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ARTICLE

# *DEXI*, a candidate gene for type 1 diabetes, modulates rat and human pancreatic beta cell inflammation via regulation of the type I IFN/STAT signalling pathway

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## Abstract

**Aims/hypothesis** The initial stages of type 1 diabetes are characterised by an aberrant islet inflammation that is in part regulated by the interaction between type 1 diabetes susceptibility genes and environmental factors. Chromosome 16p13 is associated with type 1 diabetes and *CLEC16A* is thought to be the aetiological gene in the region. Recent gene expression analysis has, however, indicated that SNPs in *CLEC16A* modulate the expression of a neighbouring gene with unknown function named *DEXI*, encoding dexamethasone-induced protein (DEXI). We therefore evaluated the role of DEXI in beta cell responses to 'danger signals' and determined the mechanisms involved.

**Methods** Functional studies based on silencing or overexpression of DEXI were performed in rat and human pancreatic beta cells. Beta cell inflammation and apoptosis, driven by a synthetic viral double-stranded RNA, were evaluated by real-time PCR, western blotting and luciferase assays.

**Results** DEXI-silenced beta cells exposed to a synthetic double-stranded RNA (polyinosinic:polycytidylic acid [PIC], a by-product of viral replication) showed reduced activation of signal transducer and activator of transcription (STAT) 1 and lower production of proinflammatory chemokines that was preceded by a reduction in IFNβ levels. Exposure to PIC increased chromatin-bound DEXI and *IFNβ* promoter activity. This effect on *IFNβ* promoter was inhibited in DEXI-silenced beta cells, suggesting that DEXI is implicated in the regulation of *IFNβ* transcription. In a mirror image of knockdown experiments, DEXI overexpression led to increased levels of STAT1 and proinflammatory chemokines.

**Conclusions/interpretation** These observations support *DEXI* as the aetiological gene in the type 1 diabetes-associated 16p13 genomic region, and provide the first indication of a link between this candidate gene and the regulation of local antiviral immune responses in beta cells. Moreover, our results provide initial information on the function of DEXI.

**Keywords** *DEXI* · Inflammation · Pancreatic beta cell · Susceptibility gene · Type 1 diabetes · Type I IFNs · Viral infection

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## Research in context

**What is already known about this subject?**

- Islet inflammation is partially regulated by the interaction between type 1 diabetes susceptibility genes and environmental factors
- Type 1 diabetes-associated SNPs in *CLEC16A* correlate with differential expression of a neighbouring gene, *DEXI*, with unknown function

**What is the key question?**

- Is the type 1 diabetes-associated candidate gene *DEXI* implicated in pancreatic beta cell inflammation and death?

**What are the new findings?**

- DEXI is involved in virus-induced pancreatic beta cell inflammation via regulation of the type I IFN–STAT signalling pathway
- DEXI is located in the chromatin of pancreatic beta cells and participates in the transcriptional regulation of virus-induced IFNβ expression
- Our findings support *DEXI* as the aetiological gene for type 1 diabetes on chromosome 16p13 and provide initial insights into the function of the protein it encodes

**How might this impact on clinical practice in the foreseeable future?**

- Molecular characterisation of the interactions between predisposing genes and environmental triggers will clarify the mechanisms by which viral infections or other 'danger signals' lead to type 1 diabetes, providing crucial information to develop therapeutic strategies that will avoid triggering autoimmunity in genetically susceptible individuals

**Abbreviations**

| | |
|---|---|
| CCL5 | Chemokine (C-C motif) ligand 5 |
| CVB5 | Coxsackievirus B5 |
| DEXI | Dexamethasone-induced protein |
| dsRNA | Double-stranded RNA |
| eQTL | Expression quantitative trait locus |
| ISRE | Interferon-stimulated response element |
| JAK | Janus kinase |
| PIC | Polyinosinic:polycytidylic acid |
| RT-PCR | Real-time PCR |
| siRNA | Small interfering RNA |
| STAT | Signal transducer and activator of transcription |

## Introduction

Type 1 diabetes is an autoimmune disease in which pancreatic beta cells are destroyed by the immune system, leading to a progressive and severe insulin deficiency. The initial stages of the disease are characterised by an islet inflammation (insulitis) that is partially driven by a 'dialogue' between the beta cells and the infiltrating immune cells [1]. Accumulating evidence suggests that the triggering of insulitis depends on an interaction between type 1 diabetes susceptibility genes [2] and environmental factors, such as viral infections [3, 4]. During viral infections, pancreatic beta cells and other islet cells release chemokines and cytokines, including type I IFNs [5, 6], that contribute to attracting and activating immune cells homing to the islets, thus contributing to the pathogenesis of type 1 diabetes [1].

Linkage and genome-wide association studies have identified 58 genomic regions showing evidence of an association with type 1 diabetes, and about 50 genes have been suggested to be potentially disease-causing genes [7]. Pathway analysis and functional studies of type 1 diabetes-associated genes expressed in human pancreatic islets indicate that many of these genes are involved in regulating antiviral responses and type I IFN signalling [2, 8–12].

Functional characterisation at the beta cell level of type 1 diabetes candidate genes related to innate immunity, for example *MDA5* (also known as *IFIH1*) [8], *PTPN2* [10] and the associated gene *USP18* [11], indicate a common theme, namely that polymorphisms leading to hyperactivation of innate immunity and inflammatory responses are usually associated with increased risk of type 1 diabetes, while decreased responses in these pathways lower the risk of developing type 1 diabetes [2].

The chromosomal region 16p13 of the human genome has been associated with several autoimmune diseases, including type 1 diabetes [13], coeliac disease [14] and multiple sclerosis [15]. This genomic region is dominated by the *CLEC16A* gene, and the most highly disease-associated SNPs,

rs12708716 and rs8062322, are located within its intron 19 [13–16]. *CLEC16A* has been considered to be the aetiological gene in this region, and experiments indicated that it regulates mitophagy in pancreatic beta cells [17]. Recent gene expression analysis followed by chromosome conformation capture experiments have, however, indicated that intron 19 of *CLEC16A* behaves as a regulatory sequence that physically interacts with the promoter of a neighbouring gene named *DEXI* (encoding dexamethasone-induced protein [DEXI]), affecting its expression [18]. The presence of the risk allele in *CLEC16A* correlates with a decrease in *DEXI* expression, whereas the presence of the protective allele is associated with higher levels of *DEXI* expression in monocytes [18], thymus [19] and B lymphoblastoid cell lines [20]. These findings suggest that *DEXI* may be the main aetiological gene in the 16p13 region and may indicate a novel and important pathway in the pathogenesis of autoimmune diseases.

*DEXI* encodes a 95 amino acid protein that is highly conserved across several species [18]. *DEXI* was identified as a differentially expressed transcript in lung tissue of individuals with emphysema [21]. The function of DEXI is unknown, but analysis of its protein sequence shows a central transmembrane domain and a repeating leucine zipper motif, which is a typical feature of many transcription factors and regulatory proteins [18].

Here we studied the role of DEXI in the beta cell response to stresses relevant to type 1 diabetes, aiming to clarify its potential involvement in the pathogenesis of the disease at the beta cell level.

## Methods

**Culture of INS-1E cells, human EndoC-βH1 cells, primary rat beta cells and human islets, and collection of human biological samples** The INS-1E cell line (research resource identifier [RRID]: CVCL_0351, kindly provided by C. Wollheim, Centre Medical Universitaire, Geneva, Switzerland] was cultured in RPMI 1640 GlutaMAX-I as previously described [22]. Antibiotic-free medium was used for transfection with small interfering (si)RNA and the double-stranded (ds)RNA analogue, polyinosinic:polycytidylic acid (PIC).

The EndoC-βH1 human beta cell line (RRID: CVCL_L909, kindly provided by R. Scharfmann, Centre de Recherche de l'Institut du Cerveau et de la Moelle Épinière, Paris, France) was cultured in plates coated with Matrigel-fibronectin (100 mg/ml and 2 mg/ml, respectively) in low-glucose DMEM as previously described [23]. For siRNA and PIC transfection, BSA- and antibiotic-free medium was used.

INS-1E and EndoC-βH1 cells were free from *Mycoplasma*, as evaluated by the MycoAlert Mycoplasma Detection kit (Lonza, Basel, Switzerland). To prevent *Mycoplasma* contamination, Plasmocin Prophylactic (Invivogen, San Diego, CA, USA) was added to the culture medium on a regular basis.

Rats were housed and used according to the Belgian Regulations for Animal Care and with permission from the local Ethical Committee of the Université Libre de Bruxelles (Brussels, Belgium). Rat islets were isolated by collagenase digestion and hand-picked under a stereomicroscope, and beta cells were purified by FACS and cultured as previously described [24] (see electronic supplementary material [ESM] Methods for details). BSA- and antibiotic-free medium was used for siRNA and PIC transfection.

Isolation of human islets from non-diabetic organ donors was carried out according to the local Ethics Committee in the University of Pisa (Pisa, Italy) as previously described [25]. Human islets were then sent to Brussels and, after overnight recovery, dispersed and cultured as previously described [12, 22]. As determined by insulin immunocytochemistry [26], the percentage of beta cells in the human islet preparations was 53% ± 14% (mean ± SEM; $n = 4$). The human islet samples have also previously been described [12].

Duodenal biopsies from individuals with coeliac disease and non-coeliac donors were collected at the Cruces University Hospital, Pediatric Gastroenterology Unit (Barakaldo, Spain) after approval by the ethics committee of Cruces University Hospital (Barakaldo, Spain). All samples were collected after informed consent had been obtained from participants or their parents (see ESM methods for details).

**RNA interference and overexpression experiments** The siRNAs targeting rat and human *DEXI* used in this study are listed in ESM Table 1. The optimal siRNA concentration (30 nmol/l) and conditions for beta cell transfection were previously established [22, 27]. Cells were transfected using the Lipofectamine RNAiMAX lipid reagent (Invitrogen, Carlsbad, CA, USA) as described [11]. After transfection, cells were cultured for a 48 h recovery period and subsequently exposed to intracellular PIC, treated with proinflammatory cytokines IL-1β plus IFNγ, or infected with coxsackievirus B5 (CVB5).

To overexpress DEXI in INS-1E and EndoC-βH1 cells, we used an overexpression plasmid encoding the human *DEXI* gene under the control of the cytomegalovirus promoter (pCMV-*DEXI*) (RC207463, Origene, Rockville, MD, USA). Cells were transfected using the Lipofectamine 2000 lipid reagent (Invitrogen). A plasmid containing only the cytomegalovirus promoter (pCMV-Control) was transfected as a negative control of overexpression.

**Cell treatments** The synthetic viral double-stranded RNA (dsRNA) analogue PIC (InvivoGen) was used at a final concentration of 1 μg/ml as previously described [11]. PIC transfection was performed under the same conditions as described

for siRNA but using Lipofectamine 2000 lipid reagent (Invitrogen).

Cells were treated with IL-1β plus IFNγ (10 and 100 U/ml, respectively) as described elsewhere [22, 28], and infection of primary rat beta cells with CVB5 (multiplicity of infection 1) was performed as previously described [5].

EndoC-βH1 cells were treated with the Janus kinase (JAK) inhibitor ruxolitinib (4000 nmol/l; Cayman Chemicals, Ann Arbor, MI, USA) as previously described [29].

**Assessment of cell viability** Cell viability was determined after incubation with the DNA-binding dyes propidium iodide (5 mg/ml; Sigma, St Louis, MO, USA) and Hoechst 33342 (5 mg/ml) as previously described [10, 28]. See also ESM Methods.

**Real-time PCR** RNA extraction was performed using the NucleoSpin RNA Kit (Macherey-Nagel, Düren, Germany), and cDNA was synthesised using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Real-time PCR (RT-PCR) was performed using TaqMan Gene Expression Assays (Thermo Fisher, Waltham, MA, USA) specific for rat and human *DEXI*, *STAT1*, *CXCL1*, *CCL5*, *CXCL9* and *IFNβ* (also known as *IFNB1*). Expression was corrected for the housekeeping genes *Gapdh* (in rat beta cells), β-actin (in human beta cells) and *RPLP0* (in intestinal biopsies). The treatments used in this study do not affect expression of these housekeeping genes (data not shown). The TaqMan Gene Expression Assays (Thermo Fisher) used are listed in ESM Table 2. *DEXI* expression was also measured in a commercially available RNA panel set of different human tissues (Human total RNA Master Panel II; Clontech, Saint-Germain-en-Laye, France).

**Western blotting** Cells were lysed in Laemmli buffer, and the immunoblot analysis was performed with the antibodies listed in ESM Table 3 (see also ESM Methods).

**ELISA** Supernatant fractions from INS-1E and EndoC-βH1 cells were collected for determination of rat chemokine (C-C motif) ligand 5 (CCL5) and human IFNβ, respectively, using commercially available ELISA kits (R&D Systems, Abingdon, UK). ELISA was performed following the manufacturer's instructions.

**Luciferase reporter assays** INS-1E cells were transfected with the siRNAs as described above. After 12 h of recovery, cells were co-transfected with pRL-CMV encoding Renilla luciferase (Promega, Madison, WI, USA) and either a firefly luciferase promoter reporter construct containing the four IFN-stimulated response element (ISRE) consensus sequences or the *IFNβ* promoter [30]. After 24 h of recovery and 16 h of PIC transfection, luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) and corrected for the luciferase activity of the internal control plasmid, pRL-CMV.

**Statistical analysis** Data are expressed as means ± SEM. A significant difference between experimental conditions was assessed by ANOVA followed by a paired Student's *t* test with Bonferroni correction. Values of $p < 0.05$ were considered statistically significant. Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA).

## Results

***DEXI* expression is upregulated by intracellular dsRNA in human and rodent pancreatic beta cells** We first evaluated the expression of *DEXI* mRNA in dispersed human islets and in the human beta cell line EndoC-βH1 compared with a set of human tissues (Fig. 1a). DEXI is expressed by several tissues, with the highest levels in heart, brain, kidney and liver, and the lowest in lung. *DEXI* mRNA expression in dispersed human islets and in human beta cells was around twofold higher than in tissues involved in the immune response (e.g. thymus and spleen). This was confirmed in human islets studied by RNA sequencing [26], compared with 16 control tissues, using the Illumina Body Map 2.0 dataset (ESM Fig. 1a). The proinflammatory cytokines IL-1β plus IFNγ did not modify *DEXI* expression in rat and human beta cells (ESM Fig. 1b, c). In line with this, RNA sequencing of human islets exposed or not exposed to IL-1β plus IFNγ [8] showed similar *DEXI* expression in control and cytokine-treated human islets, with reads per kilobase per million mapped reads of 19 ± 1 and 23 ± 3 (means ± SEM of five samples), respectively (ESM Fig. 1a). *DEXI* expression was also similar in intestinal biopsies from individuals with coeliac disease at first presentation of clinical symptoms (who were on a gluten-containing diet, and had coeliac disease-associated antibodies, atrophy of the intestinal villi and T lymphocyte infiltration), and the same individuals in remission after being treated with a gluten-free diet for over 2 years (at which time they were asymptomatic and showed negative antibodies and a return of the intestinal epithelium) (ESM Fig. 1d). Taken together, these results suggest that inflammation by itself does not alter *DEXI* expression in pancreatic beta and intestinal cells.

It has been previously shown that PIC, a synthetic viral dsRNA analogue, induces beta cell inflammation and death [8, 11, 31]. Exposure of human beta cells to PIC led to a 74-fold increase in *DEXI* mRNA expression after 2 h, which increased further at 8 h (to 179-fold) and slightly decreased at 24 h (to 148-fold) (Fig. 1b). The effect of PIC on DEXI expression was confirmed at the protein level in EndoC-βH1 cells (Fig. 1c). Exposure to PIC for 24 h increased *Dexi*

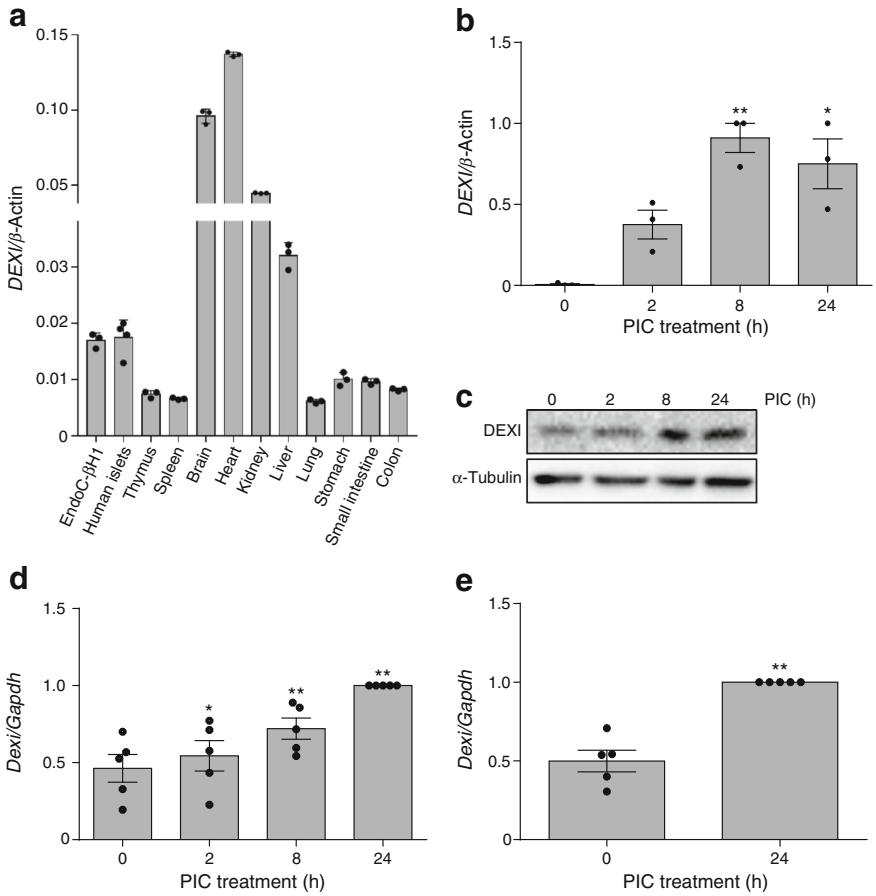



**Fig. 1** DEXI is produced by pancreatic beta cells, and its expression is upregulated by PIC. (**a**) *DEXI* mRNA expression was analysed in the human beta cell line EndoC-βH1, in dispersed human islets and in a commercially available RNA panel set of different human tissues (thymus, spleen, brain, heart, kidney, liver, lung, stomach, small intestine and colon). *DEXI* expression was determined by RT-PCR and normalised to the housekeeping gene β-actin. Results are the means of three experimental replicates. (**b**, **c**) Human EndoC-βH1 cells, (**d**) INS-1E cells and (**e**) FACS-purified primary rat beta cells were left untreated (0 h) or treated with intracellular PIC (1 μg/ml) for 2, 8 and 24 h (**b**–**d**) or 24 h (**e**). mRNA expression of *DEXI* and β-actin (**b**) or *Gapdh* (**d**, **e**) was assayed by RT-PCR, and DEXI levels were assayed by western blotting (**c**). Results are the means ± SEM of 3 (**b**) or 5 (**d**–**e**) independent experiments, and the blotting is representative of four independent experiments; $*p < 0.05$, $**p < 0.01$ vs time 0 h, Student's *t* test

mRNA expression by around 2.3-fold in INS-1E cells (Fig. 1d) and FACS-purified primary rat beta cells (Fig. 1e).

Risk alleles of autoimmune-disease associated SNPs in the *CLEC16A* gene correlate with decreased *DEXI* expression in several immune cells and tissues, including B cells, monocytes and thymus [18–20]; however, the *DEXI–CLEC16A* expression quantitative trait locus (eQTL) seems to be tissue- and context-specific [18, 32]. Available eQTL data from the GTEx Portal (https://gtexportal.org/home/, accessed December 2017) showed no correlation between rs12708716 genotype and *DEXI* expression in whole pancreas (ESM Fig. 2a). SNPs in *CLEC16A* are also associated with coeliac disease, another autoimmune disease that shares several candidate genes with type 1 diabetes. According to GTEx data, the disease-associated protective allele (G) in rs12708716 correlates with a slight decrease in *DEXI* expression in the small intestine (ESM Fig. 2b). These data were confirmed using intestinal biopsies from healthy donors; in these, biopsies from donors who were heterozygous for the protective allele (G) showed decreased *DEXI* expression compared with biopsies from donors homozygous for the risk allele A (ESM Fig. 2c).

Conversely, CVB5 infection in beta cells downregulated *Dexi* expression by 40% (ESM Fig. 3a), suggesting the potential activation of a virus-related mechanism to downregulate antiviral host defences.

**DEXI inhibition reduces viral dsRNA-induced pancreatic beta cell death** Intracellular treatment with PIC or infection with the diabetogenic CVB5 triggers beta cell death [5, 33]. To examine the role of DEXI in PIC- and CVB5-induced beta cell apoptosis, two independent siRNAs were used to knock down DEXI production in primary rat beta cells and in INS-1E cells. These different siRNAs inhibited *Dexi* mRNA

expression by 50–80% in all the conditions tested (Fig. 2a, ESM Fig. 3a). PIC transfection for 24 h increased apoptosis by 23% in si control (CTRL)-transfected primary rat beta cells, whereas DEXI inhibition reduced cell death in PIC-transfected cells by around 10% (Fig. 2b). Similarly, CVB5 infection for 24 h increased beta cell apoptosis compared with mock-infected INS-1E cells, and DEXI inhibition reduced CVB5-induced apoptosis by around 50% (ESM Fig. 3b). The decrease in PIC-induced apoptosis after DEXI knockdown was confirmed by decreased caspase 3/7 activity in INS-1E cells using a luminescence-based assay (ESM Fig. 3c).

**DEXI silencing partially inhibits PIC-induced STAT signalling pathway activation and decreases proinflammatory chemokine production in pancreatic beta cells** We next examined the effect of DEXI inhibition on the kinetics of PIC-induced activation of signal transducer and activator of transcription

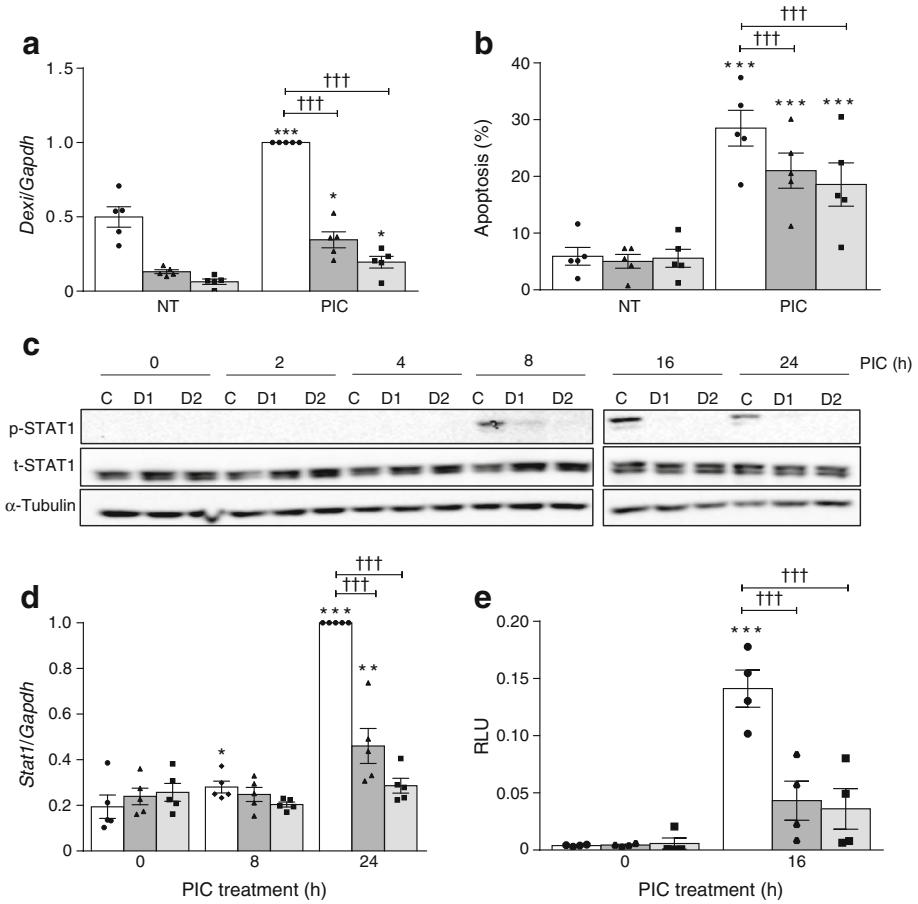



**Fig. 2** DEXI inhibition reduces PIC-induced apoptosis and STAT1 signalling pathway activity in pancreatic beta cells. Primary rat beta cells (**a**, **b**) were transfected with siCTRL (white bars) or two independent siRNAs targeting *Dexi* (si*Dexi*#1 [dark grey bars] and si*Dexi*#2 [light grey bars]). After 48 h of recovery, the cells were left untreated (NT) or treated with PIC (1 μg/ml) for 24 h. (**a**) *Dexi* mRNA expression was assayed by RT-PCR and normalised to the housekeeping gene *Gapdh*. (**b**) Apoptosis was evaluated using propidium iodide/Hoechst 33342 staining. Results are the means ± SEM of 3–5 independent experiments; $*p < 0.05$, $***p < 0.001$ vs NT and transfected with the same siRNA; $^{\dagger\dagger\dagger}p < 0.001$ vs the respective siCTRL; ANOVA followed by Student's *t* test with Bonferroni correction. (**c**) INS-1E cells were transfected with siCTRL (C) or two independent siRNAs targeting *Dexi* (si*Dexi*#1 [D1], si*Dexi*#2 [D2]). After 48 h of recovery, cells were left untreated (0 h) or treated with intracellular PIC (1 μg/ml) for 2, 4, 8, 16 or 24 h. Levels of phospho-STAT1 (p-STAT1), total STAT1 (t-STAT1) and α-tubulin (used as loading control) were evaluated by western blotting. The results are representative of three independent experiments. (**d**) INS-1E cells were transfected with siCTRL (white bars), si*Dexi*#1 (dark grey bars) or si*Dexi*#2 (light grey bars). After 48 h of recovery, the cells were left untreated (0 h) or treated with intracellular PIC (1 μg/ml) for 8 or 24 h. *Stat1* mRNA expression was assayed by RT-PCR and normalised to the housekeeping gene *Gapdh*. The results are the means ± SEM of five independent experiments; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs time 0 h (not treated) and transfected with the same siRNA; $^{\dagger\dagger\dagger}p < 0.001$ vs siCTRL treated with PIC (1 μg/ml) for 24 h; ANOVA followed by Student's *t* test with Bonferroni correction. (**e**) INS-1E cells were transfected with siCTRL (white bars), si*Dexi*#1 (dark grey bars) or si*Dexi*#2 (light grey bars) and co-transfected with an ISRE reporter construct plus a pRL-CMV plasmid (used as an internal control). After 48 h of recovery, the cells were left untreated (0 h) or treated with PIC for 16 h, and relative luciferase units (RLU) were measured. Results are the means ± SEM of four independent experiments; $***p < 0.001$ vs untreated (i.e. time 0 h) and transfected with the same siRNA; $^{\dagger\dagger\dagger}p < 0.001$ vs siCTRL treated with PIC (1 μg/ml) for 16 h; ANOVA followed by Student's *t* test with Bonferroni correction

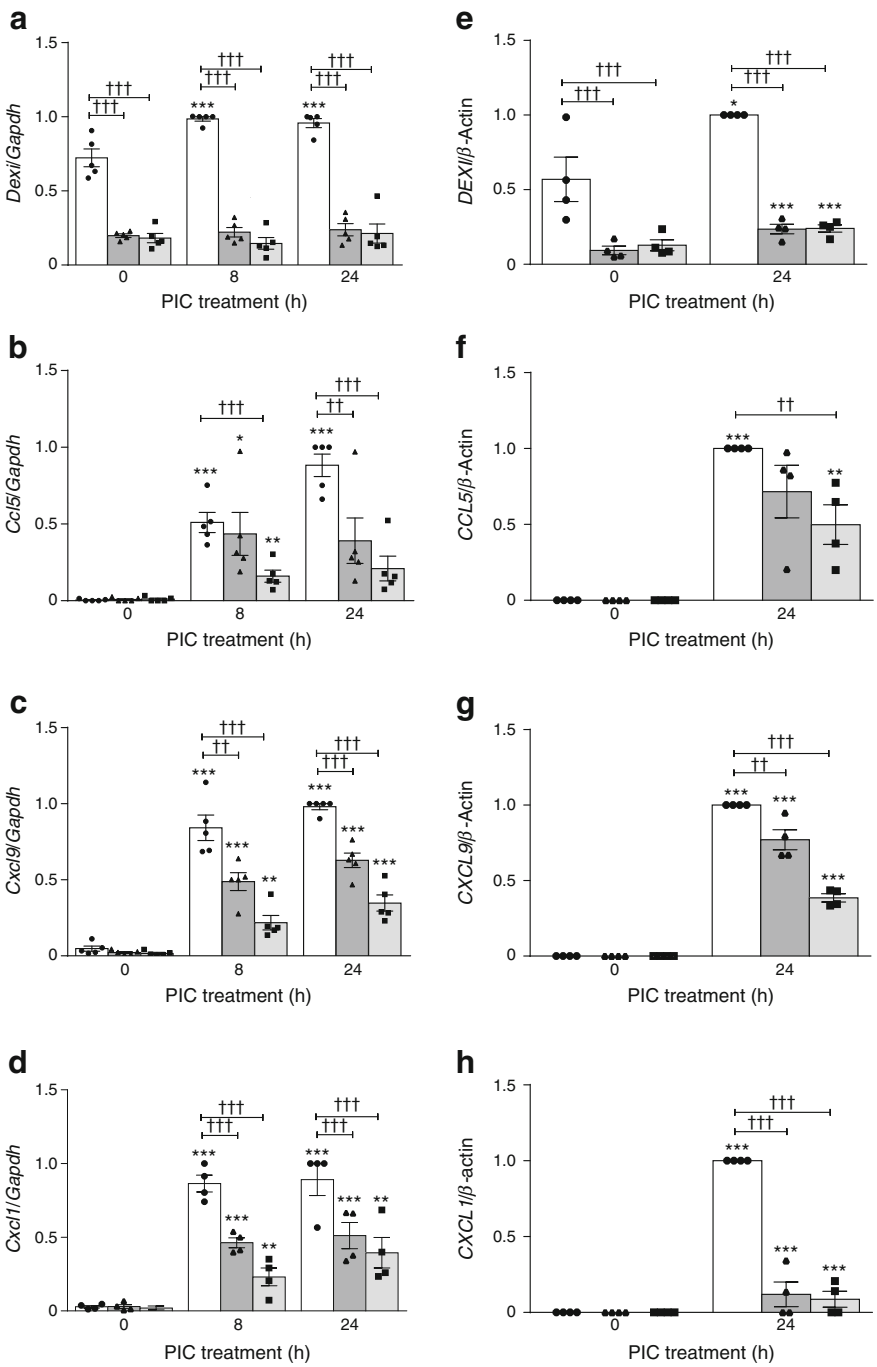



**Fig. 3** DEXI inhibition reduces PIC-induced expression of proinflammatory chemokines in rat and human beta cells. INS-1E (**a**–**d**) or EndoC-βH1 cells (**e**–**h**) were transfected with siCTRL (white bars) or two independent siRNAs targeting rat (**a**–**d**) or human (**e**–**h**) *DEXI* (si*Dexi*#1 [dark grey bars] and si*Dexi*#2 [light grey bars]), respectively. After 48 h of recovery, the cells were left untreated (0 h) or treated with intracellular PIC (1 μg/ml) for 8 or 24 h (INS-1E cells) or for 24 h (EndoC-βH1 cells). *DEXI* (**a**, **e**), *CCL5* (**b**, **f**), *CXCL9* (**c**, **g**) and *CXCL1* (**d**, **h**) expression was assayed by RT-PCR and normalised to the housekeeping gene *Gapdh* (**a**–**d**) or β-actin (**e**–**h**). Results are the means ± SEM of 4–5 independent experiments; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs time 0 h (i.e. not treated) and transfected with the same siRNA; $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs the respective siCTRL; ANOVA followed by Student's *t* test with Bonferroni correction

(STAT) signalling. STAT1 phosphorylation was markedly enhanced in INS-1E cells exposed to PIC after 8 h and up to 24 h (Fig. 2c, ESM Fig. 4a). Inhibition of DEXI abrogated STAT1 phosphorylation 8 h after PIC transfection, and this effect was prolonged for up to 24 h (Fig. 2c, ESM Fig. 4a). Although total STAT1 protein levels did not differ between siCTRL- and si*Dexi*-transfected cells 24 h after PIC transfection, PIC-induced *Stat1* mRNA expression was significantly reduced in DEXI-inhibited INS-1E cells (Fig. 2d), suggesting a potential role of DEXI in the regulation of STAT1 signalling. As PIC exposure activates the expression of several proinflammatory genes regulated by ISRE [34], we assessed the activation of an ISRE reporter in DEXI-inhibited beta cells. As shown in Fig. 2e, PIC transfection induced ISRE reporter activity in siCTRL-transfected cells by 40-fold, whereas DEXI knockdown significantly inhibited ISRE activation (around 70% less activity).

To evaluate the role of DEXI in PIC-induced chemokine production, we analysed the mRNA expression of *CCL5*, *CXCL9* and *CXCL1* in DEXI-silenced INS-1E cells (Fig.

3a–d), EndoC-βH1 cells (Fig. 3e–h) and FACS-purified primary rat beta cells (ESM Fig. 5). PIC treatment for 8 and 24 h induced *Ccl5*, *Cxcl9* and *Cxcl1* mRNA expression in siCTRL-transfected INS-1E cells, but DEXI inhibition partially prevented this effect at both time points (Fig. 3a–d). In EndoC-βH1 cells, PIC transfection for 24 h significantly increased the expression of the genes encoding proinflammatory chemokines (Fig. 3e–h). The downregulation of *Ccl5* mRNA expression in DEXI-inhibited INS-1E cells was confirmed at the protein level, as DEXI-silenced cells secreted lower amounts of CCL5 compared with siCTRL-transfected cells after PIC transfection (ESM Fig. 6). In DEXI-silenced EndoC-βH1 cells, PIC-induced *CCL5*, *CXCL9* and *CXCL1* expression was decreased by 50–65%, 30–60% and 90%, respectively (Fig. 3e–h). In FACS-purified rat primary beta cells, PIC transfection increased *Ccl5*, *Cxcl9* and *Cxcl1* expression, but only *Cxcl9* expression was significantly decreased after DEXI inhibition (ESM Fig. 5).

In a mirror image of these experiments, DEXI upregulation in INS-1E cells using an overexpression vector (220-fold increase compared with pCMV-Control-transfected cells) exacerbated PIC-induced *Stat1*, *Ccl5*, *Cxcl9* and *Cxcl1* mRNA expression by 25–40% (Fig. 4).

To confirm that DEXI affects PIC-induced proinflammatory chemokine expression through the modulation of the type IFN–STAT signalling pathway, we exposed DEXI-overproducing EndoC-βH1 cells to PIC in the absence or presence of ruxolitinib, a JAK inhibitor. As shown in Fig. 5, *DEXI* overexpression exacerbated PIC-induced *CCL5*, *CXCL9* and *CXCL1* mRNA expression, and this effect was counteracted by the presence of ruxolitinib.

**Downregulation of the STAT signalling pathway is preceded by reduced IFNβ content in DEXI-silenced pancreatic beta cells** During viral infections, pancreatic beta cells release type I IFNs that can act in an autocrine manner through the type I IFN receptor to activate proinflammatory signalling pathways [11, 12]. To evaluate the potential role of DEXI in PIC-induced type I IFN signalling pathway activation, we evaluated the expression of *IFNβ* in DEXI-silenced beta cells. *IFNβ* mRNA expression was upregulated after 24 h of PIC transfection in siCTRL-transfected beta cells, while DEXI inhibition

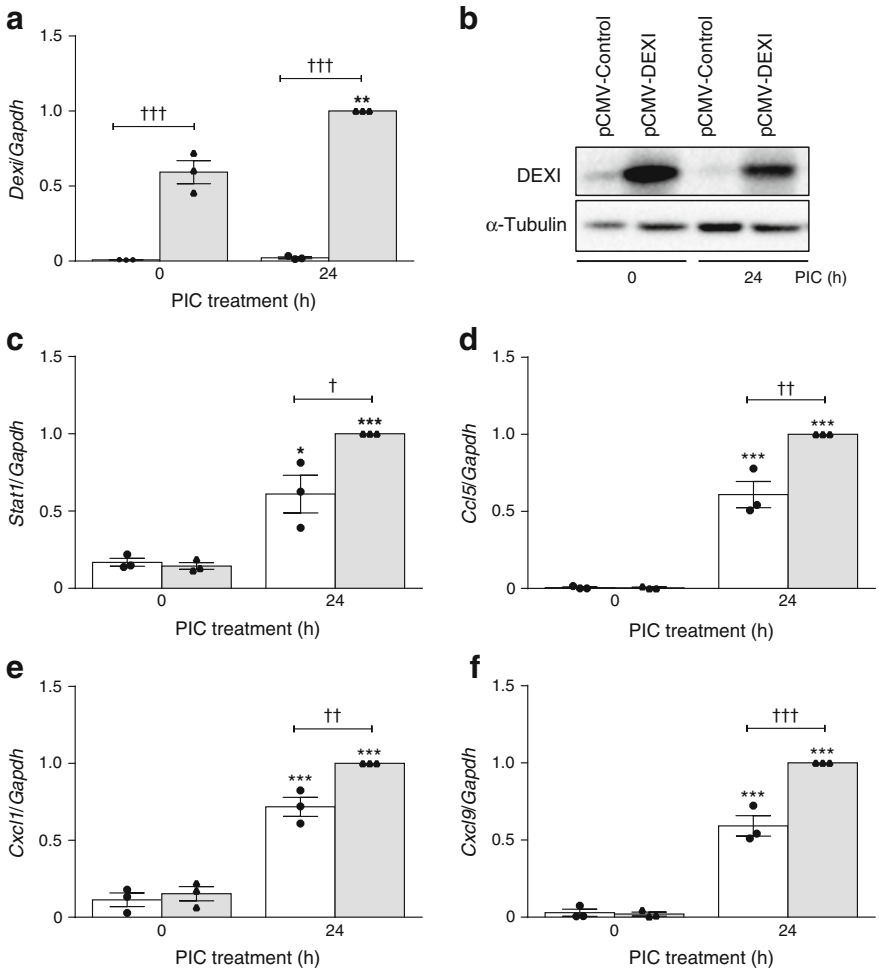



**Fig. 4** DEXI overexpression in pancreatic beta cells exacerbates PIC-induced STAT1 and chemokine expression. INS-1E cells were transfected with pCMV-Control (white bars) or with pCMV-*DEXI* (grey bars) and subsequently left untreated (0 h) or treated with intracellular PIC (1 μg/ml) for 24 h. *Dexi* mRNA (**a**) and protein (**b**) expression were determined by RT-PCR and western blotting, respectively. Results are the means ± SEM of three independent experiments; $^{**}p < 0.01$ vs time 0 h (i.e. not treated) and transfected with the same plasmid; $^{\dagger\dagger\dagger}p < 0.001$ vs the respective pCMV-Control; ANOVA followed by Student's *t* test with Bonferroni correction. (**c**–**f**) *Stat1*, *Ccl5*, *Cxcl1* and *Cxcl9* expression was assayed by RT-PCR and normalised to the housekeeping gene *Gapdh*. Results are the means ± SEM of three independent experiments; $^{*}p < 0.05$, $^{***}p < 0.001$ vs time 0 h (i.e. not treated) and transfected with the same plasmid; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs the respective pCMV-Control; ANOVA followed by Student's *t* test with Bonferroni correction

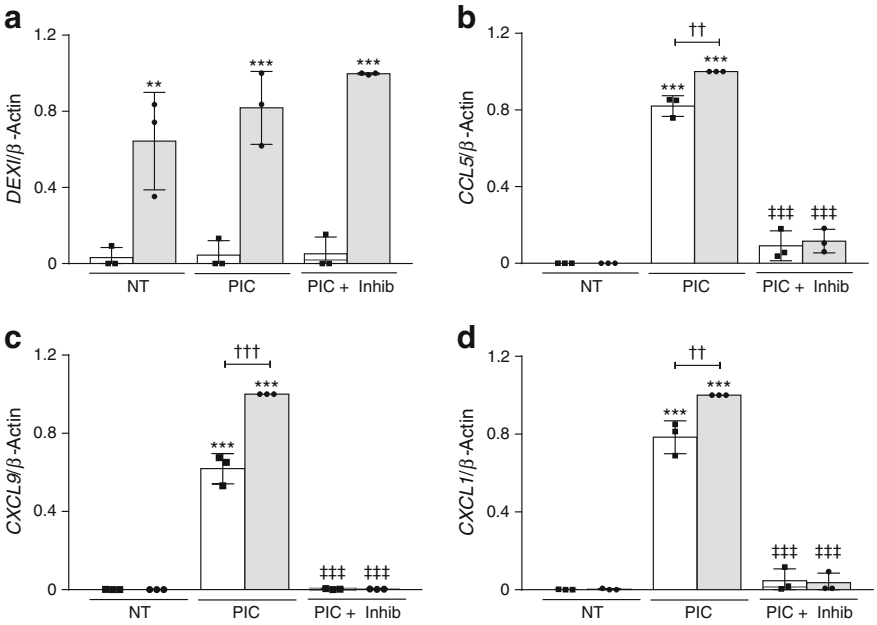



**Fig. 5** Inhibition of the STAT signalling pathway counteracts the effect of DEXI on PIC-induced production of proinflammatory chemokines. Human EndoC-βH1 cells were transfected with pCMV-Control (white bars) or with pCMV-*DEXI* (grey bars) and subsequently left untreated (NT), treated with intracellular PIC (1 μg/ml) for 24 h (PIC) or treated with PIC and ruxolitinib (4000 nmol/l) for 24 h (PIC + Inhib). mRNA expression of *DEXI* (**a**), *CCL5* (**b**), *CXCL9* (**c**) and *CXCL1* (**d**) was measured by RT-PCR and normalised to the housekeeping gene β-actin. Results are the means ± SEM of three independent experiments; $^{**}p < 0.01$, $^{***}p < 0.001$ vs NT and transfected with the same plasmid; $^{††}p < 0.01$, $^{†††}p < 0.001$ vs the respective pCMV-*DEXI*; $^{‡‡‡}p < 0.001$ vs PIC and transfected with the same plasmid; ANOVA followed by Student's *t* test with Bonferroni correction

decreased PIC-induced *IFNβ* expression in both INS-1E and EndoC-βH1 cells (Fig. 6a, b).

To clarify whether DEXI modulates viral dsRNA-induced activation of STAT signalling by regulating the early production of IFNβ, beta cells were exposed to PIC at different time points. As shown in Fig. 6c, after 4 h of PIC transfection *IFNβ* expression was already upregulated in siCTRL-transfected INS-1E cells, and inhibition of DEXI reduced this increase in *IFNβ* mRNA expression. The time course experiment revealed two peaks of upregulation of PIC-induced *Ifnβ* expression (8 and 24 h after PIC transfection), suggesting the presence of an early and a late phase of type I IFN production in response to viral infections in beta cells. At both time points (8 and 24 h), DEXI inhibition reduced *IFNβ* expression (Fig. 6a, b), confirming that the downregulation of the STAT signalling pathway in DEXI-silenced beta cells is preceded by an inhibition of IFNβ production. In line with these results, DEXI-inhibited EndoC-βH1 cells showed reduced PIC-induced IFNβ release compared with siCTRL-transfected cells (Fig. 6d).

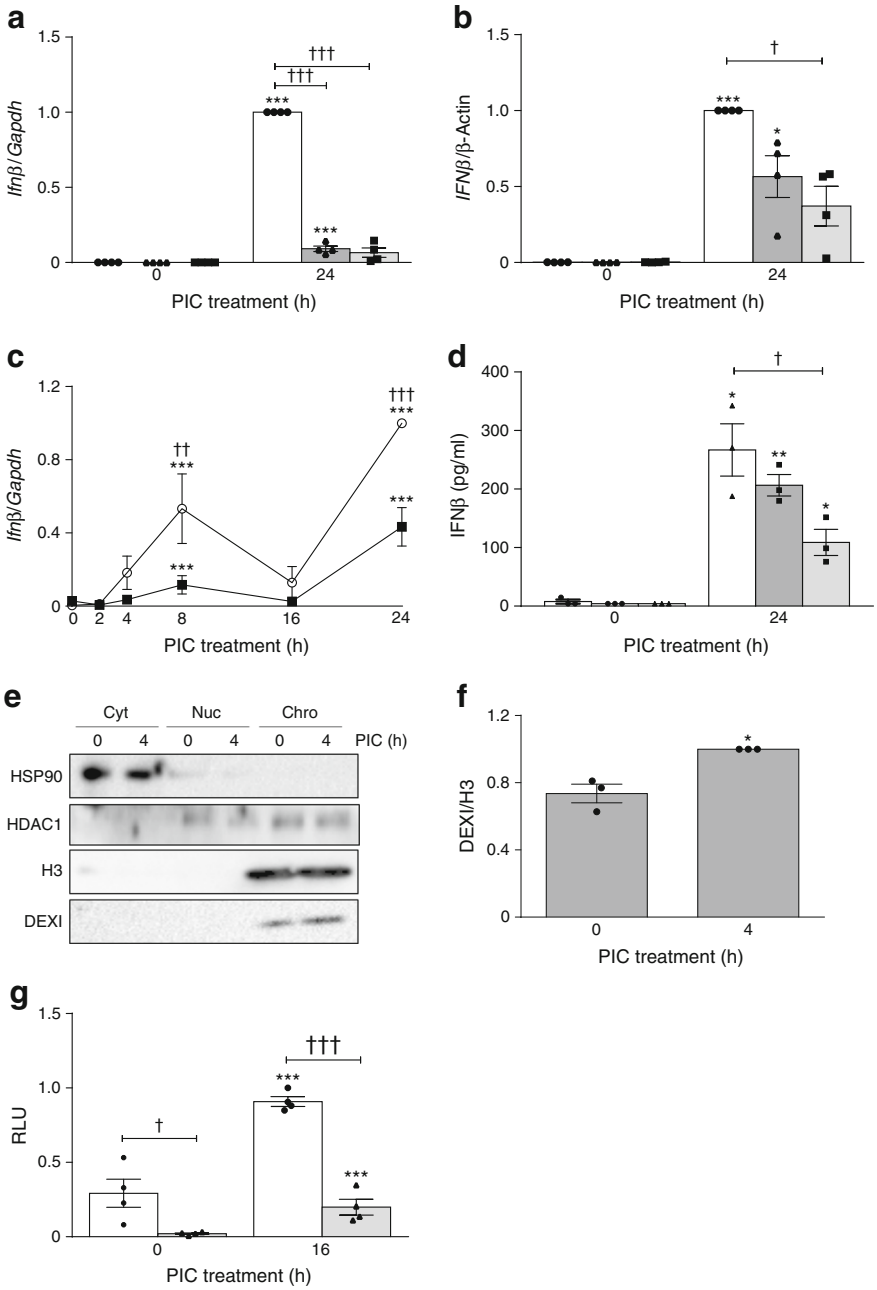
**DEXI is located in the chromatin of pancreatic beta cells and acts as a transcriptional regulator of IFNβ expression** The protein sequence of DEXI has been predicted to contain a repeating leucine pattern similar to a leucine zipper motif [18]. The leucine zipper motif is the simplest known protein-DNA recognition motif, typical of a superfamily of transcription factors that include activating transcription factor (ATF), CRE-binding protein (CREB) and Maf families [35].

As the results described above suggest that DEXI regulates the transcription of IFNβ in pancreatic beta cells, we analysed the cellular localisation of DEXI under basal condition and following 4 h of PIC exposure. DEXI was located in the chromatin of EndoC-βH1 cells before and after PIC transfection, although after 4 h of PIC transfection DEXI chromatin levels were slightly higher than in the basal condition (Fig. 6e).

To determine whether DEXI could modulate the activation of the *IFNβ* promoter, we performed a luciferase reporter assay using an *Ifnβ* promoter reporter in INS-1E cells. As shown in Fig. 6f, the *Ifnβ* promoter reporter was activated threefold by PIC. DEXI inhibition decreased *Ifnβ* promoter reporter activity by 92% and 78% under basal condition and in PIC-transfected cells, respectively, suggesting that DEXI regulates *Ifnβ* transcription.

## Discussion

The type 1 diabetes candidate gene *DEXI* encodes a 10 kDa protein with unknown function that was first identified as an upregulated transcript in the lung tissue of individuals with emphysema [21]. *DEXI* has not been positionally associated




with type 1 diabetes, but the type 1 diabetes-associated intronic SNPs in the neighbouring gene *CLEC16A* correlate with differential expression of *DEXI* [18]. Thus, *DEXI* has emerged as a potential candidate gene for type 1 diabetes and other autoimmune diseases in the 16p13 region of the human genome.

*DEXI* expression in relation to disease-associated SNPs in *CLEC16A* indicates that homozygotes for the risk alleles had a lower expression of *DEXI*, whereas the heterozygotes and homozygotes for the other allele display higher *DEXI* expression in immune-related cells [18–20]. However, as described for several other eQTLs, it seems that the *DEXI–CLEC16A* eQTL is tissue- and context-specific [18, 32], and the nature of this eQTL in pancreatic beta cells remains to be determined. The definition of eQTL signatures relevant for type 1 diabetes relies on performing genotyping and expression studies in beta cells from individuals with type 1 diabetes compared with those without it, but the very limited access to these samples makes it difficult to perform conclusive studies.

In the present study we observed that DEXI is highly expressed in human pancreatic beta cells/islets compared with other human tissues, including immune-related cells. Intracellular viral dsRNA upregulates DEXI expression in beta cells, suggesting that DEXI may play a relevant role in beta cell responses to ‘danger signals’.

◀ **Fig. 6** Downregulation of the STAT signalling pathway in DEXI-silenced beta cells is preceded by a decrease in IFNβ production. INS-1E (**a**) or EndoC-βH1 (**b**) cells were transfected with siCTRL (white bars) or two independent siRNAs targeting rat (**a**) or human (**b**) *DEXI* (si*Dexi*#1 [dark grey bars] and si*Dexi*#2 [light grey bars]), respectively, and subsequently left untreated or treated with intracellular PIC (1 μg/ml) for 24 h. Expression of *IFNβ* was assessed by RT-PCR and normalised to *Gapdh* (**a**) or β-actin (**b**). Results are the means ± SEM of four independent experiments; $^{*}p < 0.05$, $^{***}p < 0.001$ vs time 0 h (i.e. not treated) and transfected with the same siRNA; $^{\dagger}p < 0.05$, $^{\dagger\dagger\dagger}p < 0.001$ vs siCTRL treated with PIC (1 μg/ml) for 24 h; Student's *t* test with Bonferroni correction. (**c**) INS-1E cells were transfected with siCTRL (white circles) or with one siRNA targeting *Dexi* (black squares) and subsequently left untreated or treated with intracellular PIC (1 μg/ml) for 2, 4, 8, 16 and 24 h. Expression of *Ifnβ* was assessed by RT-PCR and normalised to *Gapdh*. $^{***}p < 0.001$ vs time 0 h (i.e. not treated) and transfected with the same siRNA; $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$vs siCTRL treated with PIC (1 μg/ml) at the same time point; ANOVA followed by Student's *t* test with Bonferroni correction. (**d**) EndoC-bH1 cells were transfected with siCTRL (white bars) or with si*Dexi*#1 (dark grey bars) and si*Dexi*#2 (light grey bars) and subsequently left untreated or treated with intracellular PIC (1 μg/ml) for 24 h. IFNβ protein was assayed in cell supernatant fractions using ELISA. Results are the means ± SEM of three independent experiments; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs time 0 h (i.e. not treated) and transfected with the same siRNA; $^{\dagger}p < 0.05$ vs siCTRL treated with PIC (1 μg/ml) for 24 h; ANOVA followed by Student's *t* test with Bonferroni correction. (**e**) EndoC-βH1 cells were exposed to intracellular PIC (1 μg/ml) for 4 h, and DEXI levels in different subcellular fractions were determined by western blotting. Levels of heat shock protein 90 (HSP90), as cytoplasmic marker (Cyt), histone deacetylase 1 (HDAC1), as a nuclear marker (Nuc) and histone 3 (H3) as a chromatin marker (Chro), were also determined by western blotting. (**f**) Relative chromatin-bound DEXI protein expression was determined by densitometry and normalised to H3 protein content. Densitometry results are the means ± SEM of three independent experiments; $^{*}p < 0.05$ vs time 0 h (i.e. not treated); Student's *t* test. (**g**) INS-1E cells were transfected with siCTRL (white bars) or siRNA targeting *DEXI* (grey bars) and co-transfected with an *Ifnβ* promoter reporter construct plus a pRL-CMV plasmid (used as internal control). After 48 h of recovery, they were left untreated or treated with PIC for 16 h, and relative luciferase units (RLU) were measured. Results are the means ± SEM of four independent experiments; $^{***}p < 0.001$ vs time 0 h (i.e. not treated) and transfected with the same siRNA; $^{\dagger}p < 0.05$, $^{\dagger\dagger\dagger}p < 0.001$ vs the respective siCTRL; ANOVA followed by Student's *t* test with Bonferroni correction

Our results showed that CVB5 infection downregulates DEXI expression in pancreatic beta cells. This phenomenon is probably part of the strategy that viruses use to inhibit the antiviral response of the host cell and increase their own virulence. Indeed, several studies have demonstrated that viruses are able to inhibit antiviral responses by inhibiting the activation of the IFN-induced JAK–STAT signalling pathway by different strategies [36, 37].

Here we show that DEXI inhibition reduces viral dsRNA-induced proinflammatory chemokine production via downregulation of the type I IFN–STAT signalling pathway. DEXI overexpression exacerbated PIC-induced STAT signalling and proinflammatory chemokine expression, suggesting that DEXI plays a proinflammatory role in response to viral infections at the beta cell level.

We showed here that decreased STAT1 signalling activation in DEXI-silenced beta cells is accompanied by a protective effect on PIC- and CVB5-induced beta cell apoptosis. Previous studies have reported an involvement of DEXI in the regulation of apoptotic pathways, and shown that DEXI inhibition protects against camptothecin- or hypoxia-induced apoptosis in fibroblasts [38]. In pancreatic beta cells, the implication of DEXI in virus-induced apoptosis most probably relies on its effect on the STAT1 signalling pathway activation, which is a key pro-apoptotic pathway in beta cells [11, 12, 39].

Human and rodent beta cells express several pattern recognition receptors that recognise and respond to viral components, including toll-like receptor 3 (TLR3), *MDA5* (a candidate gene for type 1 diabetes) and *RIG-I* [8, 31]. On recognition of viral dsRNA, the activation of pattern recognition receptors initiates several signal transduction cascades that include the JAK–STAT and the NF-κB-driven pathways [31]. Through activation of these pathways, viral dsRNA enhances the levels and release of proinflammatory chemokines in beta cells by promoting the production of type I IFNs, which, in an autocrine phase, activate the STAT signalling pathway; this induces local inflammation and contributes to beta cell apoptosis [10–12]. In line with these results, we observed that the decrease in STAT1 and proinflammatory chemokine expression in DEXI-inhibited cells is preceded by a decrease in *IFNβ* expression. Indeed, we already detected a decrease in *IFNβ* expression in DEXI-inhibited cells after 4 h of PIC transfection; this is 4 h before observing any increase in STAT1 phosphorylation and proinflammatory chemokine production, which starts after 8 h of PIC transfection.

Type I IFNs, including IFNβ, are important mediators of antiviral responses that have been implicated in the early stages of development of type 1 diabetes. Several studies have demonstrated the presence of type I IFNs in the islets and blood of individuals with type 1 diabetes and their relationship to the presence of Coxsackievirus B infection [3, 40, 41]. An involvement of type I IFNs in the pathogenesis of type 1 diabetes is also supported by studies in NOD mice, where type I IFNs play a crucial role in the initiation or acceleration of the autoimmune process [42]. Moreover, therapy with type I IFNs has been shown to lead to the development of type 1 diabetes in individuals with hepatitis C, multiple sclerosis or melanoma [43–45], supporting the idea that type I IFNs may be implicated in the development of type 1 diabetes.

Our results demonstrate that DEXI participates in the transcriptional activation of IFNβ upon viral infection of beta cells. Protein sequence analysis suggests that DEXI has a leucine zipper motif in its C-terminal [21]. The leucine zipper motif contains a predicted kinase

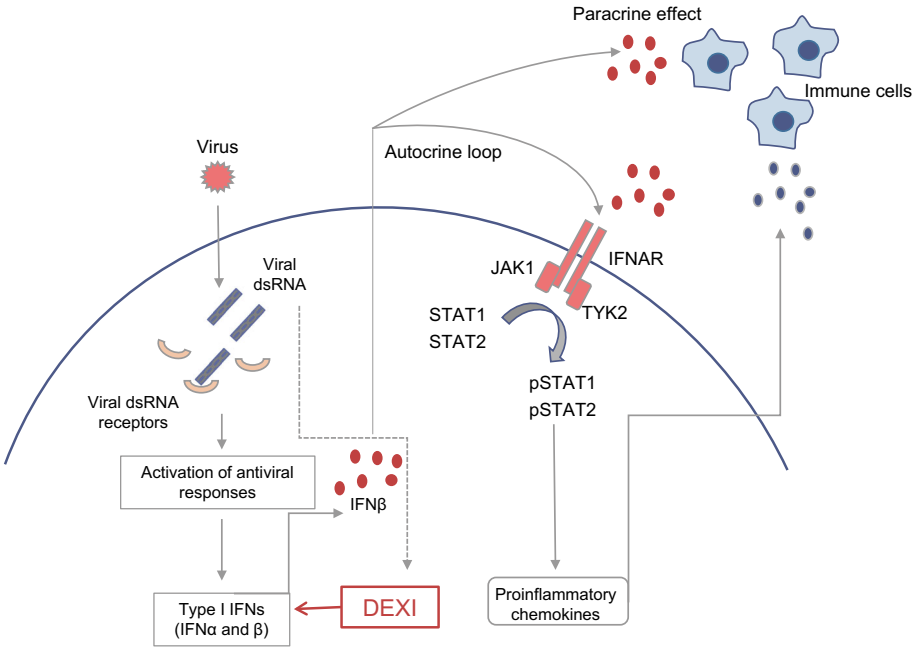



**Fig. 7** A model of the role of DEXI in the modulation of virus-induced proinflammatory pathways in pancreatic beta cells. On recognition of viral dsRNA by cytoplasmic receptors, the antiviral response is activated in pancreatic beta cells, leading to the production of type I IFNs. Viral dsRNA upregulates the production of chromatin-bound DEXI, which acts as a transcriptional activator of *IFNβ* expression. Together with other proinflammatory mediators (e.g. chemokines), IFNβ is released by beta cells and participates in the attraction of immune cells. At the same time, IFNβ acts in an autocrine manner, activating the STAT signalling pathway and thus promoting the production of proinflammatory chemokines and increasing local inflammation. In genetically susceptible individuals, type 1 diabetes-associated SNPs may affect the function of DEXI, leading to an excessive inflammatory response to viral infections that may contribute to local inflammation and eventual beta cell destruction. IFNAR, interferon α/β receptor; TYK2, tyrosine kinase 2

phosphorylation site, suggesting that interactions of DEXI with itself or other proteins may be regulated via phosphorylation [21]. We observed that DEXI is bound to chromatin, and that the amount of chromatin-associated DEXI is augmented after 4 h of PIC transfection, supporting the potential role of DEXI as a transcriptional regulator activated in response to 'danger signals' in beta cells. Further studies are needed to clarify the mechanisms by which DEXI regulates IFNβ levels and to determine whether DEXI acts as a transcriptional regulator by itself or in combination with other proteins regulating gene expression.

In summary, the present study provides evidence that the protein encoded by the type 1 diabetes candidate gene *DEXI* participates in the activation of the local antiviral immune response through regulation of the type I IFN–STAT signalling pathway in pancreatic beta cells (Fig. 7). Our findings support *DEXI* as the aetiological gene for type 1 diabetes on chromosome 16p13 and provide initial insights into the mechanisms involved. Further molecular characterisation of the interactions between predisposing genes and environmental triggers will allow a clarification of the mechanisms by which a viral infection or other danger signal leads to autoimmunity and type 1 diabetes in genetically susceptible individuals.

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**Data availability** All the data generated during this study have been included in this article. The data regarding RNAseq of human islets have been previously published in Eizirik et al [26] and are available from D. L. Eizirik.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

**Contribution statement** RSS, LM, TV, AOG, AJM and ACR researched data, and revised and edited the manuscript. DLE contributed to the design and interpretation of the experiments, and to discussion, and wrote, revised and edited the manuscript. LC contributed to interpretation of the experiments and discussion, and revised and edited the manuscript. IS contributed to the original idea, design and interpretation of experiments, researched data, contributed to discussion, and wrote, revised and edited the manuscript. All authors have read and approved the manuscript and given informed consent. IS the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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