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Interferon alpha impairs insulin production in human beta cells via endoplasmic reticulum stress

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ABSTRACT

Despite substantial advances in the research exploring the pathogenesis of Type 1 Diabetes (T1D), the pathophysiological mechanisms involved remain unknown. We hypothesized in this study that interferon alpha (IFNα) participates in the early stages of T1D development by triggering endoplasmic reticulum (ER) stress. To verify our hypothesis, human islets and human EndoC-βH1 cells were exposed to IFNα and tested for ER stress markers, glucose stimulated insulin secretion (GSIS) and insulin content. IFNα treatment induced upregulation of ER stress markers including Binding immunoglobulin Protein, phospho-eukaryotic translation initiation factor 2α, spliced- X-box binding protein-1, C/EBP homologous protein and activating transcription factor 4. Intriguingly, IFNα treatment did not impair GSIS but significantly decreased insulin production in both human islets and EndoC-βH1 cells. Furthermore, IFNα decreased the expression of both proinsulin convertase 1 and proinsulin convertase 2, suggesting an altered functional state of the beta cells characterized by a slower proinsulin-insulin conversion. Pretreatment of both human islets and EndoC-βH1 cells with chemical chaperones 4-phenylbutyric acid and tauroursodeoxycholic acid completely prevented IFNα effects, indicating an ER stress-mediated impairment of insulin production. We demonstrated for the first time that IFNα elicits ER stress in human beta cells providing a novel mechanistic role for this virus-induced cytokine in the development of T1D. Compounds targeting molecular processes altered in ER-stressed beta cells could represent a potential therapeutic strategy to prevent IFNα-induced beta cell dysfunction in the early onset of T1D.

1. Introduction

Type 1 Diabetes (T1D) is an autoimmune disease characterized by pancreatic beta cell destruction and its incidence has been rapidly rising worldwide in the past several decades. Environmental factors such as viruses have been implicated as possible causes of this rise [1–3]. Moreover, growing evidence suggests that the virally-induced cytokine interferon alpha (IFNα) is a key trigger of T1D. Indeed, therapies that neutralize IFNα are able to suppress the beta cell dysfunction that precedes the disease, although the underlying molecular mechanisms are still unclear [4].

All the major beta cell autoantigens, including insulin, traffic through the endoplasmic reticulum (ER), a key organelle specialized in a number of important cellular tasks including protein folding, calcium and redox regulation, and the activation of survival/death pathways. Evidence is accumulating that defective folding and rapid degradation of mutant proteins is among the causes of various pathologic conditions including metabolic disease, neurodegenerative disease, inflammatory disease, and cancer. In all cases the disorders are affecting the folding of exportable proteins that tend to accumulate within the ER [5–8]. In professional secretory cells like beta cells, the rapidly changing demand for insulin production and secretion in response to serum glucose levels relies greatly on ER stability to ensure proper synthesis and folding of proinsulin [9]. Recently, ER stress has been proposed to play a critical role in autoimmune-mediated beta cell destruction. Indeed ER-stressed beta cells in early T1D could eventually die or produce neo-autoantigens which could be targets of the autoimmune response in T1D, initiating a self-perpetuating vicious cycle of failing beta cells and autoimmune attack in genetically susceptible individuals [10–12].

Interestingly, high levels of IFNα have been linked with ER stress

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associated apoptosis in human urothelial cancer cells [13] connecting IFNα to ER stress in non-pancreatic cells. Moreover, the α subunit of eukaryotic translation initiation factor 2α (eIF2α) connects ER stress and interferon responses, indeed the production of IFNα triggered by viral infection upregulates double-stranded RNA-dependent protein kinase expression which phosphorylates and activates eIF2α [14].

The key role of IFNα in the etiology of T1D and the link between ER stress and beta cell autoimmunity let us to hypothesize that IFNα could trigger T1D by inducing ER stress in beta cells. Here we show strong evidence to support this hypothesis.

2. Materials & methods

2.1. Cell cultures and reagents

Human islets were isolated from deidentified donors by the Integrated Islet Distribution Program (IIDP) as per IIDP protocols (iidp.coh.org/), and transported to the principal investigator within 24 h. Human pancreata were obtained from 15 non-diabetic organ donors (45 ± 10 years, range 28–60 years). There were 7 female and 7 male donors. (For one donor the gender was not documented). Characteristics of human islet donors are also shown in Supplementary Table 1. Upon receipt, human islets were cultured in RPMI 1640 medium (Thermo Scientific) containing 5.5 mM glucose, 10% FBS (Sigma) and 1% antibiotic antimycotic solution (HyClone) and maintained at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h before starting experiments. The protocol was approved as exempt by Albert Einstein College of Medicine institutional review board. Insulin-producing EndoC-βH1 cells, a human beta cell line, were cultured as previously described [15]. For different experiments cells were seeded on 10-cm dishes, 6-cm dishes or on 12-mm diameter glass coverslips in 24-well plates. 24 h later, cells were vehicle treated or treated with the indicated drugs at the indicated experimental settings. Thapsigargin and Tunicamycin were purchased from Sigma, IFNα, 4-phenylbutyric acid (PBA) and tauroursodeoxycholic acid (TUDCA) were purchased respectively from Millipore, Sigma and Calbiochem.

2.2. Immunofluorescence

1.5×10^5 EndoC-βH1 cells were seeded on Matrigel and fibronectin coated 12-mm glass coverslips in 24-well plates and allowed to attach for 5 days at 37 °C. Cells were then vehicle treated or treated with the indicated drugs at the indicated experimental settings and fixed in 4% PFA (Polysciences, Inc.) in PBS for 15 min at room temperature. After being permeabilized by 0.5% Triton X-100 (Bio-Rad) in PBS for 10 min, cells were blocked for 1 h with 1% normal goat serum (Cell Signaling) in PBS (both steps were done at room temperature). Cells were subsequently immunostained overnight at 4 °C with the primary antibodies diluted in blocking buffer. Indirect immunofluorescence was performed using guinea pig polyclonal anti insulin, rabbit polyclonal anti C-peptide antibody, and rabbit monoclonal anti PDX-1 as described in Supplementary Table 2. Secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 568 (Life Technologies, 1:500) were used for 1 h at room temperature. After final washes with PBS, the coverslips were mounted on microscope slides with ProLong Gold Antifade Reagent containing DAPI to counterstain the nuclei (Cell Signaling). Samples were examined with a Leica DM6000 microscope.

2.3. RNA isolation, reverse transcription, semi-quantitative and real-time RT-PCR

RNA isolation, reverse transcription and real-time reverse transcription polymerase chain reaction (RT-PCR) were performed as previously described [16]. In brief, total RNA from human islets or EndoC-βH1 cells was isolated using TRIzol reagent (Thermo Scientific) in combination with the RNeasy Mini kit (Qiagen) followed by DNase treatment. Five hundred nanograms of total RNA were retrotranscribed using the Superscript III kit (Thermo Scientific) following the manufacturer's instructions. The cDNAs obtained after retrotranscription were used as templates for quantitative PCR, run on an AbiPRISM 7300 fast real-time cycler using the power SYBR Green real-time PCR master mix kit and quantified by built-in SYBR Green Analysis (all from Applied Biosystems). The relative amount of specific mRNA was normalized to Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). To amplify spliced and unspliced X-box binding protein-1 (*Xbp1*) mRNA, PCR products were electrophoresed on 2.5% agarose gel and *GAPDH* was used as a loading control. Primer sequences (Integrated DNA Technologies) used for PCR are shown in Supplementary Table 3.

2.4. Western blot assay

Total proteins from adult human islets and EndoC-βH1 cells were extracted as previously described [17,18]. Supernatants were collected and equal amount of proteins (50 μg per sample) were subjected to electrophoresis on SDS- polyacrylamide gel and transferred to Immobilon-P membranes (Millipore) for 2 h at 100 V on ice. Membranes were incubated in Odyssey Blocking Buffer (LI-COR) for 1 h at room temperature and then reacted overnight at 4 °C with specific primary antibodies for the protein of interest. The antibodies used in this study are described in Supplementary Table 2. All immunoblots were developed with the LI-COR Odyssey system, using infrared-labeled anti-rabbit and anti-mouse IgG (LI-COR 1:5000) secondary antibodies.

2.5. Insulin secretion and insulin/proinsulin contents

For insulin secretion analysis, insulin content, and proinsulin:insulin ratio we used 50 size-matched islets from each individual for each experimental condition. All experiments were repeated at least 3 times using islets from different donors (at least 3 donors). Human islets with or without IFNα were left overnight in glucose starving medium (RPMI without glucose, supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin) and subsequently incubated for 1 h at 37°C with 1 ml Krebs-Ringer bicarbonate buffer solution supplemented with 2 mM glucose or 20 mM glucose. (Krebs-Ringer bicarbonate buffer solution: 120 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L MgSO4, 1.2 mmol/L KH2PO4, 2.4 mmol/L CaCl2, and 20 mmol/L NaHCO3, supplemented with 10 mmol/L HEPES and 0.2% BSA and gassed with a mixture of 95% O2 and 5% CO2). Islets were then pelleted by centrifugation at 1500 rpm for 5 min at 4 °C and supernatants were collected and stored at −20 °C for insulin secretion. Insulin secretion in EndoC-βH1 cells was performed as previously described [4]. For insulin and proinsulin content measurements, human islets and EndoC-βH1 cells were lysed in ice-cold acid ethanol (75% ethanol, 1.5% HCl) and incubated overnight in this extracting solution at 4 °C. Lysates were then sonicated and centrifuged for 2 min at 20,000*g*. Samples were kept frozen at −20 °C before use. In both systems insulin intracellular content was measured using the Human Insulin kit (Mercodia) and proinsulin was measured using the Human Proinsulin kit (Millipore).

2.6. Statistics

All experiments were performed at least in triplicate. Data are presented as means ± SEM. Statistical differences were determined