Original article

MOLECULAR METABOLISM

IL-13 improves beta-cell survival and protects against IL-1beta-induced beta-cell death

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ABSTRACT

Objectives: IL-13 is a cytokine classically produced by anti-inflammatory T-helper-2 lymphocytes; it is decreased in the circulation of type 2 diabetic patients and impacts positively on liver and skeletal muscle. Although IL-13 can exert positive effects on beta-cell lines, its impact and mode of action on primary beta-cell function and survival remain largely unexplored.
Methods: Beta-cells were cultured for 48 h in the presence of IL-13 alone or in combination with IL-1β or cytokine cocktail (IL-1β, IFNγ, TNFα).
Results: IL-13 protected human and rat beta-cells against cytokine induced death. However, IL-13 was unable to protect from IL-1β impaired glucose stimulated insulin secretion and did not influence NFκB nuclear relocalization induced by IL-1β. IL-13 induced phosphorylation of Akt, increased IRS2 protein expression and counteracted the IL-1β induced regulation of several beta-cell stress response genes.
Conclusions: The prosurvival effects of IL-13 thus appear to be mediated through IRS2/Akt signaling with NFκB independent regulation of gene expression. In addition to previously documented beneficial effects on insulin target tissues, these data suggest that IL-13 may be useful for treatment of type 2 diabetes by preserving beta-cell mass or slowing its rate of decline.

Keywords Beta-cells; Apoptosis; Cytokines; Gene expression; Akt

1. INTRODUCTION

A decrease in functional beta-cell mass is a key feature of type 2 diabetes [1,2]. Increasing evidence indicates that alterations in the balance between anti- and pro-inflammatory cytokines may play a role in the pathogenesis not only of type 1 but also type 2 diabetes in humans and may possibly underlie decreased beta-cell function/mass in the latter condition. Type 2 diabetes is thus associated with chronic low-grade inflammation and immune cell infiltration in islets, though this occurs at a lesser degree than in islets from type 1 diabetic patients [3]. Additionally, it is known that islet endocrine cells are able to produce certain cytokines, both pro- and anti-inflammatory [4,5]. The detrimental effects of pro-inflammatory pathways triggered by T-helper-1 (Th1) cytokines (e.g. IL-1β, TNFα, IFNγ) in islets have been widely studied and are implicated in beta-cell failure [6]. By contrast, the effects of Th2 cytokines (e.g. IL-4 and IL-13) on islets and more specifically on beta-cells have been investigated only in few studies [7] and mainly on transformed rodent beta-cell lines.
IL-13 is secreted by activated Th2 cells and classified as an anti-inflammatory cytokine due to its ability to suppress the secretion of several macrophage and monocyte-derived inflammatory cytokines. IL-13 mediates its effects by interacting with a complex receptor system comprised of IL-4Rα and IL-13Rα1 with downstream activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling cascade and the PI3kinase pathway through recruitment of insulin receptor substrate members [8,9].
IL-13 plays a key role in the regulation of hepatic glucose production in mice [10]. Moreover, IL-13 plasma levels are reduced in type 2 diabetic patients compared to healthy individuals and IL-13 exerts an autocrine effect on glucose metabolism in skeletal muscle [11]. Most recently, it has been found that IL-13 is induced in adipose tissue of obese humans and high-fat fed mice, and that the source of such IL-13 is primarily adipocytes [6].
IL-13 is able to impact positively on transformed rodent beta-cell lines and on intact rat islets [12–14]. Indeed, IL-13 increases Rinm5F cell viability and improves that of INS1E cells following serum starvation or when challenged with palmitate. IL-13 has also been found to counteract the suppression of rat islet glucose oxidation induced by IL-1β [14].
Against this background, we wanted to explore the impact of IL-13 on primary human or rat beta-cell function and survival and the mechanism underlying these putative effects. Our study shows for the first time that IL-13 protects primary beta-cells against IL-1β and mixed cytokine induced cell death without affecting proliferation or insulin secretion. These effects are mediated through the IRS2/Akt signaling cascade, and IL-13 further regulates the expression of a small number of specific genes involved in the beta-cell response to stress, albeit independently of NFκB.

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2. MATERIALS AND METHODS

2.1. Human islets, sorted beta-cells

Human islets were kindly provided by the Cell Isolation and Transplant Centre of the University of Geneva (JDRF award 31-2008-413, ECIT Islet for Basic Research Program). Human islets were dispersed by Acutase (PAA Laboratories, Austria) and beta-cells were sorted by FACS after labeling with Newport Green using a FACSVantage (Becton Dickinson, Franklin Lakes, NJ, USA) as previously described [15].

For functional analysis and immunostaining, human islets, non sorted single cells or sorted beta-cells were cultured in CMRL-1066 medium containing 5 mM glucose, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamax, 250 μg/ml gentamycin, 10 mM Hepes and 10% fetal calf serum (FCS) (Invitrogen, Switzerland) on plastic dishes coated with extracellular matrix secreted by 804G rat bladder cancer cells (804G ECM), as described elsewhere [15] and were left in islet medium for 24 h to adhere and spread before initiation of the experiments.

2.2. Rat islets, sorted beta-cells

Islets were isolated by collagenase digestion of pancreas from adult male Wistar rats. Beta-cells were separated from non beta-cells by autofluorescence using a FACSVantage as described previously [15]. Islet dispersed cells, sorted beta-cells and sorted non beta-cells were cultured in DMEM medium containing 11 mM glucose, 0.05 mg/ml gentamicin, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% FCS (Invitrogen, Switzerland). Cells were cultured on 804G ECM-coated plates as described elsewhere [15] and were left for 24 h to adhere and spread before initiation of the experiments.

2.3. Western blotting

Beta-cell protein lysate was separated by SDS-PAGE, transferred to Immobilon-P membranes (Merck Millipore, Germany) and probed with anti-NFkB and anti-phospho-NFkB (p65) (Santa Cruz Biotechnology, USA), anti-IRS2, Akt1, Akt2, anti-phospho Akt (Serine 473) (Cell Signaling Technology, USA) and anti-actin (Merck Millipore, Germany).

2.4. RNA extraction, library preparation, sequencing, mapping and expression quantification

Total RNA was prepared according to the manufacturer's instructions (ReliaPrep RNA Cell Miniprep System, Promega, USA). PolyA + RNA library construction and sequencing were performed as described elsewhere [16]. The 49 bp paired-ends reads were mapped with gemtools v1.7.1 [17,18] onto the Rnor_5.0 genome and onto the Rnor_5.0.73 gene annotation. A maximum of 5 mismatches was allowed for the alignment and reads having a mapping quality score below 150 were filtered out.

The differential expression analysis was performed with the DESeq2 software [19] by taking into account the batch and the GC content of each sample.

2.5. Insulin secretion

For acute insulin release in response to glucose, islets and beta-cells were preincubated for 2 h (2.8 mM glucose) and incubated in Krebs–Ringer bicarbonate Hepes buffer, 0.5% BSA (KRB) containing 2.8 mM glucose for 1 h followed by 1 h incubation in KRB containing 16.7 mM glucose. Total insulin was extracted with 0.18 M HCl in 70% ethanol for determination of insulin content. Insulin was measured by radioimmunoassay.

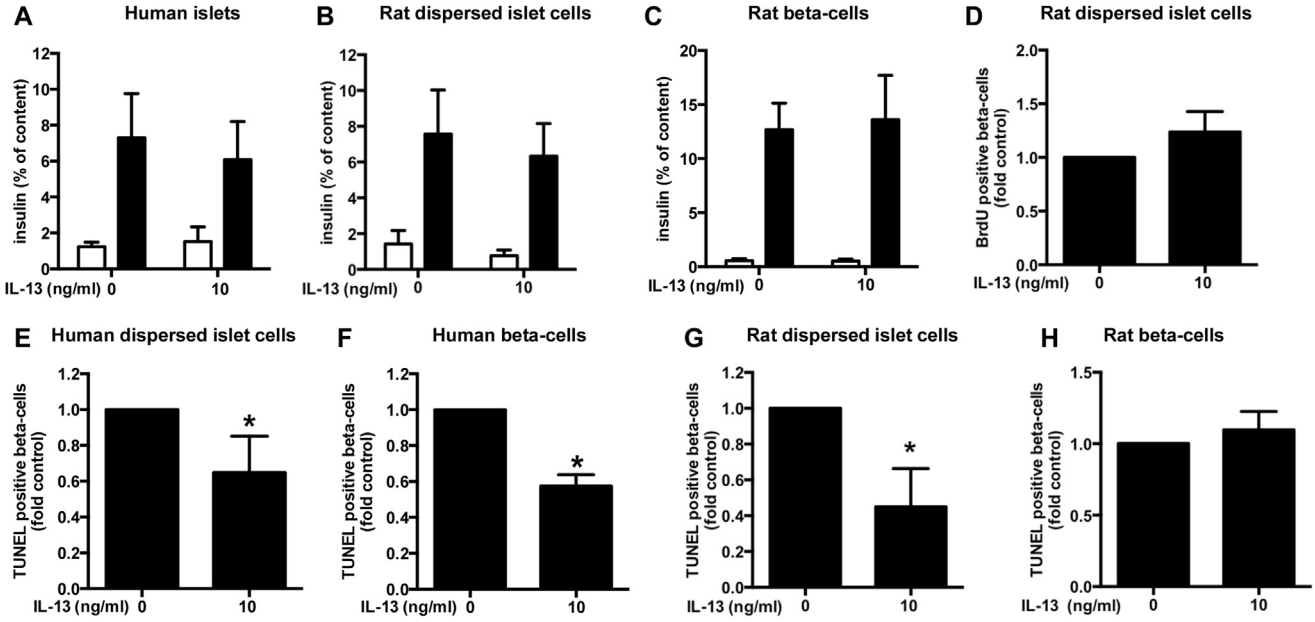

Figure 1: IL-13 decreases human and rat beta-cell death. Human islets, human or rat dispersed islets or sorted human or rat beta-cells were cultured for 48 h with IL-13 (10 ng/ml). A–C: Insulin secretion. A: Human islet insulin secretion: 2.8 mM glucose (open bars), 16.7 mM glucose (closed bars); n = 5. Rat dispersed islet cells (B; n = 5) or sorted beta-cells (C; n = 7) insulin secretion: 2.8 mM glucose (open bars), 16.7 mM glucose (closed bars). D: Rat beta-cell proliferation. BrdU-positive beta-cells in dispersed rat islet cells (normalized to control = 9.98 ± 1.93% BrdU-positive beta-cells); n = 5. E-H Beta-cell death. E: TUNEL-positive beta-cells among dispersed human islet cells (normalized to control = 0.47 ± 0.1% TUNEL-positive beta-cells); n = 5. F: TUNEL-positive sorted human beta-cells (normalized to control = 1.06 ± 0.32% TUNEL-positive beta-cells); n = 4. G: TUNEL-positive beta-cells in dispersed rat islet cells (normalized to control = 0.12 ± 0.03% TUNEL-positive beta-cells); n = 3. H: TUNEL-positive sorted rat beta-cells (normalized to control = 0.06 ± 0.02% TUNEL-positive beta-cells); n = 5. *p < 0.05 *vs.* control as tested by Student's t-test.

