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Original Research

Cellular Advanced Glycation End Products Aggravate the Immune Response in Mononuclear Cells from Patients with Type 1 Diabetes

Liang Yang[1], Yuxuan Qian[2], Shixin Lei[1], Dongping Sun[3,*]

[1]School of Basic Medical Science, Gansu Medical College, 744000 Pingliang, Gansu, China
[2]Department of Biology, School of Arts & Sciences, University of Rochester, Rochester, NY 14627, USA
[3]Department of Clinical Medicine, The Affiliated Hospital of Gansu Medical College, 744000 Pingliang, Gansu, China
*Correspondence: GSsundongping@163.com (Dongping Sun)

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by immune response mediated islet beta cells destruction. However, the mechanisms that cause immune response in TIDM are still under investigation. Therefore, the goal of this study was to investigate the role of advanced glycation end products (AGEs) in the regulation of the immune response in peripheral blood mononuclear cells (PBMCs) from patients with T1DM. **Methods**: PBMCs isolated from T1DM patients and control subjects were used in the current study. Cytokines, AGEs related to glyoxalase 1 (GLO1), methylglyoxal (MG)-derived AGEs were assessed longitudinally. **Results**: The results of published T1DM PBMC microarray datasets using random-effects meta-analysis models revealed immune responses in the PBMCs of patients with T1DM compared with control subjects. Moreover, the activity of GLO1, which is the key MG-metabolizing enzyme, was significantly reduced in PBMCs from T1DM patients. We confirmed that, compared to the control subjects, GLO1 expression and activity were markedly decreased and MG-derived AGEs were significantly accumulated in the PBMCs from T1DM patients. In addition, phytohemagglutinin stimulated the secretion of tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) was positively correlated with the accumulation of cellular AGEs. Therefore, the exposure of PBMCs from control subjects to MG and a GLO1 inhibitor enhanced the accumulation of cellular MG-derived AGEs and the secretion of TNF-α and IFN-γ. **Conclusions**: The results of this study showed that the accumulation of cellular AGEs causes a decline in the immune response of patients with T1DM.

Keywords: type 1 diabetes; peripheral blood mononuclear cells; pro-inflammatory cytokines; advanced glycation end products

1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the destruction of pancreatic beta cells [1]. In the context of T1DM, peripheral blood mononuclear cells (PBMCs) play a role in the autoimmune destruction of pancreatic beta cells [2]. Insulitis, the infiltration of PBMCs in islets, is an indicator of beta cell destruction in T1DM [3]. The exact mechanism by which PBMCs contribute to the destruction of beta cells is not fully understood. However, it may involve pro-inflammatory cytokines such as interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), which can activate inflammatory pathways and trigger the production of islet reactive oxygen species and islet beta cell apoptosis [4,5]. In addition to directly damaging beta cells, pro-inflammatory cytokines also contribute to the development and progression of insulitis [6]. Pro-inflammatory cytokines are mainly secreted from inflammatory T helper cells and M1 macrophages [7,8]. Understanding the role of PBMCs in the disease process is a key area of investigation.

Methylglyoxal (MG), a highly reactive and toxic compound, is one of the precursors of advanced glycation end products (AGEs) [9]. Glyoxalase 1 (GLO1) is an enzyme that plays a critical role in the detoxification of MG [10] by preventing the formation of AGEs and reducing oxidative stress [11]. In patients with type 1 diabetes mellitus (T1DM), 67.5% showed preferentially high binding to MG–Lys–Cu2+ modified human DNA [12]. Peppa *et al*. [13] showed that MG-mediated protein structural alterations enhance immunogenicity in T1DM. In addition, inhibition of MG-mediated AGEs in non-obese diabetic mice was shown to significantly reduce insulitis and delay the onset of diabetes [13]. Those studies indicate that MG-mediated AGEs increase the progress of T1DM.

AGEs can activate the PBMCs of the immune system, promoting the immune response and the release of pro-inflammatory cytokines and autoantibodies [14]. Dietary AGEs increase circulating B cells and natural killer cells, as well as serum levels of C-reactive protein in healthy subjects [15]. AGEs functionally stimulate immune reactions in macrophages, mast cells, and B cells. The inflammatory effects of AGEs are through activation of the nuclear factor kappa B (NF-κB) pathway [16,17]. However, the role of cellular AGEs in triggering the immune response in T1DM is largely unknown. Therefore, understanding the complex

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interactions among the immune response, PBMCs, and glycation in T1DM is important for the development of novel treatments and approaches to managing the disease.

In this study, the results of microarray meta-analysis of circulating PBMCs showed that patients with T1DM exhibited an increase of immune response and reduction of GLO1 expression, compared with that of control subjects. Our experimental data further confirmed that diabetic patients had increased AGE levels in PBMCs, which was indicated by a lower expression of GLO1 and higher levels of intercellular MG-derived AGEs. Furthermore, pro-inflammatory cytokines, such as IFN-γ and TNF-α, showed a positive correlation with the levels of MG-H1 and GLO1. Finally, exposure of PBMCs from healthy subjects with MG and a GLO1 inhibitor also enhanced the secretion of pro-inflammatory cytokines. In conclusion, our results indicate that MG-derived AGEs may contribute to the induction of pro-inflammatory cytokine release from the PBMCs from patients with T1DM.

2. Materials and Methods

2.1 Materials

Ficoll was purchased from Shanghai Chemical Company (Shanghai, China). MG, phytohemagglutinin (PHA), and S-p-bromobenzylglutathione cyclopentyl diester (Br-BzGCp2) were from Sigma-Aldrich (St. Louis, MO, USA). TRIzol was purchased from Invitrogen (Carlsbad, CA, USA). MG-H1 antibody was obtained from Novus Biologicals (Littleton, CO, USA). The BeyoRT™ III First Strand cDNA Synthesis Kit; BeyoFast™ SYBR Green qPCR Mix; anti-mouse IgG horseradish peroxidase (HRP)-linked secondary antibody; and enzyme-linked immunosorbent assay (ELISA) kits for pro-inflammatory cytokines IL-1β, TNF-α, and IFN-γ were purchased from Beyotime Biotech Ltd. (Beijing, China). Fetal bovine serum (FBS) was obtained from Sijiqing Co. (Hubei, China). RPMI 1640 medium was purchased from Genom Biotech Pvt. Ltd. (Shanghai, China).

2.2 Subjects

The study was approved by the Affiliated Hospital of Gansu Medical College (Lanzhou, Gansu province, China) and conducted in accordance with the principles of the Declaration of Helsinki. Diabetic patients were recruited from the Affiliated Hospital of Gansu Medical College. Informed consent was obtained from the patients. A total of 12 males and 7 females between the ages of 9 and 45 years old with newly diagnosed T1DM ($<$1 year) according to the criteria of the American Diabetes Association, were recruited to this study. Patients were excluded from the study if they had an active infection, acute diabetic complications, or autoimmune disease. Control subjects were 35 volunteers without diabetes or other autoimmune diseases. No infectious diseases were detected during the 1-month period prior to the study.

2.3 Isolation of PBMCs

Fasting human venous blood was collected in sterile heparinized tubes between 08:00 and 10:30 am. PBMCs were isolated using Ficoll gradients within 4 h after the blood was drawn, and then washed with 1$\times$ phosphate-buffered saline (PBS).

2.4 Cell Viability Using Trypan Blue Assay

Isolated PBMCs from control subjects were added to RPMI 1640 containing 10% FBS. Cells (2×10^5/mL) were seeded in 96-well plates and treated with different concentrations of MG and a GLO1 inhibitor. After 24 h of incubation, cells were resuspended and trypan blue dye was added. Cell viability was calculated based on the ratio of stained cells and total cells.

2.5 GLO1 Activity

The measurement of GLO1 activity followed the protocol described by Qian *et al*. [10]. Cellular lysate from PBMCs was added to a hemithioacetal solution, which was prepared by pre-incubation with methylglyoxal and glutathione. The production of S-lactoylglutathione mediated by GLO1 was determined at an absorbance of 235 nm. GLO1 activity was calculated by the change in S-lactoylglutathione concentration.

2.6 Cytokine Expression after Treatment of PBMCs with PHA

Isolated PBMCs (2×10^5/mL) were cultured in RPMI 1640 medium supplemented with 10% FBS, and treated with 10 µg/mL PHA for 24 h [18]. The supernatant was collected for the measurement of pro-inflammatory cytokines.

2.7 Measurement of IL-1β, TNF-α, and IFN-γ by ELISA

The concentrations of IL-1β, TNF-α, and IFN-γ in the supernatant were measured by the protocol described by the commercial supplier. In brief, 25 µL supernatant and 75 µL dilution buffer were added to individual wells and incubated at room temperature for 2 h, washed with 1X Wash Buffer, and then incubated with capture antibodies and substrate. The results were obtained using absorbance at 450 nm by a microplate reader.

2.8 Determination of MG-H1 Level by Indirect ELISA

Indirect ELISAs with anti-MG-H1 antibody were carried out following the protocol described by Moraru *et al*. [19]. In brief, cell lysate (20 µL) in ammonium bicarbonate 100 mM pH 9.6 into a 96-well plate. The plate was incubated at room temperature for 2 h and the wells were washed with PBS. Then the wells were blocked in 5% (w/v) bovine serum albumin and incubated at room temperature for 2 h. The wells were incubated overnight with an anti-MG-H1 antibody, followed by washing and then incubation with anti-rabbit IgG, HRP-linked secondary antibody at room temperature for 1 h. The absorbance at 450 nm was measured by a microplate reader.

2.9 qPCR for mRNA

RNA from PBMCs was isolated by TRIzol according to the manufacturer's instructions. RNA concentration was measured following cDNA synthesis using the BeyoRT™ III First Strand cDNA Synthesis Kit. The PCR reactions contained 10 pM sense and 10 pM antisense oligonucleotides with BeyoFast™ SYBR Green qPCR Mix. The human GLO1 forward primer was 5′-AGC AGA CCA TGC TAC GAG TG-3′, and the reverse primer was 5′-GAG AGC GCC CAG GCT ATT T-3′; the human β actin forward primer was 5′-GGT GGC TTT TAG GAT GGC AAG-3′, and the reverse primer was 5′-ACT GGA ACG GTG AAG GTG ACA G-3′.

2.10 Collection of microarray data from the PBMCs of patients with T1DM

The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) was used to explore the expression profiles in microarray datasets. The keywords 'type 1 diabetes', 'autoimmune diabetes', 'mononuclear', and 'PBMC' were applied for this search. The inclusion criteria were: (1) gene expression profiling of circulating PBMCs; (2) patients with newly diagnosed T1DM and suitable control subjects; and (3) clear microarray platforms. In summary, the current study identified three GEO datasets (GSE55098, GSE29142, and GSE9006). The study included a total of 64 newly diagnosed patients with T1DM and 44 control subjects. The microarray platforms were Affymetrix Human Genome U133 Plus 2.0 Array, Phalanx Human OneArray, and Affymetrix Human Genome U133A Array.

2.11 Meta-Analysis of Microarray Data from the PBMCs of Patients with T1DM

All microarray datasets were downloaded from the NCBI GEO datasets using the R package GEOquery. Individual datasets were re-analyzed using the R package limma version 3.42.2 (Parkville, Victoria, Australia). The results of log fold change (logFC), p values, and the left and right limits of the confidence interval for logFC from individual datasets were exported for further meta-analysis. Meta-analysis was performed with the MetaVolcanoR package [20] by combining the P values using the random method. Differentially expressed genes (DEGs) from random analysis between patients with T1DM and control subjects were used for the functional analysis. For the enrichment analysis, the R package, clusterProfiler (Guangzhou, China) was used for the analysis of Gene Ontology (GO) biological process [21].

2.12 Statistical Analyses

Statistical analyses were performed with GraphPad Prism 8 (Dotmatics, Boston, MA, USA). Due to the limitation of patients, the significant analysis was conducted with the Mann–Whitney U Test. Correlation analysis was performed by simple linear regression analysis. $p < 0.05$ was considered statistically significant.

Table 1. General profile in recruited subjects.

	Control	T1DM
Sex (male/female)	35 (23/12)	19 (12/7)
Age (years)	24.45 ± 11.90	20.84 ± 10.53
Fasting glucose (mM/L)	4.87 ± 0.72	9.90 ± 1.75*
HbA1c (%)	5.07 ± 0.63	6.77 ± 0.78*
GAD+ (N)		14
IAA+ (N)		7

* $p < 0.05$, compared with control subjects. HbA1c, Hemoglobin A1c; GAD, glutamic acid decarboxylase; IAA, insulin autoantibodies.

3. Results

3.1 Microarray Data Collection and Meta-Analysis of PBMCs from Patients with T1DM

Three studies were recruited for this meta-analysis study. GSE55098 [22] included 12 patients with newly diagnosed T1DM (defined as treatment with insulin for <12 weeks) and 10 control subjects. GSE29142 [23] included 9 patients newly diagnosed T1DM (considered patients with positive glutamic acid decarboxylase 65-kilodalton isoform, islet antigen-2, and insulin autoantibodies) and 10 control subjects. GSE9006 [24] had 43 patients with newly diagnosed T1DM and 24 control subjects. Those studies were conducted in China, the Czech Republic, and the United States. As shown in Fig. 1A, the upregulated DEGs in PBMCs from patients with T1DM compared with control were 715 (GSE55098), 969 (GSE29142), and 642 (GSE9006); and the down-regulated DGEs were 783 (GSE55098), 1008 (GSE29142), and 701 (GSE9006).

Then we performed random analysis based on the results of limma analysis from individual studies. Fig. 1B shows the top 1% of both up- and downregulated DEGs of PBMCs from patients with T1DM. To identify DEGs related to the functional biological group, using GO biological process analysis, the results from upregulated DEGs in T1DM further confirmed that the development of T1DM was related to the immune response (Fig. 1C). The top pathways of downregulated DEGs in T1DM showed the inhibition of mitochondrial electron transport chain activity (Fig. 1D).

3.2 Augmentation of Cellular Glycation in the PBMCs of Patients with T1DM

As shown in Table 1, patients with T1DM had significantly higher levels of fasting blood glucose and hemoglobin A1C compared with control subjects. The correlation between fasting blood glucose and glycated hemoglobin was positive (r = 0.7081, $p < 0.05$). The results of Fig. 2A also demonstrated that patients with T1DM

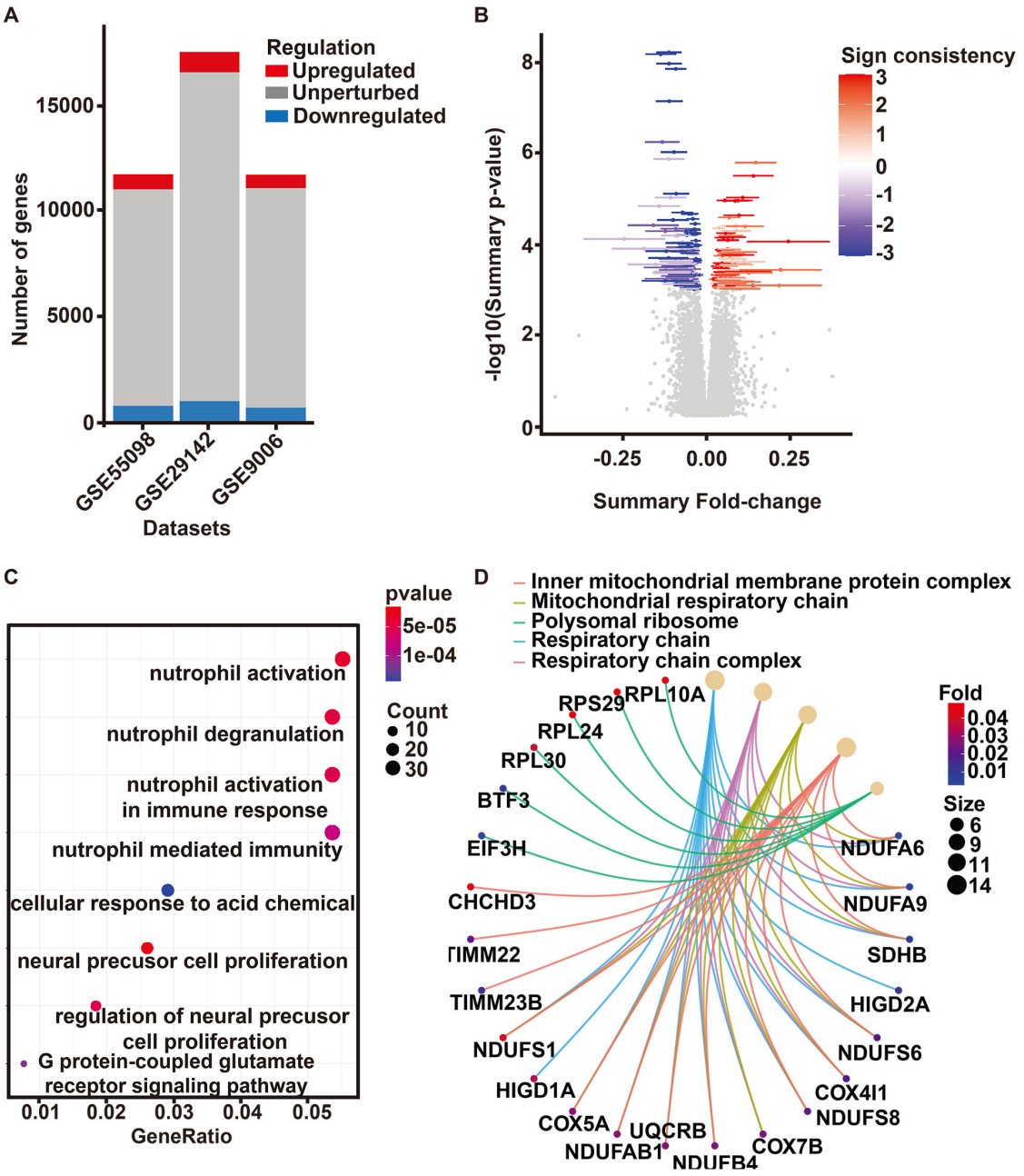

Fig. 1. Activation of the immune response in peripheral blood mononuclear cells (PBMCs) from patients with type 1 diabetes mellitus (T1DM). (A) Differentially expressed genes (DEGs) of published microarray datasets. Red: upregulated DEGs; blue: downregulated DEGs; grey: no significant difference genes. (B) Forest plot of top 1% DEGs in PBMCs between patients with T1DM and control subjects using meta-analysis random effect analysis. (C) Gene Ontology (GO) term biological process analysis of upregulated DEGs of PBMCs from T1DM patients compared with control subjects. (D) GO term biological process analysis of downregulated DEGs of PBMCs from T1DM patients compared with control subjects.

had significantly increased MG-H1 (MG-derived AGEs) in PBMC lysate, compared to control subjects. Both results indicated that there was significant accumulation of systemic AGEs, including circulating PBMCs in patients with T1DM. The increase in AGE accumulation is also related to the detoxification of MG by the ubiquitous cellular enzyme, GLO1. Fig. 2B shows that the expression of GLO1 was markedly lower ($p = 0.001096$) in patients with T1DM based on the results of random analysis. In the current study, we also confirmed the previous microarray data. Fig. 2C,D show that GLO1 expression and activity were significantly reduced in the PBMCs of patients with T1DM compared to those of control subjects.

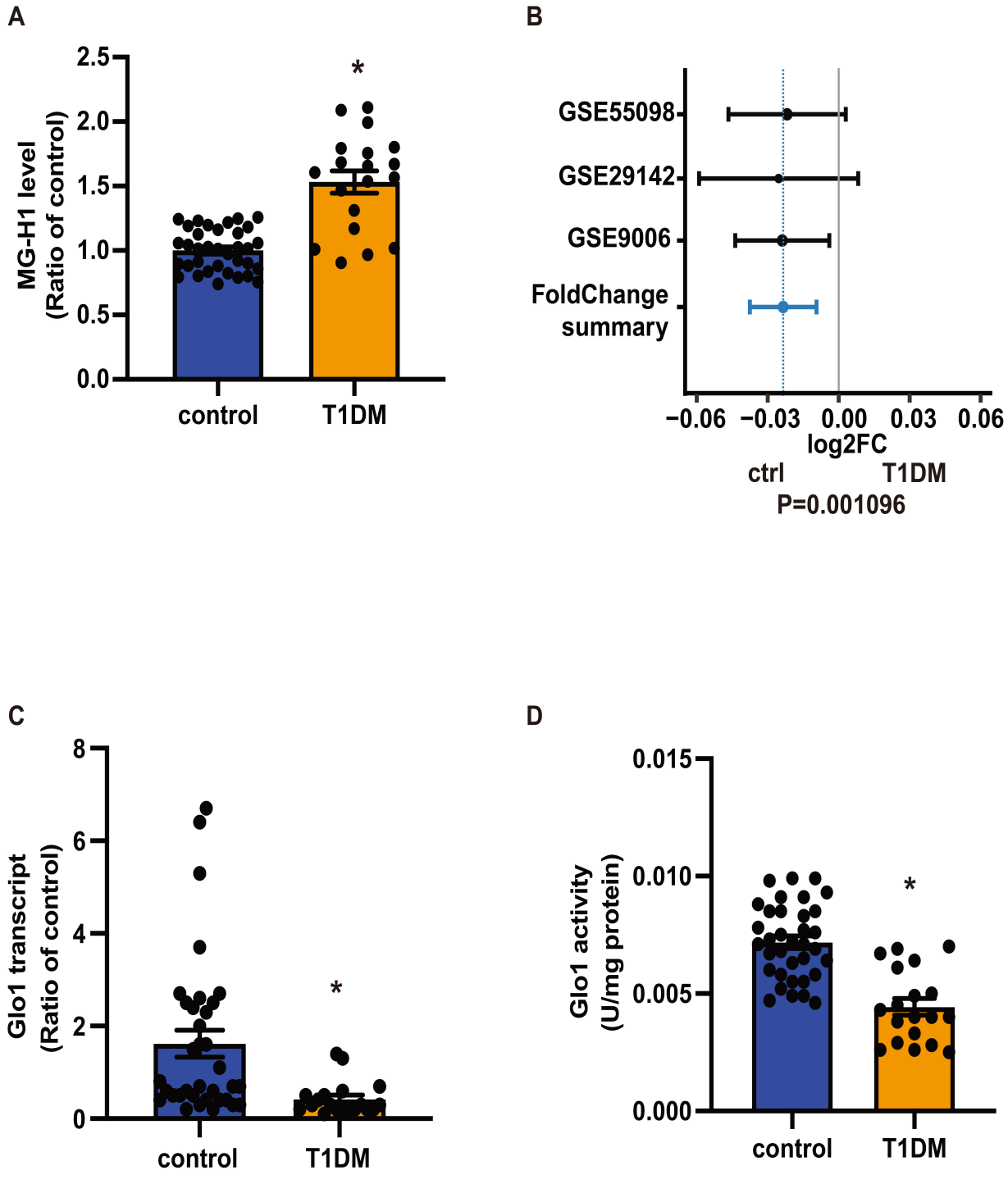

Fig. 2. Accumulation of cellular Advanced glycation end products (AGEs) in peripheral blood mononuclear cells (PBMCs) from patients with type 1 diabetes mellitus (T1DM). (A) Cellular levels of methylglyoxal (MG)-derived AGEs were measured by indirect enzyme-linked immunosorbent assay (ELISA), as described in *Materials and Methods*. * indicates the comparison with control subjects, as determined by Mann–Whitney U Test. $p < 0.05$. (B) The expression of Glyoxalase I (GLO1) in PBMCs from published Gene Expression Omnibus (GEO) datasets were analyzed based on random effect analysis. (C) GLO1 transcript in PBMCs was determined by qPCR. * indicates the comparison with control subjects, as determined by Mann–Whitney U Test. (D) GLO1 activity was measured in PBMCs from patient with T1DM and control subjects. * indicates the comparison with control subjects, as determined by Mann–Whitney U Test.

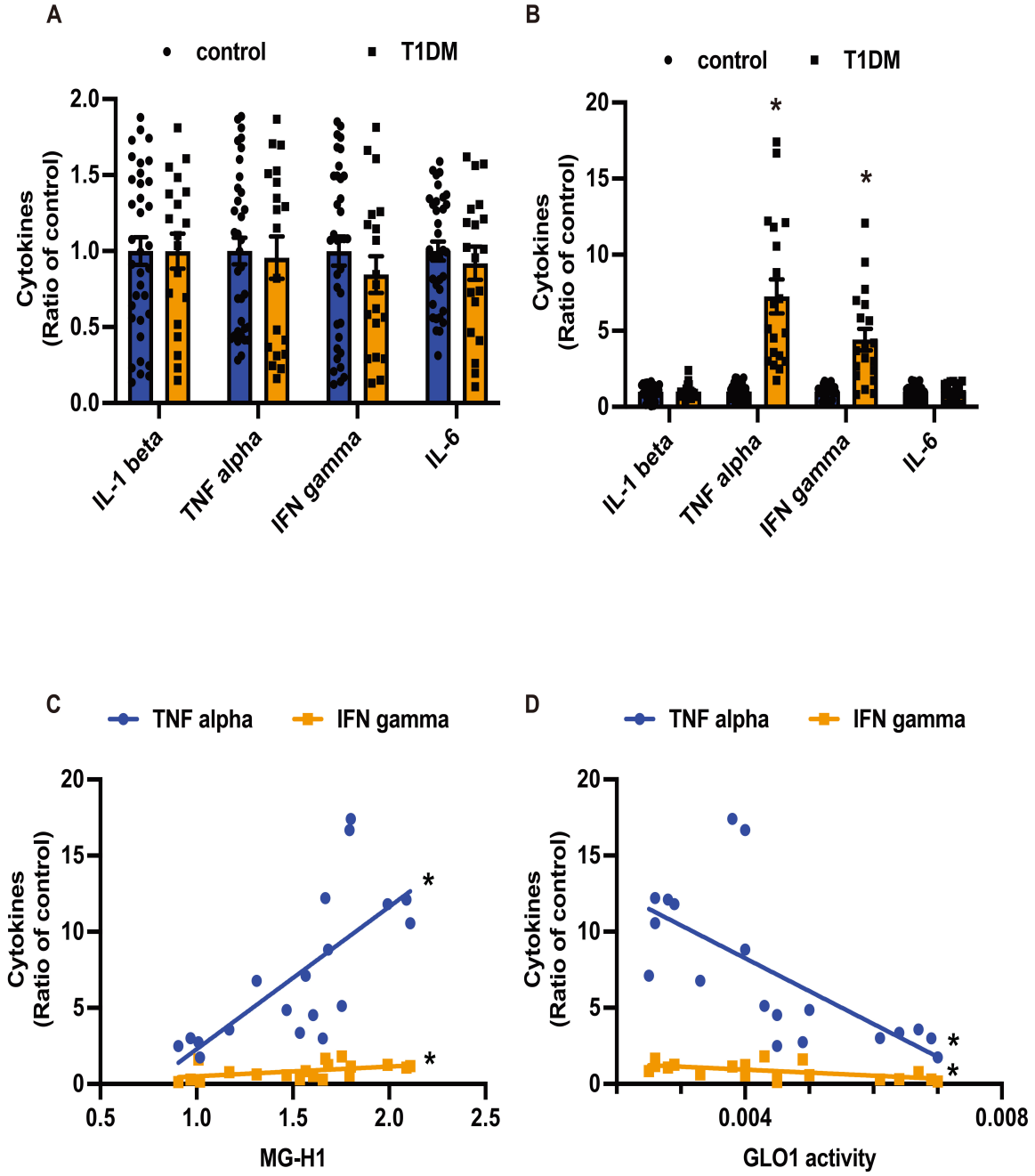

Fig. 3. The activation of immune response in PBMCs from patients with T1DM. (A) Serum cytokine levels from patients with T1DM and control subjects were determined by commercial ELISA kits. (B) Supernatant cytokine levels were measured in phytohemagglutinin (PHA)-treated PBMCs from patients with T1DM and control subjects by commercial ELISA kits. * indicates the comparison with control subjects, as determined by Mann–Whitney U Test. The correlation of secreted cytokines induced by PHA in PBMCs from T1DM and cellular MG-derived AGEs (C), GLO1 activity (D) were analyzed. * indicates the comparison with control subjects, as determined by simple linear regression analysis.

3.3 Increase of Pro-Inflammatory Cytokines Release in PBMCs from T1DM Patients was Positively Correlated with AGEs

T1DM is considered an autoimmune disease. Therefore, the pro-inflammatory cytokines were determined in the serum of patients with T1DM. As shown in Fig. 3A, there was no significant difference in circulating levels of IL-1β, TNF-α, IFN-γ, and IL-6 between patients with T1DM and control subjects. To further explore immune dysfunction in T1DM, PHA, a nonspecific T cell activator, was used to enhance the immune reaction. Fig. 3B shows that IFN-γ and TNF-α levels were markedly higher

in PHA-stimulated PBMCs from T1DM compared with those from control subjects. However, there was no significant difference in the levels of IL-1β and IL-6 in PHA-stimulated PBMCs from patients with T1DM and control subjects.

To explore whether cellular AGEs aggravate the immune response in T1DM, correlation analysis was performed between pro-inflammatory cytokines such as IFN-γ and TNF-α and MG-derived AGEs in patients with T1DM. Fig. 3C shows that PHA-induced TNF-α and IFN-γ secretion were positively correlated with an increase of MG-H1 level in PBMCs from T1DM. However, cytokine secretion showed a negative correlation with GLO1 activity (Fig. 3D). In summary, our results indicated that the accumulation of AGEs in T1DM also contributes to exacerbating immune dysfunction in immune cells.

3.4 MG-Derived AGEs Enhance Pro-Inflammatory Cytokine Secretion in PBMCs

To further evaluate the role of MG-derived AGEs on pro-inflammatory secretion, MG and a GLO1 inhibitor, BrBzGCp2, was applied in the current study. Fig. 4A shows that treatment of PBMCs with 20 µM MG significantly reduced cell viability compared with vehicle. To further explore the effect of MG on the accumulation of cellular AGEs, the results showed that 10 µM MG markedly enhanced the accumulation of MG-derived AGEs (Fig. 4B). The effect of BrBzGCp2 on GLO1 activity was also determined in PBMCs from control subjects. The results of Fig. 4C showed that 1.5 µM BrBzGCp2 led to a significant reduction of PBMC viability compared with that of vehicle. However, we did not observe a significant difference in cell viability between 0.5 and 1 µM BrBzGCp2. To determine whether BrBzGCp2 induces cellular AGE accumulation, PBMCs were treated with the vehicle, 0.5 and 1 µM BrBzGCp2 for 24 h. The 0.5 µM BrBzGCp2 effectively increased cellular MG-derived AGE accumulation (Fig. 4D). Therefore, in the current study, 10 µM MG and 0.5 µM BrBzGCp2 were applied to PBMCs.

Finally, we examined the effects of MG and BrBzGCp2 on the regulation of the immune response in PBMCs from control subjects. Fig. 4E shows that treatment of PBMCs with 10 µM MG or 0.5 µM BrBzGCp2 led to an increase in TNF-α and IFN-γ secretion compared to vehicle. In conclusion, the results showed that the accumulation of AGEs in PBMCs contributed to an immune response in PBMCs.

4. Discussion

It is well-known that T1DM is an autoimmune disease [25,26]; however, the immune response in T1DM is still under investigation. Therefore, in this study, we explored the role of AGEs in the regulation of the immune response in the PBMCs from patients with T1DM. Our results showed the following. (1) Using GO biological process analysis based on the meta-analysis of published GEO datasets, upregulated DEGs in PBMCs from patients with T1DM showed an increased inflammatory response. The downregulated DEGs indicated the inhibition of mitochondrial electron chain transport in patients with T1DM. (2) In agreement with the results of meta-analysis, our results further confirmed that MG-derived AGE level significantly increased and the expression of GLO1, which is the key enzyme that detoxicates MG into S-2-hydroxyacylglutathione [10], was markedly reduced in PBMCs from T1DM. Moreover, the enhancement of AGEs was positively correlated to inflammatory cytokines secretion. (3) Exposure to MG and a GLO1 inhibitor in PBMCs from control subjects increased inflammatory cytokine secretion. In summary, our study indicated that in the PBMCs of patients with T1DM, the accumulation of cellular AGEs enhances the immune response. This may decrease the destruction of islet beta cells.

In our study, we first performed meta-analysis based NCBI GEO datasets using random effect analysis. The results from upregulated DEGs showed that the activation of neutrophils (Fig. 1C), one of the main cell types involved in T1DM [27]. The downregulated DEGs also demonstrated the inhibition of the mitochondrial electron chain transport (Fig. 1D). The mitochondrial electron chain transport, including complex I, III and IV, is the major focus of immunometabolism [28]. Exposure of rats to rotenone, a mitochondrial complex I inhibitor, causes systemic oxidative stress and the release of pro-inflammatory cytokines [29]. The mitochondrial complex III inhibitor, antimycin A, also contributes to the pro-inflammatory response [30]. In summary, the results from our meta-analyses further supported the hypothesis that T1DM is an immune response disease.

Cellular AGEs and the immune response are strongly interconnected [31,32]. AGEs can function as inflammatory stimulators that trigger inflammatory signaling [33], including the secretion of pro-inflammatory cytokines [34]. A study revealed that the AGE receptor binds to high mobility group box 1 (HMGB1) and macrophage antigen-1 (Mac-1). The activation of HMGB1 and Mac-1 propagates inflammation [35,36]. Overexpression of GLO1 improves the pro-inflammatory response in experimental diabetic nephropathy [37]. Results of Fig. 2A confirmed the accumulation of MG-derived AGEs in PBMCs from patients with T1DM. Fig. 2C,D shows that GLO1 expression and activity in PBMCs from patients with T1DM were significantly reduced, which were in agreement with single gene expression from random analysis (Fig. 2B). Furthermore, PHA-stimulated pro-inflammatory cytokine release was positively correlated with the levels of MG-H1, and negatively correlated with GLO1 activity in PBMCs from T1DM (Fig. 3B–D).

The effect of the accumulation of cellular AGEs on the immune response is also detected in different diseases. Exposure of neutrophils to MG or inhibition of GLO1 activity

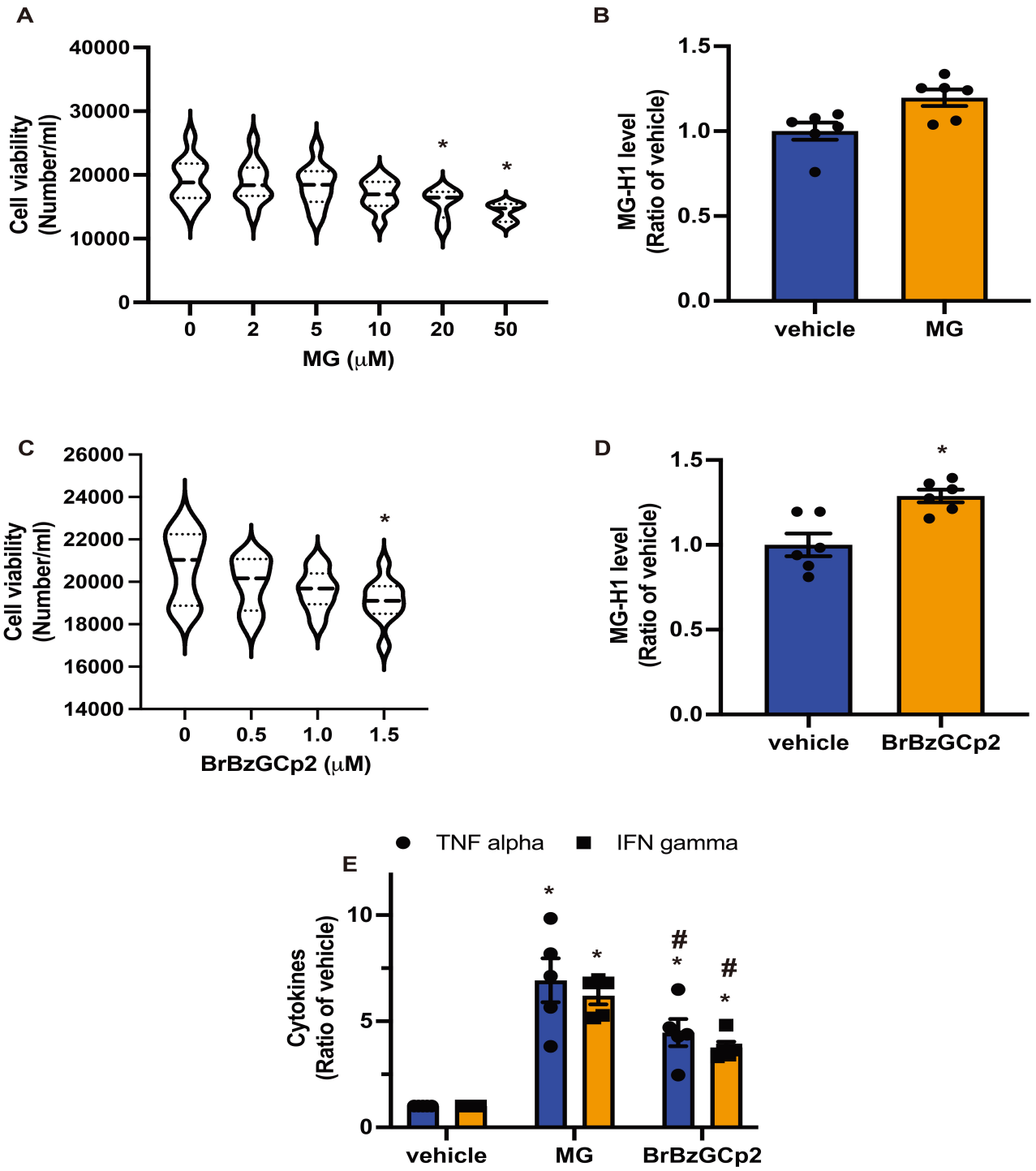

Fig. 4. Accumulation of cellular AGEs by MG and GLO1 inhibitor enhanced immune response in mononuclear cells. (A) Cell viability of MG-treated mononuclear cells from control subjects (n = 10) was determined. * indicates the comparison with control subjects, as determined by the Mann–Whitney U Test. (B) Cellular MG-derived AGEs in MG-treated PBMCs were determined by indirect ELISA. * indicates the comparison with control subjects, as determined by Mann–Whitney U Test. (C) Cell viability of GLO1 inhibitor – S-4-Bromobenzylglutathione cyclopentyl diester (BrBzGCp2) treated mononuclear cells from control subjects (n = 10) was determined. * indicates the comparison with control subjects, as determined by the Mann–Whitney U Test. (D) Cellular MG-derived AGEs in BrBzGCp2-treated PBMCs were determined by indirect ELISA. * indicates the comparison with control subjects, as determined by Mann–Whitney U Test. (E) TNF-α and IFN-γ levels in supernatant from MG or BrBzGCp2 PBMCs were determined by commercial ELISA kits. * indicates the comparison with the vehicle, # indicates the comparison with MG treatment, as determined Mann–Whitney U Test.

using RNA interference in healthy keratinocytes or using GLO1 inhibitor, BrBzGCp2 in rat bone marrow mesenchymal stem cells also demonstrated that impaired GLO1 activity enhanced the expression of TNF-α, IL-1β, IL-6 [38–40]. Therefore, in this study, to further explore whether cellular AGEs directly enhanced immune response in PBMCs, MG and BrBzGCp2 were applied to isolated PBMCs from control subjects. According to the previous studies [10,41,42], 20 µM MG and 2 µM BrBzGCp2 started to show a significant reduction in cell viability, compared to the vehicle. Therefore, 10 µM MG and 0.5 µM BrBzGCp2 were used in the current study. Fig. 4C,D shows that exposure of isolated PBMCs to 10 µM MG or 0.5 µM BrBzGCp2 resulted in a significant increase in cellular MG-derived AGE accumulation. Furthermore, treatment with MG and BrBzGCp2 led to an increase in pro-inflammatory cytokine TNF-α and IFN-γ secretion (Fig. 4E). Therefore, our results indicated that cellular AGE accumulation aggravated the autoimmune response in T1DM. The mechanism causing immune response by AGEs may be due to the activation of inflammatory-related pathways. An increase in cellular AGEs accumulation activates the NF-κB pathway, and the activation of NF-κB is one of the key pathways for stimulating pro-inflammatory cytokines including TNF-α, IFN-γ, and IL-1β [43]. The analysis of brains from Alzheimer's disease showed the co-localization of AGEs and inducible nitrogen synthase (iNOS), one of the key markers of inflammation [44]. The induction of iNOS by AGEs is due to activation of the p38 mitogenactivated protein kinase-dependent pathway [45]. In summary, our results showed that the accumulation of cellular AGEs weakens the immune response, which may contribute to the destruction of islet beta cells or diabetic chronic complications.

5. Conclusions

In summary, our results showed that the accumulation of AGEs by hyperglycemia in diabetic patients was positively correlated with the immune response in PBMCs. The exposure of MG or inhibition of GLO1 also stimulated the secretion of pro-inflammatory cytokines. After the onset of T1DM, the accumulation of AGEs caused by hyperglycemia and inhibition of GLO1 increased the secretion of pro-inflammatory cytokines. The joint effects of glycation and the immune response in T1DM may accelerate the progression of T1DM and related complications. Therefore, the treatment of T1DM may include alleviating hypoglycemia and improving the immune response. However, there are several limitations in the current study (1) the limited numbers of T1DM patients: we only recruited 19 T1DM patients. (2) The age difference between T1DM. In current study, the age of patients was from 9 to 45 years old. Therefore, the increase of recruited T1DM patients will contribute to understand the correlation between AGEs and immune response in T1DM.

Availability of Data and Materials

Data of meta-analysis from T1DM patients (GSE55098, GSE29142, and GSE9006) are from GEO datasets, National Center for Biotechnology Information. Clinical data were not available due to patients' information.

Author Contributions

LY performed the research. YQ and LY performed meta-analysis and analyzed the data. SL provided scientific advice and patients collection. DS designed the study, collected patients for this study, and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The ethical committee of Affiliated Hospital of Gansu Medical College has approved this study [2023]IEC (027). And all patients have been formed consent.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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