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Effect of High Glucose on Cytokine production by Human Peripheral Blood Immune Cells and Type I Interferon Signaling in Monocytes: Implications for the Role of Hyperglycemia in the Diabetes Inflammatory Process and Host Defense against Infection

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Abstract

The major metabolic feature of diabetes is hyperglycemia which has been linked to the diabetes inflammatory processes, and diabetes-related vulnerability to infection. In the present study, we assessed how glucose affected PBMCs in type I interferon (IFN) production and subsequent signaling. We found that the moderately elevated glucose promoted, and high glucose suppressed type I IFN production, respectively. Pre-exposure to high glucose rendered monocytes more sensitive to IFN- α stimulation with heightened signaling, whereas, instantaneous addition of high glucose did not exhibit such effect. Consistent with this finding, the mRNA levels of IFN- α -induced IRF-7 in PBMCs were positively correlated with HbA1c levels of diabetes patients. Additionally, we found that high glucose promoted the production of other proinflammatory cytokines/chemokines. This study suggests that hyperglycemia may affect the inflammatory process in diabetes via promoting proinflammatory cytokines, as well as the host defense against microbial infections through impeding type I IFN production and signaling.

Keywords

hyperglycemia; glucose; cytokine; interferon; inflammation; diabetes

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Conflict of interest

The authors declare there is no conflict of interest.

Introduction

Although type I diabetes (T1D) and type II diabetes (T2D) occur by different pathophysiologic mechanisms, they share a common major metabolic feature, hyperglycemia. One of the treatment goals for diabetes is to normalize blood glucose to avoid or delay diabetes-related complications [1–4]. Both T1D and T2D are thought to develop via distinct and separate inflammatory responses that may share some common features, such as proinflammatory cells and cytokines [5–7]. It has been established that hyperglycemia plays a major part in this inflammatory process [8–14], which might adversely affect diabetes disease progression and complications. It has been documented that diabetes patients are vulnerable to various infections and the control of hyperglycemia is critical for managing infections in diabetes patients [15–21]. Therefore, it is conceivable that hyperglycemia often occurring in diabetes patients may interfere with the body's defensive mechanism against pathogens. However, how hyperglycemia affects these inflammatory processes once diabetes develops or how this may influence diabetes-related vulnerability to infection is not well understood.

In the present study, we utilize human peripheral blood mononuclear cells (PBMCs) and a human monocyte cell line, THP-1 to study how glucose affects type I IFN or other inflammatory cytokine/chemokine production stimulated by TLR3 agonist, as well as how glucose affects type I IFN signaling. We found that glucose levels significantly affected the production of type I IFN and other cytokines/chemokines. Furthermore, glucose levels also significantly influence type I IFN signaling. This study may provide important information for understanding diabetes-related complications and vulnerability to infections.

Materials and Methods

1. Experimental cells

Human blood buffy coat samples and blood samples were obtained from the Life South Blood Center at Gainesville, Florida, or UF Health clinic under the protocol approved by the Institutional Review Board. All patients' blood samples were drawn after the informed consent forms were signed by the patients. The THP-1 cell line was originally obtained from ATCC and kindly provided by Dr. Mark Wallet's laboratory at the University of Florida, and maintained in the medium recommended by ATCC.

2. PBMC processing

Buffy coat blood samples were diluted with phosphate-buffered solution (PBS) at approximately 1:1 ratio. Then, the diluted blood was subjected to Ficoll-hypaque gradient centrifugation at 1500 rpm for 20 min. The PBMCs were collected from the interface between Ficoll and plasma, washed once with PBS, then resuspended into complete DMEM (glucose 5.5 mM) medium containing 10% fetal bovine serum (FBS).

3. Preparation of supernatants for cytokine assay

PBMCs (1×10^6) were stimulated with poly I:C (Invitrogen) (20 μ g/ml) in 1 ml DMEM containing 10% FBS in the presence of different concentrations of glucose (5.5 mM, 8 mM,

16 mM, 24 mM) for 24 h. Thereafter, the cells were harvested and centrifuged at 1500 rpm for 10 min. The supernatants were collected and frozen at -80°C for later cytokine analysis. In the other experimental setting, PBMCs (1×10^6) were pre-incubated with different concentrations of glucose as described above for 48 h. Then, poly I:C (20 $\mu\text{g/ml}$) was added to the cultures for additional 24 h. The supernatants were saved for cytokine assay.

4. Type I IFN bioactivity assay

The human type I IFN sensor cell line, HEK-BlueTM cell was purchased from Invivogen and maintained in the medium recommended by the company. HEK-BlueTM cells (50,000/well) in 180 μl DMEM complete media were placed in a flat bottom 96-well plate. 20 μl of each sample was added to each well. The cells were allowed to be incubated in a CO₂ cell culture incubator for 20 h. Thereafter, 20 μl of the culture supernatant of each incubation was collected and along with 180 μl of Quanti-Blue reagent (Invivogen) was added to a new flat bottom 96-well plate. The plate was incubated at room temperature until the appropriate color change was reached (2-6 h), and then read on a Biotek Plate Reader at wavelength of 630 nm.

5. Luminex assay for cytokines in the culture supernatants

The supernatants prepared above were measured using the 22 human cytokine multiplex kit (Millipore) and analyzed by Luminex 100 (Luminex) according to the instruction from the manufacturer (Millipore).

6. Flow cytometric assay for expression of Siglec I (CD169) on monocytes

PBMCs were stimulated with polyI:C (20 $\mu\text{g/ml}$) or IFN- α (PBL Biotech) (1000 U/ml) with different concentrations of glucose (5.5 mM, 8 mM, 16 mM, 24 mM) for 24 h. Thereafter, the cells were harvested and stained with fluorescence-conjugated CD14 and CD169 antibodies (BioLegend). Flow cytometric analyses were performed using BD LSRFortessa. The data were analyzed using the Flowjo software (version 10.0.8). The mean fluorescence intensity (MFI) of CD169 on monocytes was calculated after gating on CD14+ cells.

7. qRT-PCR

RNA samples were prepared using PBMCs treated with different conditions according to the RNA extraction protocol from the manufacturer (Qiagen). The quality and quantity of the RNA samples were checked before qRT-PCR. The qRT-PCR for IRF-7 and β -actin gene expression was performed using qRT-PCR kits from Qiagen following the instruction from the manufacturer. The quantification of IRF-7 gene expression was determined relative to β -actin gene expression for a given sample. The ratio of IRF-7 expression level between IFN- α -treated and medium only conditions was calculated and expressed as the relative level of IRF-7 gene expression induced by IFN- α .

8. Western blotting for assessment of phosph-STAT1 in monocytes induced by IFN- α

Monocyte cell line THP-1 cells (1×10^6) were incubated with or without IFN- α (1000 U/ml) in DMEM-complete medium for 1 h in the presence of different concentrations of glucose (time-course study showed that phosphorylation of STAT1 reached the maximal level after 1

h incubation with IFN- α (Supplemental Fig 1)). The second set of experiments were performed the same way but using THP-1 cells pre-incubated with different concentrations of glucose for 48 h. The cells in the above cultures were centrifuged and the cell lysates were prepared by adding 300 μ l Laemmli sample buffer mixed with 2-mercaptoethanol (2-ME 1:20 v/v) and appropriate amount of loading dye (Bio-Rad). The cell lysates were mixed with phosphatase inhibitor and protease inhibitor cocktail following the instruction from the manufacturer (Thermo Scientific). The cell lysates were denatured by heating at 99 °C for 3 min. Then the lysates were loaded onto 4-20% gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) Criterion TMTGXTM Precast Gels (Bio-Rad Laboratories, Inc. USA), and run at 30 V for 10 min, then 110 V for 1.5 h. The proteins in the gel were transferred to a PVDF membrane (Invitrogen) using a 7-min iBlot Transfer system (Thermo Fisher Scientific). The membrane was blocked in 5% non-fat milk overnight, then incubated with the primary antibody, rabbit-anti-human phosph-STAT1 (Phosphor-Tyro 701) or STAT1 (Cellsignaling) (1:1000) for 1 h at room temperature, or overnight at 4°C. After thorough wash, the membrane was incubated with secondary anti-rabbit Ig antibody-HRP (1:2000) for 1 h. The membrane was washed and the protein bands were detected by adding enhanced chemiluminescence (ECL) solution (Thermo Fisher Scientific). Images were captured with an Alpha Innotech FluorChem HD2 imaging system. The β -actin (Cellsignaling) blots were performed to serve as internal controls following the same procedure.

9. Statistical analysis

Graphpad Prism software (version 7) was used for graph preparation and data analysis. Student's paired *t* test, unpaired *t* test or correlation test was chosen depending on nature of the data set. *P*<0.05 was considered statistically significant.

Results

1. Effect of Glucose on type I IFN production by PBMCs stimulated with TLR3 agonist, polyI:C, and the subsequent type I IFN responding marker, CD169 expression on monocytes

TLR3 agonist, polyI:C is a double-stranded RNA, and a potent type I IFN stimulator [22, 23]. To determine how glucose affects type I IFN production by peripheral blood immune cells, PBMCs from healthy blood donors were stimulated with polyI:C in the presence of different concentrations of glucose. A glucose concentration of 5.5 mM is equivalent to normal human fasting blood glucose. We found that different concentrations of high glucose differentially influenced type I IFN production by PBMCs in response to poly I:C stimulation. Moderate increase over 5.5 mM, such as 8 mM promoted type I IFN production, whereas very high level of glucose, such as 24 mM significantly suppressed type I IFN production (Fig 1A). These changes of type I IFN production were well correlated with the changes of CD169 expression on monocytes (Fig 1B and C). CD169 is a sensitive type I IFN responding marker on human monocytes [24].

2. The influence of pre-incubation with different concentrations of glucose on type I IFN production stimulated by polyI:C as well as the subsequent type I IFN signaling

Hyperglycemia frequently occurs in patients with diabetes that is not well managed. Thus, it is of interest to study how those hyperglycemia pre-experienced immune cells respond to inflammatory stimuli. To this end, we attempted to mimic this clinical scenario by incubating PBMCs with different concentrations of glucose for 48h, and then the cells were stimulated with TLR3 agonists, polyI:C for an additional 24h. The results showed that the pre-incubation with glucose appeared to have an impact on type I IFN production similar to concurrent incubation with glucose. Unlike the findings shown in Figure 1, the subsequent type I IFN signaling, CD169 expression on monocytes was slightly declined at the moderately elevated level (8 mM) but insignificant (Fig 2A and B) in all tested subjects.

3. Type I IFN signaling induced by IFN- α in monocytes cultured with different concentrations of glucose

In the above experiments, we demonstrated type I IFN bioactivities produced by PBMCs responding to poly I:C stimulation at different glucose concentrations. Given that there are many subtypes of type I IFN, the type I IFN bioactivity assay employed in the present study measured all type I IFNs induced by poly I:C shown in Figure 1 and 2. These subtypes of type I IFNs may affect type I IFN signaling differently, and the factors other than type I IFN induced by poly I:C might also influence type I IFN signaling. In the following experiments, to more specifically investigate how glucose affects type I IFN signaling, we employed one subtype of type I IFN, IFN- α , to incubate with PBMCs in the presence of different concentrations of glucose. We found that glucose did not show significant impact on type I IFN signaling in response to IFN- α stimulation in terms of CD169 expression on monocytes (Fig 3A and B). Additionally, we found that glucose itself slightly suppressed CD169 expression with increasing concentrations (supplemental Fig 2). Furthermore, we employed western blot experiments using the THP-1 monocyte cell line to assess how glucose affected phospho-STAT1 levels induced by IFN- α . Consistent with the above findings, we found that instantly adding glucose at different concentrations had little impact on phosphorylation of STAT1 in THP-1 cells (Fig 4C).

4. Pre-incubation with high concentration of glucose significantly enhances type I IFN signaling in monocytes induced by IFN- α

Despite the results shown in Figure 3 that glucose appears to not significantly interfere with type I IFN signaling, it is of interest to assess how glucose pre-exposure affects immune cells to respond to type I IFN stimulation, which would more closely echo the situation of diabetes patients. To this end, we incubated PBMCs with different concentrations of glucose for 48 h, then the cells were stimulated with IFN- α as indicated. Of interest, PBMCs pre-incubated with glucose responded to IFN- α differently from PBMCs without pre-incubation with glucose shown in Figure 3, demonstrating that increased concentrations of glucose significantly promoted IFN- α -induced CD169 expression on monocytes (Fig 4A and B). These findings were further confirmed in our western blot experiments using high glucose pre-exposed THP-1 cells stimulated by IFN- α (Fig 4C).

5. HbA1c levels are positively correlated with expression levels of IRF7 induced by IFN- α

The level of HbA1c usually indicates how well the hyperglycemia in diabetes was controlled during the last 3 months. Uncontrolled hyperglycemia results in elevated levels of HbA1c. Thus, the elevated levels of HbA1c would suggest that the blood immune cells have lived in a hyperglycemic environment for a relatively long period of time. Given that IRF7 has been shown to be a reliable important marker for type I IFN response[25–29], we chose to use IRF7 expression level to demonstrate type I IFN response. To study the relationship between hyperglycemia and type I IFN signaling, we enrolled 11 T1D patients with known HbA1c levels (Table 1). We stimulated PBMCs with IFN- α for 18 h, and the levels of interferon-responding factor 7 (IRF-7) was measured by qRT-PCR (Table 1). Then, the correlation between HbA1c and IRF7 mRNA levels was analyzed. As shown in Figure 5, a significant positive correlation between the levels of HbA1c and IRF7 was observed, which is in agreement with the findings shown in Figure 4.

6. Influence of glucose on the production of cytokines/chemokines by PBMCs stimulated with polyI:C

Hyperglycemia in diabetes patients may influence the inflammatory process through affecting the production of pro-inflammatory cytokines/chemokines. To determine whether glucose levels affect the production of pro-inflammatory cytokines/chemokines other than type I IFN demonstrated above, we treated PBMCs with polyI:C in the presence of different concentrations of glucose for 24 h. Another group was pre-treated with different concentrations of glucose for 48 h, then stimulated with polyI:C for an additional 24 h. Thereafter, the supernatants from all cultures were measured for cytokines/chemokines using the 22 human cytokine multiplex Luminex kit. In both experimental settings, we found that glucose did not exhibit a significant impact on most cytokines/chemokines measured until it reached a very high level, 24 mM (Fig 6A and B). When PBMCs were stimulated with polyI:C in the presence of 24 mM glucose, they produced significantly higher levels of multiple cytokines/chemokines except for IL-8 and MCP-1 with certain discrepancies between both experimental settings (Fig 6A and B). Consistent with the type I IFN bioactivity assay results shown in Figure 1 and 2, the production of interferon-induced protein-10 (IP-10) was significantly reduced in the cultures with 24 mM glucose in both experimental settings (Fig 6A and B). The rest of the cytokines/chemokines in the 22 multiplex kit either were not detectable or there was no difference among different glucose conditions (data not shown).

Discussion

In the present study, we show that glucose levels differentially affect human peripheral blood PBMCs to produce type I IFN depending on the concentrations. Pre-incubation with high concentration of glucose appears to reprogram PBMCs to be less capable of producing type I IFN upon TLR agonist stimulation. Given that type I IFN and its induced signals are the major innate immunity against pathogens [30, 31], this study may shed light on and provide a deeper understanding about the host defense against microbial infections in diabetes patients, particularly the patients with uncontrolled hyperglycemia.

Hyperglycemia often occurs in diabetes patients if the blood glucose is not well managed, and many diabetes-related complications are caused by hyperglycemia directly or indirectly, such as the complications in heart, kidney, blood vessels, and nerves [32–34]. In addition, diabetes patients also are vulnerable to various infections if hyperglycemia is not well controlled [17, 35–37]. Type I IFN signaling plays an important role in many inflammatory processes, such as lupus or T1D autoimmune process [6, 38], as well as in host immune defense against microbial infections [30]. Thus, hyperglycemia occurring in T1D may aberrantly affect the autoimmune process and diabetes-related inflammation, as well as host anti-microbial defense through impeding type I IFN signaling. However, how glucose affects type I IFN production and type I IFN signaling is not well investigated. In the current study, we investigated different concentrations of glucose on the production of type I IFN in response to stimulation with TLR3 agonist, poly I:C. PolyI:C is a double-stranded RNA and potently stimulates various types of immune cells to produce large quantities of type I IFN [22, 23]. In the present study, it is of interest to learn that glucose at moderately elevated levels promotes type I IFN production whereas highly elevated glucose suppresses type I IFN production. Type I IFN signaling induced at different conditions in terms of CD169 expression levels on monocytes is well correlated with the type I IFN bioactivities induced by the polyI:C stimulation. Differential response of PBMCs to TLR agonists in terms of the induction of type I IFN signaling, such as CD169 expression on monocytes was also displayed when PBMCs were pre-incubated with different concentration of glucose. Similar findings were obtained when PBMCs were stimulated with TLR9 agonist, CpG as well (data not shown). It is noteworthy that the baseline level of CD169 expression on monocytes is low and does not change when monocytes are cultured in medium alone for 48 h (Supplemental Fig 3), suggesting that the up-regulation of CD169 was solely attributed to TLR agonist-induced production of type I IFN. However, based on the above findings it is difficult to determine whether glucose levels have a direct effect on type I IFN-induced type I IFN signaling (CD169 expression on monocytes) because incubation with different concentrations of glucose lead to different amounts of type I IFN production. Nevertheless, the differential impact of different concentrations of glucose on type I IFN production triggered by TLR agonists suggests that diabetes patients with uncontrolled hyperglycemia may have distinct capacity to defend microbial infections, particularly viral infections. It would be of interest to directly assess how glucose affects type I IFN signaling induced by type I IFN, such as IFN- α .

Very few studies investigate the direct effect of glucose on type I IFN-induced signaling in immune cells. A recent report demonstrated that a high concentration of glucose (25 mM) enhanced type I IFN-induced signaling with up-regulation of IFN-stimulated genes (ISGs) [39]. It is noted that in this report the authors employed two concentrations of glucose in their experiments, normal (5.5 mM) and high glucose (HG) (25 mM) to study the effect of glucose on IFN- α -induced ISGs at 72 h incubation. Although it was found that HG promoted the expression of ISGs induced by type I IFN, 72 h incubation would be too long to reflect the changes solely induced by type I IFN and HG because HG would lead to a variety of other inflammatory changes within 72 h, such as the production of advanced glycation end products (AGEs), which in turn might bind to the receptors of AGEs (RAGEs) on monocytes to potentially influence type I IFN signaling [40, 41]. In our current study, we

shortened the incubation time to 24 h with escalating concentrations of glucose from normal (5.5 mM) to HG (24 mM) as described in the Methods. Our system would be more pertinent to the assessment of glucose's effect on type I IFN-induced signaling. In contrast to the reported results [39], in our system, the concurrent incubation with different concentrations of glucose appears to have no significant impact on IFN- α -induced type I IFN signaling in terms of CD169 expression on monocytes and phospho-STAT1 in THP-1 cells. However, similar to the reported results [39], pre-incubation with elevated levels of glucose for 48 h significantly enhances IFN- α -induced type I IFN signaling. This enhanced type I IFN signaling is not attributed to the direct effect from glucose, because it appears that high glucose alone actually slightly down-regulates CD169 expression on monocytes (supplemental Fig 2). We ruled out the possibility that high osmolality or IFN- α might induce cell death (supplemental Fig 4). Slight down-regulation of CD169 by high glucose suggest that high glucose may affect intrinsic basal level of ISGs expression in the absence of exogenous type I IFN. A recent elegant study shows that the intrinsic expression of ISGs in stem cells without exogenous trigger from type I IFN plays an important role for stem cells to defend themselves from viral infections [42]. Given that no type I IFN bioactivity was detected in the cultures with different concentrations of glucose in the absence of TLR agonist stimulation (data not shown), it is likely that high glucose may dampen the host defense against infections through down-regulating intrinsic ISGs.

It is highly likely that the immune cells of diabetes patients have experienced hyperglycemia frequently occurring in diabetes, and are reprogrammed with different gene expression profiles. It has been demonstrated that aberrant regulation of glucose metabolism participates in an epigenetic modulation process to alter gene expression patterns in a variety of cell types [43–45]. Therefore, this glucose pre-conditioning may lead to altered functional properties of immune cells through epigenetic modification, including modification of the type I IFN-induced signaling pathway. Indeed, pre-incubation with different concentrations of glucose endows monocytes with a differential response to type I IFN stimulation in terms of the level of CD169 expression and phosphorylated STAT1. Further research is required to study glucose modulation of epigenetics in immune cells. To study the effect of long-term presence of hyperglycemia on type IFN signaling, we evaluated IRF-7 mRNA levels after IFN- α stimulation in diabetes patients with different levels of HbA1c whose levels indicate how well the blood glucose is controlled within the last 3 months. Higher HbA1c indicates a higher level of long-term blood glucose. Consistent with the results of in vitro experiments, IRF-7 levels are positively correlated with the levels of RBC HbAc, suggesting that prolonged exposure to different levels of pre-existing hyperglycemia may program immune cells to differentially respond to type I IFN stimulation. These findings also suggest that diabetes patients may hold different anti-viral capabilities depending on the degree of hyperglycemia. A recent elegant study demonstrates that hyperglycemia drives intestinal barrier dysfunction with systemic infectious consequences of diabetes, such as *Salmonella* and *Citrobacter* [46]. In this study, the authors also found that these systemic infections were dependent on glucose transportation in intestinal epithelial cells because the glucose transporter GLUT2 deletion protected mice from pathogen invasion [46]. Hence, hyperglycemia might impede host anti-microbial activities at multiple levels in diabetes patients.

The relationship between hyperglycemia and inflammation has been heavily studied. It has been demonstrated that hyperglycemia can lead to inflammatory processes through a variety of pathways [47–50]. It is believed that the advanced glycation end products (AGEs) actively participate in these inflammatory processes. AGEs are rapidly accumulated with constant hyperglycemia in diabetes. AGEs promote inflammation in many different ways, one of which is through activating NF- κ B after binding their receptors on various types of cells [51–53]. It can also bind to other inflammatory mediators, such as HMGB1 to aggravate the inflammatory process [54, 55]. The cytokines or inflammatory mediators produced by the inflammatory cells in tissues or peripheral blood triggered by various stimuli under hyperglycemic condition may contribute to diabetes complications or influence the treatment of hyperglycemia. Despite the indisputable evidence that hyperglycemia causes inflammatory processes in diabetes, few studies cover the extended cytokine/chemokine panel (22 human cytokine multiplex kit) used in the current study. The impact of the instant incubation and pre-incubation with a serial escalating glucose concentration on cytokine/chemokine production by immune cells will provide important information for understanding how glucose levels in diabetes influence the diabetes inflammatory process. Our findings in the present study demonstrate that when glucose reaches very high levels, such as 24 mM, several proinflammatory cytokines are produced at markedly increased levels by PBMCs under TLR stimulation. Glucose transporter levels such as GLUT1 on immune cells may affect this pro-inflammatory process, as it has been shown that GLUT1 overexpression results in macrophages with inflammatory phenotype through enhancing glucose metabolism[56]. It is possible that poly I:C or IFN- α used in the current study affects monocyte-macrophages in glucose metabolism and subsequent pro-inflammatory cytokine production through regulating GLUT1 expression, which needs to be further investigated. Our above findings are also supported by a study showing that macrophages stimulated with *Mycobacterium tuberculosis* in vitro in the presence of high glucose secretes increased levels of pro-inflammatory cytokines [57]. However, the difference exists between the two studies in terms of the concentration of high glucose to enhance pro-inflammatory cytokine production. In Lachmandas' study [57], they only observed this effect when glucose was used at an extremely high level (40 mM), whereas in the current study, we observed this effect at the glucose concentration of 24 mM. This difference may be due to the different experimental systems employed, and/or the stimulus used (*mycobacterium tuberculosis* versus double-stranded virus-like poly I:C) in these two studies. Nonetheless, these enhanced inflammatory activities under the influence of high glucose implies that diabetes patients with uncontrolled hyperglycemia may display enhanced responsiveness to the components of infectious agents to produce increased levels of proinflammatory cytokines thereby leading to more severe diabetes-related tissue damages and diabetes-related complications. To minimize these pathological processes, it is necessary that hyperglycemia be well managed to control blood glucose in a proper range.

In summary, hyperglycemia in diabetes depending on the levels of blood glucose may differentially impact type I IFN production and type I IFN signaling, as well as the production of proinflammatory cytokines/chemokines during microbial infection. These changes may not only actively participate in the diabetes inflammatory process in both T1D and T2D to accelerate the disease process and the development of diabetes-associated

complications, but also interfere with the host defense against microbial infections. Thus, diabetes patients may be benefited in many ways from controlling hyperglycemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Different elevated levels of glucoses differentially affect type I IFN production by TLR agonist-triggered immune cells.
- High concentration of glucose significantly suppresses type I IFN production.
- Pre-exposure of high glucose renders monocytes more sensitive to IFN- α stimulation.
- IFN- α -induced IRF7 by PBMCs of diabetes patients are positively correlated with HbA1c levels.
- High glucose promotes PBMCs to produce multiple proinflammatory cytokines/chemokines.

Figure 1. Concurrent incubation of PBMCs with different concentrations of glucose on type I IFN production induced by poly I:C and the subsequent type I IFN signaling

A. PBMCs (1×10^6) were stimulated with poly I:C (20 μ g/ml) in the presence of different concentrations of glucose as shown for 24 h. Then, the supernatants from the cultures were measured for type I IFN activities using the HEK-blue type I IFN activity assay cell line. The data from four different healthy subjects were individually shown and statistically analyzed. **B.** The cells from the above cultures were harvested and stained with fluorescent CD14 and CD169 antibodies. The expression levels of CD169 on CD14+ monocytes were

examined by flow cytometry (upper panel). The data from four different healthy subjects were shown individually (lower panel). Paired t test was used for data analysis. *: $p < 0.05$; **: $p < 0.01$. Similar results were obtained from an additional independent experiment using the same number of healthy subjects.

Figure 2. Pre-incubation of PBMCs with different concentrations of glucose on poly I:C-induced type I IFN production and the subsequent type I IFN signaling

A. PBMCs (1×10^6) were pre-incubated with different concentrations of glucose for 48 h, then stimulated with poly I:C (20 μ g/ml) for an additional 24 h. Thereafter, the supernatants were measured for type I IFN bioactivities using the HEK-Blue cell assay system. The data from four different healthy subjects were individually shown and statistically analyzed. **B.** The cells from the above cultures were harvested and stained with fluorescent CD14 and CD169 antibodies. The expression levels of CD169 on CD14+ monocytes were examined by flow cytometry. The data from four different healthy subjects were shown individually.

Paired t test was used for data analysis. *: $p < 0.05$. Similar results were obtained from an additional independent experiment using the same number of healthy subjects.

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Figure 3. IFN- α -induced type I IFN signaling in the presence of different concentrations of glucose

A. PBMCs (1×10^6) were incubated with IFN- α (1000 U/ml) in the presence of different concentrations of glucose for 24 h. The expression of CD169 on CD14 $^{+}$ monocytes were examined by flow cytometry. The expression levels (MFI) of CD169 on monocytes (gate 1) were analyzed and shown below the plots correspondingly. **B.** The summary of triplicates of the above cultures with each condition is shown. Paired t test was used for data analysis. An additional independent experiment was performed using the same numbers of healthy subjects and Similar results were obtained. **C.** THP1 cells were incubated with IFN- α in the

presence of different concentrations of glucose for 1 h. Then, the cells were harvested and prepared for western blot samples. The western blots were performed to examine phosphorylated STAT1 and β -actin. The same results were obtained in three additional independent experiments.

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Figure 4. Effect of glucose pre-incubation on IFN- α -induced type I IFN signaling

A. PBMCs (1×10^6) were incubated with different concentrations of glucose as indicated for 48 h. Thereafter, IFN- α (1000 U/ml) was added to the above cultures for an additional 24 h. The expression levels of CD169 on CD14 $^{+}$ monocytes (MFI) were examined by flow cytometry. The MFIs of CD169 on monocytes (gate 1) at different conditions were analyzed and shown below the plots correspondingly. **B.** The summary of the triplicates of the above cultures with each condition was shown. **C.** THP1 cells were incubated with different concentrations of glucose for 48 h. Then, IFN- α (1000 U/ml) was added to the cultures for 1 h. Thereafter, the cells were harvested and prepared for western blot samples. The western

blots were performed to examine phosphorylated STAT1 and β -actin. The same results were obtained in three additional independent experiments

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Figure 5. Correlation between HbA1c and the level of IRF-7 expression in PBMCs induced by IFN- α

PBMCs of 11 T1D patients with different levels of HbA1c were stimulated with IFN- α (1000 U/ml) for 18 h. The cells were harvested and RNA samples were prepared for qRT-PCR to detect mRNA levels of IRF-7. The IFN- α -stimulated IRF-7 mRNA level was designated as a ratio value (IFN- α -stimulated/medium only). The relationship between IFN- α -stimulated IRF-7 mRNA levels and HbA1c values was analyzed using the correlation test.

Figure 6. Proinflammatory cytokines and chemokines produced by PBMCs with or without pre-incubated with glucose in response to poly I:C stimulation

A. PBMCs (1×10^6) from healthy blood donors were stimulated with poly I:C (20 μ g/ml) in the presence of different concentrations of glucose for 24 h. Then, the supernatants from the cultures were measured for cytokines/chemokines using the 22 human cytokine multiplex kits. A representative of four independent experiments is depicted. **B.** PBMCs (1×10^6) were pre-incubated with different concentrations of glucose for 48 h, then stimulated with poly I:C (20 μ g/ml) for an additional 24 h. Thereafter, the supernatants were measured for

cytokines/chemokines using the 22 human cytokine multiplex kits. A representative of four independent experiments is depicted. *: $p<0.05$; **: $p<0.01$.

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Table 1

Demographic data of the 11 T1D patients

Patient#	Age	Sex	HbA1c	IRF-7
1	11	M	8.6	9.3
2	25	M	6.4	7
3	18	M	5.8	6.4
4	12	M	11	11
5	15	F	7.5	8.8
6	15	F	7.1	7.8
7	12	M	9.6	12.7
8	14	F	8.6	12.3
9	6	F	8.6	9.2
10	11	F	9.6	11.1
11	12	F	7.1	11.4