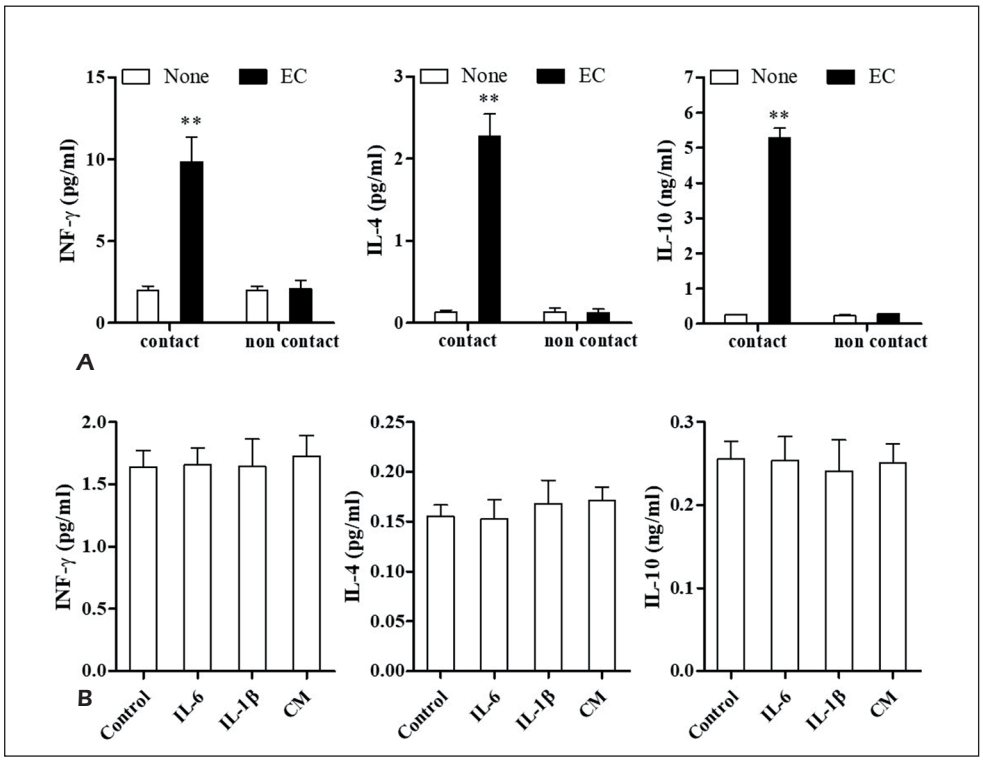

Figure 3. Cell-cell contact is necessary for HG and AGEs inducing T cells inflammatory response. **A**, ELISA assay showing the protein levels of IFN-γ, IL-4, and IL-10 secreted by T-lymphocytes under non-contact or contact condition. **B**, ELISA assay showing the protein levels of IFN-γ, IL-4, and IL-10 secreted by T-lymphocytes after treatment with IL-6, IL-1β and condition medium (CM) from HUVECs. $^{**}p<0.01$.

that the ICOS/ICOSL expression level was significantly increased in T-lymphocytes and HUVECs after treatment of HG or AGEs, we tested the hypothesis that ICOS/ICOSL interaction may be involved in the regulation of T cells inflammatory response in CD3 and HUVECs co-culture system. The result of ELISA assay uncovered that the level of cytokines including IFN-γ, IL-4, and IL-10 in supernatants of contact co-culturing system increased respectively at 5.1, 2.2, and 5.1-fold compared to control cells ($p<0.01$), while no significant difference was observed in the comparison between the non-contact and control T cells (Figure 3A), which demonstrated that the contact co-culture had an enhanced ability to induce the secretion of cytokines from T cells. In the following experiments, IL-6 and IL-1β secreted from HUVECs and the condition medium (CM) from HUVECs was used to treat CD3 cells. IFN-γ, IL-4, and IL-10 expressed on all treated T cells did not differ from that on the control cells (Figure 3B), which confirmed more exactly that the direct cell-cell contact between T-lymphocytes and HUVECs is necessary for the activation of T cells. These findings provided evidence for the important role of cell contact between CD3 and HUVECs in HG or AGEs induced-T cells inflammatory response.

Cell-Cell Contact is Not a Requirement for HG and AGEs Inducing Endothelial Dysfunction

Next, we explored the impact of HG or AGEs treatment in the co-culture system of CD3 and HUVECs on endothelial function. Interestingly, we found a significant increase of IL-8 and IL-6 production from HUVECs under both contact and non-contact condition compared to the control cells ($p<0.01$, Figure 4A). Besides, there is a significant difference of cytokines secretion from HUVECs treated with CM1 (T cell condition medium) and CM2 (co-culture condition medium) compared to the control group ($p<0.01$, Figure 4B). These findings confirm that the presence of contact co-culture is not necessary for inducing endothelial dysfunction. Besides, a remarkable weakness of cell viability ($p<0.01$, Figure 4C) and a well-marked increase of caspase-3 expression ($p<0.01$, Figure 4D) were found in CM1- and CM2-inducted HUVECs-, both of which were

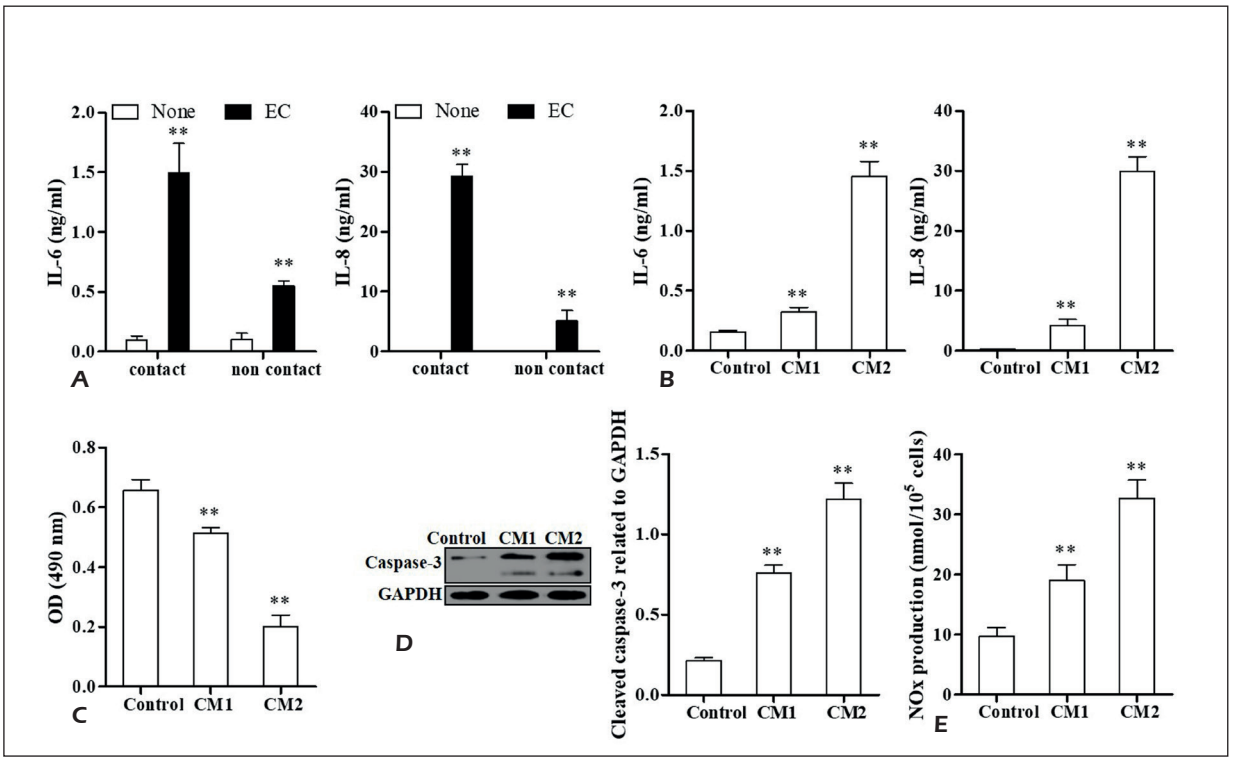

Figure 4. Cell-cell contact is not a requirement for HG and AGEs inducing endothelial dysfunction. **A**, ELISA assay showing the protein levels of IL-6 and IL-8 secreted by HUVECs under non-contact or contact condition. **B**, ELISA assay showing the protein levels of IL-6 and IL-8 secreted by HUVECs after treatment with control medium, CM1, and CM2. **C**, MTT assay showing the cell viability of HUVECs after treatment with control medium, CM1 and CM2. **D**, Western blot analysis showing the protein level of caspase-3 in HUVECs after treatment with control medium, CM1 and CM2. **E**, NOx production measurement showing the NOx production from HUVECs after treatment with control medium, CM1 and CM2. CM1, T cell condition medium; CM2, co-culture condition medium. $^{**}p<0.01$.

represented in the result of MTT and Western blot assays. The above findings reveal that the regulatory effect of T cells on HUVECs growth and apoptosis is not dependent on direct cell-cell contact. The NOx level, an important biochemical marker in endothelial dysfunction, was also detectable[21]. Unsurprisingly, treatment with CM1 and CM2 alone caused an important increase in NOx production compared with the control ($p<0.01$, Figure 4E). We showed that the endothelial dysfunction induced by HG or AGEs did not require the cell-cell contact between CD3 and HUVECs.

Blockage of ICOS/ICOSL Pathway Inhbits HG and AGEs Induced-T Cells Inflammatory Response and Endothelial Dysfunction

Based on our prior results, we tested the hypothesis that the molecular mechanism underlying HG or AGEs induced-T cells inflammatory response and endothelial dysfunction may be associated with the activation of the ICOS/ICOSL signaling pathway. To verify this, we blocked the ICOS/ICOSL pathway by using an antagonist anti-ICOS antibody. Compared to the control cells, the level of IFN-γ, IL-4, IL-10, IL-6, IL-8 was remarkably decreased when anti-ICOSL mAbs were added in co-culture ($p<0.01$, Figure 5A), revealing a key role of the ICOS/ICOSL pathway in inducing cytokines secretion. As a result of the MTT assay, CM2 administration contributed to significant suppression of HUVECs viability ($p<0.01$), while this inhibitory effect was reversed effectively by anti-ICOSL mAbs ($p<0.01$, Figure 5B). In contrast with cell growth data, we observed an important increase of caspase-3 protein expression in CM2-treated cells *vs.* those cells treated with a control medium ($p<0.01$). This high level was almost restored to the normal in antibody treatment groups ($p<0.01$, Figure 5C). Similar results were also observed in the NOx production ($p<0.01$, Figure 5D). These data suggest that the ICOS/ICOSL interaction is involved in the

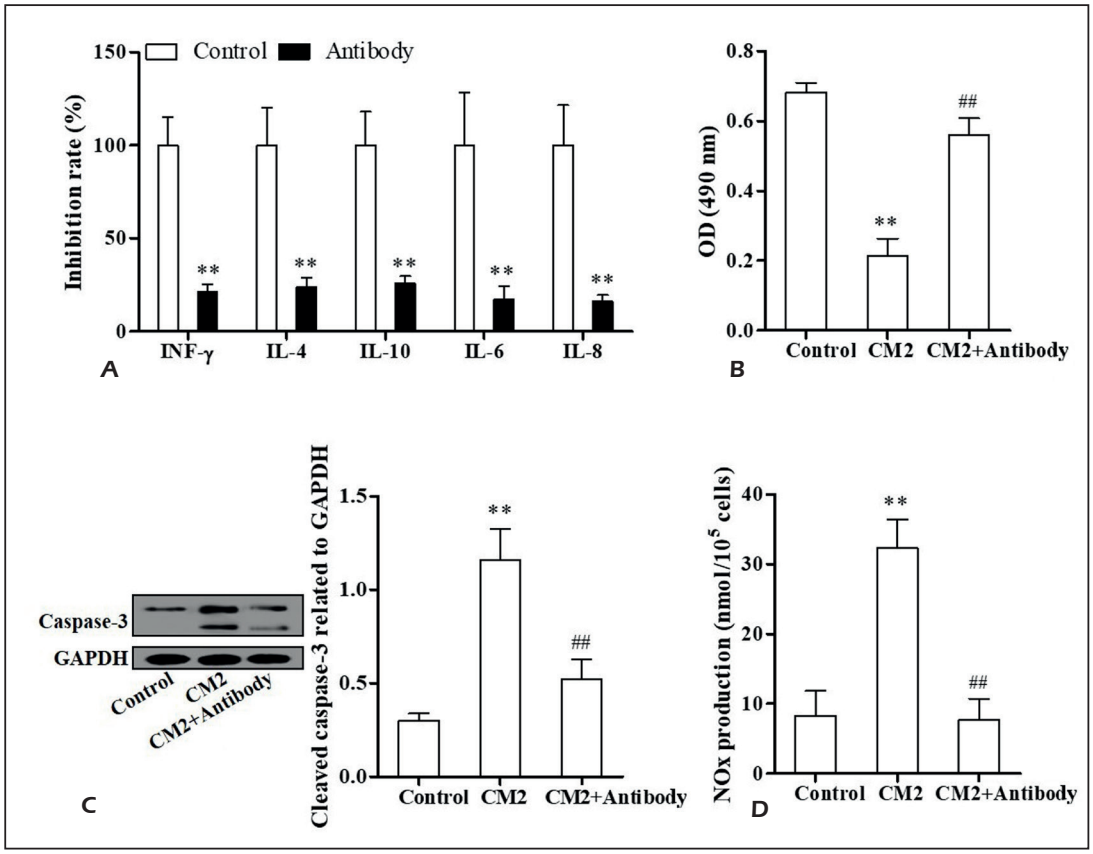

Figure 5. Blockage of ICOS/ICOSL pathway inhibits HG and AGEs induced-T cells inflammatory response and endothelial dysfunction. **A**, ELISA assay showing the inhibition rate of IFN-γ, IL-4, IL-10, IL-6 and IL-8 secretion from T cells after treatment with CM2 plus ICOS antibody. **B**, MTT assay showing the cell viability of HUVECs after treatment with control medium, CM2 and CM2 plus ICOS antibody. **C**, Western blot analysis showing the protein level of cleaved caspase-3 in HUVECs after treatment with control medium, CM2 and CM2 plus ICOS antibody. **D**, NOx production measurement showing the NOx production from HUVECs after treatment with control medium, CM2 and CM2 plus ICOS antibody. CM1, T cell condition medium; CM2, co-culture condition medium. $^{**}p<0.01$ vs. control group, $^{\#\#}p<0.01$ vs. CM2 group.

regulation of vascular endothelial function. The above results indicate that HG or AGEs-mediated inflammatory response and endothelial dysfunction strongly rely on the ICOSL/ICOS pathways.

Discussion

Type 2 diabetes mellitus, a common metabolic disease with high morbidity worldwide, is characterized by hyperglycemia, hyperlipidemia, glucose intolerance and insulin resistance[22]. These metabolic disturbances induce serious inflammatory responses and endothelial dysfunction[8,23] and further contribute to various cardiovascular complications. ICOS, an inducible co-stimulator, plays an important role in immune regulatory function[16,17]. The ICOS/ICOSL interaction has been found to be in connection with the pathogenesis of diversified disorders[18-20]. However, the underlying function of the ICOS/ICOSL pathway in the development of T2DM has not been fully explored thus far. In this work, our main objective was to investigate the expression of ICOS/ICOSL in diabetic patients and confirm its role in T2DM-associated inflammatory response and endothelial dysfunction, which may provide novel direction for the treatment of type 2 diabetes mellitus at the molecular level. Chronic hyperglycemia is the most common symptom in diabetes development, which has been revealed to stimulate various vascular complications[5]. Under the environment of long-term hyperglycemia, the advanced glycosylation end products in diabetic vasculature would increase significantly[6]. Different clinical studies present different results in terms of the serum or plasma levels of AGEs because of varying detection procedures or conditions. The

recognized standpoint, however, is that AGEs level is increased significantly in diabetic patients compared with normal subjects2[4]. In our work, we treated human peripheral blood T-lymphocytes and human umbilical vein endothelial cells (HUVECs) with glucose at a concentration of 0, 5.5, 16.7, 33.3 mmol/L, or AGEs at the concentration of 50, 100, 200 μg/mL. Following this, our team detected ICOS protein levels using a west blot analysis. The choice for glucose and AGE concentration is, according to prior research, that has explored the potential AGEs functions *in vitro* endothelial cells[25,26]. We found that HG and AGEs treatment contributed to a significant upregulation of ICOS in CD3 cells derived from both diabetic patients and normal donors in a concentration-dependent manner. Meanwhile, a significant enhancement of ICOSL levels in HUVECs has also shown AGEs dose increasing. These findings display evidence that high-glucose or AGE treatment can upregulate the ICOS/ICOSL expression in T cells or HUVECs.

Type 2 diabetes mellitus is a chronic inflammatory disorder and a variety of inflammatory reactions would occur after the onset. Some studies[27,28] supported that inflammation plays an important role in the development of hyperglycemia-mediated vascular damage. Therefore, T2DM is generally accompanied with the abnormal expression of inflammatory factors[29]. Herein, we measured the expression of several common inflammatory factors, including the pro-inflammatory IFN-γ secreted from Th1 cells and the anti-inflammatory cytokine IL-4, IL-10 produced from Th2 cells[30]. Compared to non-contact cells, T cells from contact co-culturing system secreted more inflammatory cytokines; however, no well-marked differences of these cytokines were observed between the control cells and IL-6-, IL-1β- or condition medium-treated cells, which revealed that the cell contact between T-lymphocytes and HUVECs is necessary for the activation of T cells.

Hyperglycemia acts in concert to target endothelial cells in diabetic patients, contributing to oxidative stress and endothelial dysfunction[31]. Endothelial dysfunction is considered to be the earliest event in the development of vascular complications which can later induce vascular dysfunction in diabetes. Therefore, we explored the effect of HG or AGEs treatment in a co-culture system of CD3 and HUVECs on endothelial function in the following experiments. The elevation of inflammatory factors indirectly reveals the high prevalence rate of inflammatory reactions, which has the ability to impair the vascular endothelial wall and cause endothelial dysfunction[32,33]. Among the usual inflammatory cytokines, interleukin-6 (IL-6) and interleukin-8 (IL-8) are regarded as the key contributors to endothelial dysfunction in type 2 diabetes[34,35]. Therefore, the expression level of IL-6 and IL-8 were determined herein using ELISA assay. Compared to the untreated cells, a significant difference of IL-8 and IL-6 secretion was presented regardless of the direct contact between HUVECs with CD3 cells or not; besides, these cytokines secreted from HUVECs treated with CM1 and CM2 were all much more than the control group. Thus, the above observation confirms that the presence of contact co-culture is not necessary for the endothelial dysfunction of HUVECs. The findings have extended the previous observations and have shown that serum AGEs level were correlated with endothelial dysfunction in diabetes[21]. Furthermore, CM1 and CM2 treatment also induced a remarkable weakening of cell viability, whereas a significant enlargement of cell apoptosis revealed the kill effect of the activated T cells on HUVECs. To identify the mechanisms underlying the negative effect of T cells on endothelial function, we next assessed the production of NOx from control, CM1- and CM2-treated HUVECs. The high NOx level is an important reason which causes endothelial dysfunction and tissue damage[36]. Treatment with CM1 and CM2 alone both caused a significant increase in NOx production, compared with the controls in HCAECs. Considering this with our prior findings, it can be concluded that the cell-cell contact between T cells and HUVECs leads to an increase in the secretion of cytokines and causes endothelial dysfunction. The activation of T cells is contact-dependent, while cell-cell contact is not a requirement for the regulation of activated T cells on HUVECs. Collectively, cell contact-mediated T cells activation enhances the killing effect of T cells on HUVECs.

ICOS is an inducible co-stimulator with its positive effect on cell proliferation, survival, and differentiation having been confirmed by continual research[37,38]. ICOS/ICOSL interaction was involved in the induction of cytokines secretion, T-lymphocytes proliferation and upregulation of cell surface molecules[39,40]. Considering the high expression of ICOS/ICOSL in T-lymphocytes and HUVECs under the treatment of HG or AGEs, we tested the hypothesis that the molecular underlying HG or AGE induced-inflammatory response

and endothelial dysfunction may be associated with the activation of the ICOS/ICOSL signaling pathways. To verify this, we specifically blocked ICOS/ICOSL pathways by using an antagonist anti-ICOS antibody. We observed that ICOS/ICOSL blockage contributed to a significant increase of IFN-γ, IL-4, IL-10, IL-6, and IL-8 production, revealing a key role of the ICOS/ICOSL pathway in HG- and AGEs-induced inflammatory response and endothelial dysfunction. This finding was partly consistent with a prior study[41] showing that ICOS blockade reduces IFN-γ secretion and weakens pro-inflammatory abilities of the activated CD8 memory T cells. Furthermore, CM2 administration resulted in significant suppression of HUVECs viability as well as a well-marked increase of caspase-3 level and NOx production, while this effect was reversed effectively by anti-ICOSL mAbs. This data confirms the important role of the ICOS/ICOSL pathways in HG or AGEs-mediated inflammatory responses and endothelial dysfunction. ICOS/ICOSL pathways have been found to be associated with the anti-tumoral mechanism of the activated CD8+ T-cells-as described in the report of Nelson et al[39]. To our knowledge, this work has been the first to explore the role of ICOS/ICOSL in the inflammatory response and endothelial dysfunction in T2DM.

Conclusions

We discovered a positive correlation between the dose of HG or AGEs and ICOS/ICOSL expression level T lymphocytes and HUVECs and provided evidence that HG or AGEs treatments have an ability to induce T cell inflammatory responses and endothelial dysfunctions. Cell-cell contact is necessary for HG and AGEs inducing an inflammatory response, whereas it is unnecessary, contrastingly, for inducing endothelial dysfunctions. Further experiments confirmed the involvement of ICOS/ICOSL interaction in the development of diabetes-associated inflammatory response and endothelial dysfunction. This work provides insight into the biological functions and molecular mechanisms of the ICOS/ICOSL signaling pathway in T2DM.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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