PTPN2, a Candidate Gene for Type 1 Diabetes, Modulates Pancreatic β-Cell Apoptosis via Regulation of the BH3-Only Protein Bim

Izortze Santin,[1] Fabrice Moore,[1] Maikel L. Colli,[1] Esteban N. Gurzov,[1] Lorella Marselli,[2] Piero Marchetti,[2] and Decio L. Eizirik[1]

OBJECTIVE—Genome-wide association studies allowed the identification of several associations between specific loci and type 1 diabetes (T1D). However, the mechanisms by which most candidate genes predispose to T1D remain unclear. We presently evaluated the mechanisms by which *PTPN2*, a candidate gene for T1D, modulates β-cell apoptosis after exposure to type I and II interferons (IFNs), cytokines that contribute to β-cell loss in early T1D.

RESEARCH DESIGN AND METHODS—Small interfering RNAs were used to inhibit PTPN2, STAT1, Bim, and Jun NH_2-terminal kinase 1 (JNK1) expression. Cell death was assessed by Hoechst and propidium iodide staining. BAX translocation, Bim phosphorylation, cytochrome c release, and caspases 9 and 3 activation were measured by Western blot or immunofluorescence.

RESULTS—PTPN2 knockdown exacerbated type I IFN–induced apoptosis in INS-1E, primary rat, and human β-cells. PTPN2 silencing and exposure to type I and II IFNs induced BAX translocation to the mitochondria, cytochrome c release, and caspase 3 activation. There was also an increase in Bim phosphorylation that was at least in part regulated by JNK1. Of note, both Bim and JNK1 knockdown protected β-cells against IFN-induced apoptosis in PTPN2-silenced cells.

CONCLUSIONS—The present findings suggest that local IFN production may interact with a genetic factor (*PTPN2*) to induce aberrant proapoptotic activity of the BH3-only protein Bim, resulting in increased β-cell apoptosis via JNK activation and the intrinsic apoptotic pathway. This is the first indication of a direct interaction between a candidate gene for T1D and the activation of a specific downstream proapoptotic pathway in β-cells. ***Diabetes*** **60:3279–3288, 2011**

Type 1 diabetes (T1D) is a chronic autoimmune disease during which pancreatic β-cells are specifically damaged by an aberrant immune response. Susceptibility to T1D is linked to genetic factors, but T1D-predisposing genes have low penetrance and only a small proportion of individuals genetically at risk will develop the disease. During the past few years, genome-wide association studies allowed the identification of a large number of robust associations between specific chromosomal loci and T1D development (1). Well-known susceptibility genes include *HLA-DR*, *CTLA-4*, *IFIH1* (*MDA5*), and *PTPN22* (2). However, these genes only account for part of interindividual differences in disease predisposition or phenotypic diversity, and the pathophysiologic mechanisms by which most candidate genes predispose to T1D remain unclear.

It is likely that an interplay between T1D susceptibility genes and environmental factors contributes to the triggering and progression of the disease (3). In this context, a better understanding of the functional effects of the susceptibility genes and their interaction with putative environmental causalities would help to understand the pathogenesis of T1D (4). In animal models for other autoimmune diseases (e.g., Crohn's disease), there is a striking interaction between a mutation in the *Atg16L1* candidate gene and the Murine norovirus, resulting in pathologic abnormalities similar to Crohn's disease (5). In the context of T1D, a single nucleotide polymorphism (SNP) in the susceptibility gene *PTPN22* and early introduction of cow's milk in the diet are associated with the induction of islet autoantibodies and diabetes development in the Finnish population (6).

Approximately 30% of the T1D candidate genes are expressed in β-cells (7) (M.L.C., F.M., D.L.E., unpublished data), suggesting that β-cells play a role in their own demise in T1D. The reduction of β-cell mass in T1D is preceded by an inflammatory process (insulitis) driven in part by a "dialog" between β-cells and infiltrating immune cells, mediated by the local release of cytokines and chemokines (8). Viruses are potential environmental factors contributing to the triggering of insulitis (9,10). During viral infections, β-cells release several chemokines and cytokines, including type I interferons (IFNs) (IFNα and IFNβ) (7,11), which contribute to T1D pathogenesis (12,13). In this respect, the T1D candidate gene *IFIH1* (*MDA5*) is involved in the recognition of double-stranded RNA (dsRNA), a by-product of viral replication (14), and we previously observed that knocking down MDA5 in pancreatic β-cells prevents dsRNA-induced expression of key cytokines and chemokines (7).

PTPN2 (also known as *TC-PTP* or *PTP-S2*) is another candidate gene for T1D (1). Known risk alleles for T1D in the *PTPN2* gene are noncoding, and noncoding variants that may affect splicing have been identified by resequencing (1). One T1D risk variant of *PTPN2* is associated with decreased PTPN2 expression in $CD4^+$ T cells and transformed B-cell lines (15). The *PTPN2* gene encodes a phosphatase that is ubiquitously expressed (16). The cytokines IFNγ and tumor necrosis factor-α increase PTPN2 expression in human colonic intraepithelial cells, and an upregulation of PTPN2 expression has been observed in

From the [1]Laboratory of Experimental Medicine, Université Libre de Bruxelles, Brussels, Belgium; and the [2]Department of Endocrinology and Metabolism, Metabolic Unit, University of Pisa, Pisa, Italy.
Corresponding author: Decio L. Eizirik, deizirik@ulb.ac.be.
Received 5 June 2011 and accepted 31 August 2011.
DOI: 10.2337/db11-0758
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-0758/-/DC1.

intestinal biopsies from patients with active celiac disease (17,18). PTPN2 is highly expressed in immune-related cells, and its expression is modified in $CD4^+$ T cells from patients with T1D when compared with $CD4^+$ T cells from healthy control subjects (15).

PTPN2 is an important negative regulator of the Janus kinase-STAT signaling pathway that is activated downstream of type I (IFNα and IFNβ) and II (IFNγ) IFN receptors. We recently described that this phosphatase is induced by IFNγ and a synthetic dsRNA, polyinosinic-polycitidilic acid (PIC), in β-cells and exacerbates IFNγ- and PIC-induced β-cell apoptosis by modulating STAT1 activation (7,19). However, the mechanisms connecting this candidate gene to actual β-cell death remain unclear.

We presently observed that PTPN2 also regulates type I IFN–induced apoptosis in β-cells. By systematically knocking down genes putatively involved in the apoptosis pathway of β-cells (20), we identified the apoptotic mechanisms by which PTPN2 knockdown exacerbates type I and II IFN–induced β-cell death. This clarifies the interaction between a candidate gene for T1D and the activation of specific proapoptotic pathways in β-cells and broadens our understanding of the molecular mechanisms involved in the gene/environment interactions triggering insulitis and β-cell apoptosis.

RESEARCH DESIGN AND METHODS

Culture of primary fluorescence-activated cell sorting–purified rat β-cells, human islets, and INS-1E cells. Rat islets were isolated by collagenase digestion and handpicked under a stereomicroscope, and β-cells were fluorescence-activated cell sorting (FACS)–purified and cultured as previously described (11,19). The β-cell preparations used in this study contained 96 ± 2% β-cells (n = 12). For cytokine treatment, cells were cultured in the medium without serum. For small interfering (si)RNA transfection, BSA- and antibiotic-free medium was used.

Human islets were isolated from four nondiabetic organ donors (age 70 ± 3 years; BMI 25.7 ± 0.3 kg/m^2) in Pisa, Italy, with the approval of the local ethics committee. Islets were isolated by enzymatic digestion and density-gradient purification (21), and cultured in M199 medium containing 5.5 mmol/L glucose. The human islets were shipped to Brussels within 1–5 days of isolation. After overnight recovery in Ham's F-10 containing 6.1 mmol/L glucose, 10% FBS, 2 mmol/L GlutaMAX, 50 μmol/L 3-isobutyl-1-methylxanthine, 1% BSA, 50 units/mL penicillin, and 50 μg/mL streptomycin, islets were dispersed for treatment and viability assays as previously described (19). The percentage of β-cells, examined in the four dispersed islet preparations by staining with anti-insulin antibody (1:1,000; Sigma-Aldrich, Bornem, Belgium) and donkey anti-mouse IgG rhodamine (1:200; Lucron Bioproducts, De Pinte, Belgium), was 71 ± 2%.

The INS-1E cell line (a gift from Dr. C. Wollheim, Centre Medical Universitaire, Geneva, Switzerland) was cultured in Roswell Park Memorial Institute 1640 GlutaMAX-I (22).

RNA interference. The siRNAs used in the study are described in Supplementary Table 1. The optimal concentration of siRNA used for cell transfection (30 nM) was established previously (19). Cells were transfected using the Lipofectamine 2000 lipid reagent (Invitrogen, Carlsbad, CA) as previously described (23). For dose-response experiments, the following concentrations of the siRNA targeting *PTPN2* gene were used: 1, 5, 10, and 30 nM. After transfection, cells were cultured for a 48-h recovery period and subsequently exposed to cytokines. All siRNAs used in the current study have been validated for use in rodent and human cells, leading to 60–80% inhibition of the target genes (7,19,24) (J. Barthson, E.N.G., D.L.E., unpublished data).

Cell treatment, dsRNA transfection, and assessment of cell viability. The following cytokine concentrations were used on the basis of dose-response experiments previously performed by our group (11) (I.S., F.M., D.L.E. unpublished data): recombinant rat IFNα (specific activity: 1×10^8 units/mg; PBL Biomedical Laboratories, Piscataway, NJ) at 1,000 units/mL and human IFNα (specific activity: 1.8×10^8 units/mg; PeproTech Inc., Rocky Hill, NJ) at 2,000 units/mL, rat IFNβ at 1,000 units/mL (specific activity: 8×10^7 units/mg; PBL Biomedical Laboratories), and IFNγ at 100 units/mL and 1,000 units/mL for rat and human (specific activity: 2×10^7 units/mg; R&D Systems, Abingdon, U.K.). The synthetic dsRNA analog PIC (Invivogen, San Diego, CA) was used at the final concentration of 1 μg/mL. PIC transfection was performed in the same conditions described for siRNA, but using 0.15 μL Lipofectamine 2000 per condition (7,11).

The percentage of living, apoptotic, and necrotic cells was determined using the DNA-binding dyes propidium iodide (PI, 5 μg/mL; Sigma-Aldrich, Bornem, Belgium) and Hoechst dye 33342 (HO, 5 μg/mL; Sigma-Aldrich) as described (7,19,25). Results are expressed as percentages (i.e., number of apoptotic cells/total number of cells × 100). Apoptosis was confirmed by Western blot analysis of cleaved caspases 9 and 3 and cytochrome c release as previously described (26,27).

Promoter studies and determination of protein and mRNA expression. For promoter studies, INS-1E cells were plated in 24-well plates and transfected with siRNAs as mentioned above. After 12 h of recovery, cells were cotransfected with pRL-CMV encoding *Renilla* luciferase (Promega, Madison, WI) and a firefly luciferase promoter-reporter construct containing three IFNγ-activated sequence (GAS) consensus sequences (GAS reporter–LR0075; Panomics, Fremont, CA) or four IFN-stimulated response element (ISRE) consensus sequences (ISRE reporter–LR0040). After 24 h of recovery and 4 h of treatment with IFNα or IFNγ, 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 (28).

Immunofluorescence, Western blot analysis, mRNA extraction, and real-time PCR were performed as described (27,29). The antibodies used in Western blot analysis are described in Supplementary Table 2.

Statistical analysis. Data are presented as means ± SEM. Comparisons were performed by ANOVA followed by Student t test with Bonferroni correction or by paired t test as indicated. A P value <0.05 was considered statistically significant.

RESULTS

PTPN2 silencing increases type I IFN–induced apoptosis in INS-1E cells and primary β-cells. INS-1E cells were left untransfected or transfected with a control siRNA (siCTRL) or an siRNA targeting PTPN2 (siPTPN2), previously shown to efficiently inhibit PTPN2 expression (19). Cells were subsequently treated for 24 h with IFNα or IFNβ (Fig. 1*A*). None of the treatments significantly affected cell viability in nontransfected or siCTRL-transfected cells, whereas PTPN2 inhibition exacerbated apoptotic cell death in all IFN-treated cells (Fig. 1*A*). Additional experiments using a higher concentration of IFNα and IFNβ (2,000 units/mL) confirmed the proapoptotic activity of siPTPN2 (Supplementary Fig. 1*A*), and the concentration of 1,000 units/mL was chosen for subsequent experiments in INS-1E cells and primary rat β-cells. Because the effects of IFNα and IFNβ were similar (Fig. 1), most subsequent experiments were performed with IFNα. Similar results were observed in primary rat β-cells (Fig. 1*B*). Thus, inhibition of PTPN2 by two different siRNAs (siPTPN2 and siPTPN2#1) increased apoptosis after a 48-h treatment with type I or II IFNs. Of note, the necrotic component of cell death was negligible (<2% of cells, data not shown) in all experiments.

We next performed viability experiments using different concentrations of siPTPN2 (1, 5, 10, and 30 nM). PTPN2 expression gradually decreased with increasing concentrations of siPTPN2 (Supplementary Fig. 1*B*), whereas IFNα- and IFNγ-induced β-cell apoptosis progressively increased (Supplementary Fig. 1*C*).

During viral infections, β-cells release type I IFNs (7,11) that act in an autocrine/paracrine fashion through the type I IFN receptor (IFNAR). To evaluate the potential role of IFNAR in PIC-induced apoptosis, INS-1E cells were transfected with siRNAs targeting PTPN2, IFNAR1, or PTPN2 and IFNAR1 and subsequently transfected with PIC (Fig. 1*C*). Inhibition of PTPN2 increased PIC-induced apoptosis. IFNAR silencing decreased PIC-induced cell death, whereas the double knockdown of PTPN2 and IFNAR abolished the exacerbating effect of PTPN2 inhibition on PIC-induced apoptosis (Fig. 1*C*). These data suggest that PIC-induced β-cell apoptosis in PTPN2-silenced cells is at

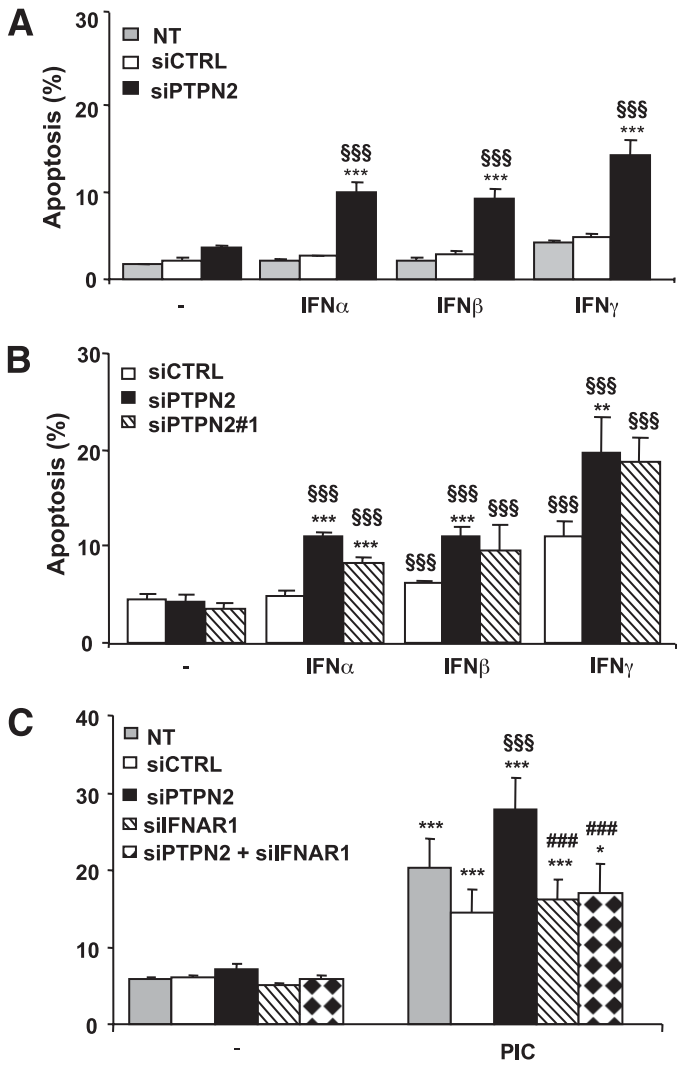

FIG. 1. PTPN2 inhibition exacerbates type I and II IFN–induced apoptosis in INS-1E cells and primary rat β-cells. *A*: INS-1E cells were left untransfected (NT) (gray bars) or transfected with siCTRL (white bars) or siPTPN2 (black bars). After 48 h of recovery, cells were left untreated (-) or treated for 24 h with IFNα (1,000 units/mL), IFNβ (1,000 units/mL), or IFNγ (100 units/mL) as indicated. Apoptosis was evaluated using Hoechst and propidium iodide staining. Results are means ± SEM of four independent experiments; §§§*P* < 0.001 vs. untreated (i.e., not treated with cytokines) siPTPN2; ***P* < 0.001 vs. NT or siCTRL treated with the same cytokine; ANOVA. *B*: Primary rat β-cells were treated as in *A*, but cells were exposed to cytokines for 48 h and two independent siRNAs against PTPN2 were used. Results are means ± SEM of three to five independent experiments; §§§*P* < 0.001 vs. untreated (i.e., not treated with cytokines) siCTRL or siPTPN2; ***P* < 0.01 and ****P* < 0.001 vs. siCTRL treated with the same cytokine; ANOVA. *C*: INS-1E cells were left NT or transfected with siCTRL, siPTPN2, siRNA against IFNα/β receptor α chain, or a combination of siPTPN2 and siRNA against IFNα/β receptor α chain. After 48 h of recovery, cells were left NT (-) or transfected with PIC as described in RESEARCH DESIGN AND METHODS. Results are means ± SEM of five independent experiments; §§§*P* < 0.001 vs. NT + PIC, **P* < 0.05 and ****P* < 0.001 vs. untreated (i.e., not transfected with PIC) transfected with the same siRNA; ###*P* < 0.001 vs. siPTPN2 + PIC; ANOVA.

least partially mediated by type I IFNs that are released by the β-cells themselves in response to the synthetic dsRNA.

PTPN2 inhibition increases IFNα- and IFNγ-induced apoptosis via STAT1 activation. Type I IFNs preferentially induce STAT1/2 heterodimers that activate ISREs, whereas type II IFNs favor the activation of STAT1 homodimers that bind to the GAS (30). Inhibition of PTPN2 in INS-1E cells significantly increased IFNα-induced ISRE reporter activity (1.7-fold), whereas IFNγ stimulation led to poor ISRE reporter activation, even after PTPN2 silencing (Fig. 2*A*). The GAS reporter activity was increased in untransfected and siCTRL-transfected INS-1E cells after IFNγ treatment, and PTPN2 silencing further exacerbated activation of the GAS reporter (twofold induction), whereas IFNα did not modify GAS reporter activity (Fig. 2*B*). Double transfection of PTPN2- and STAT1-targeting siRNAs potently inhibit both target proteins (19), which was confirmed in the present series of experiments (data not shown). PTPN2 knockdown significantly increased IFNα- and IFNγ-induced apoptosis, whereas double knockdown of PTPN2 and STAT1 prevented the proapoptotic effect of PTPN2 inhibition in INS-1E cells exposed to IFNα or IFNγ (Fig. 2*C*). These results suggest that STAT1 activation plays a key role in both type I and II IFN–induced β-cell apoptosis in PTPN2-deficient cells.

PTPN2 inhibition exacerbates type I and II IFN–induced β-cell apoptosis through the intrinsic mitochondrial pathway. To clarify the death pathways by which PTPN2 deficiency exacerbates type I and II IFN–induced cell death, we first analyzed genes induced by the endoplasmic reticulum stress response, previously shown to be associated with interleukin (IL)-1β +IFNγ-induced apoptosis of β-cells (31). The mRNA expression of C/EBP homologous protein (CHOP), immunoglobulin heavy chain-binding protein (BIP), or X-box binding protein 1 spliced (XBP-1s) was not induced in siPTPN2-transfected INS-1E cells after 24 h of treatment with IFNα or IFNγ compared with siCTRL (Supplementary Fig. 2). There were also no changes in BIP expression after 2, 4, 8, and 16 h of treatment with IFNα or IFNγ and siPTPN2 exposure (data not shown), making it unlikely that endoplasmic reticulum stress response contributes to IFN-induced apoptosis after PTPN2 knockdown.

We next examined the intrinsic mitochondrial pathway of apoptosis, because IFNγ activates this cell death pathway when associated with the proinflammatory cytokines IL-1β or tumor necrosis factor-α (25,27). Immunofluorescence analysis of INS-1E cells in which PTPN2 was silenced demonstrated diffuse BCL2-associated X protein (BAX) staining in untreated cells (Fig. 3, *top*), whereas it colocalized with the mitochondrial marker ATP synthase after 16 h of treatment with IFNα (Fig. 3, *middle*) or IFNγ (Fig. 3, *bottom*), demonstrating that BAX translocates to the mitochondria in siPTPN2-silenced cells. In line with our initial viability experiments (Fig. 1), fewer apoptotic cells were observed in untreated or IFN-treated siCTRL-transfected cells (data not shown).

Western blot evaluation of mitochondrial and cytoplasmic fractions from siCTRL- and siPTPN2-transfected INS-1E cells demonstrated that IFNα and IFNγ induced mitochondrial cytochrome c release to the cytoplasm in PTPN2-deficient cells but not in siCTRL-transfected cells (Fig. 4*A* and *B*). In line with these findings, PTPN2 knockdown also induced caspase 3 activation after 16 and 24 h of IFNα and IFNγ treatment (Fig. 4*C* and *D*). Taken together, the data from Figures 3 and 4 confirm the results observed in viability assays (Fig. 1) and indicate that apoptosis occurs mainly through the mitochondria-driven intrinsic pathway of cell death in IFN-treated PTPN2-deficient cells.

The BH3-only protein Bim mediates β-cell death in PTPN2-silenced cells. The members of the B-cell lymphoma 2 (BCL-2) family, death protein 5 (25), BCL2 binding component 3 (p53 up-regulated modulator of apoptosis [PUMA]) (27), and BCL2-like 11 (Bim) (32), have been shown to contribute to β-cell apoptosis after exposure to cytokines (IL-1β + IFNγ) or metabolic stress. IFNα or IFNγ

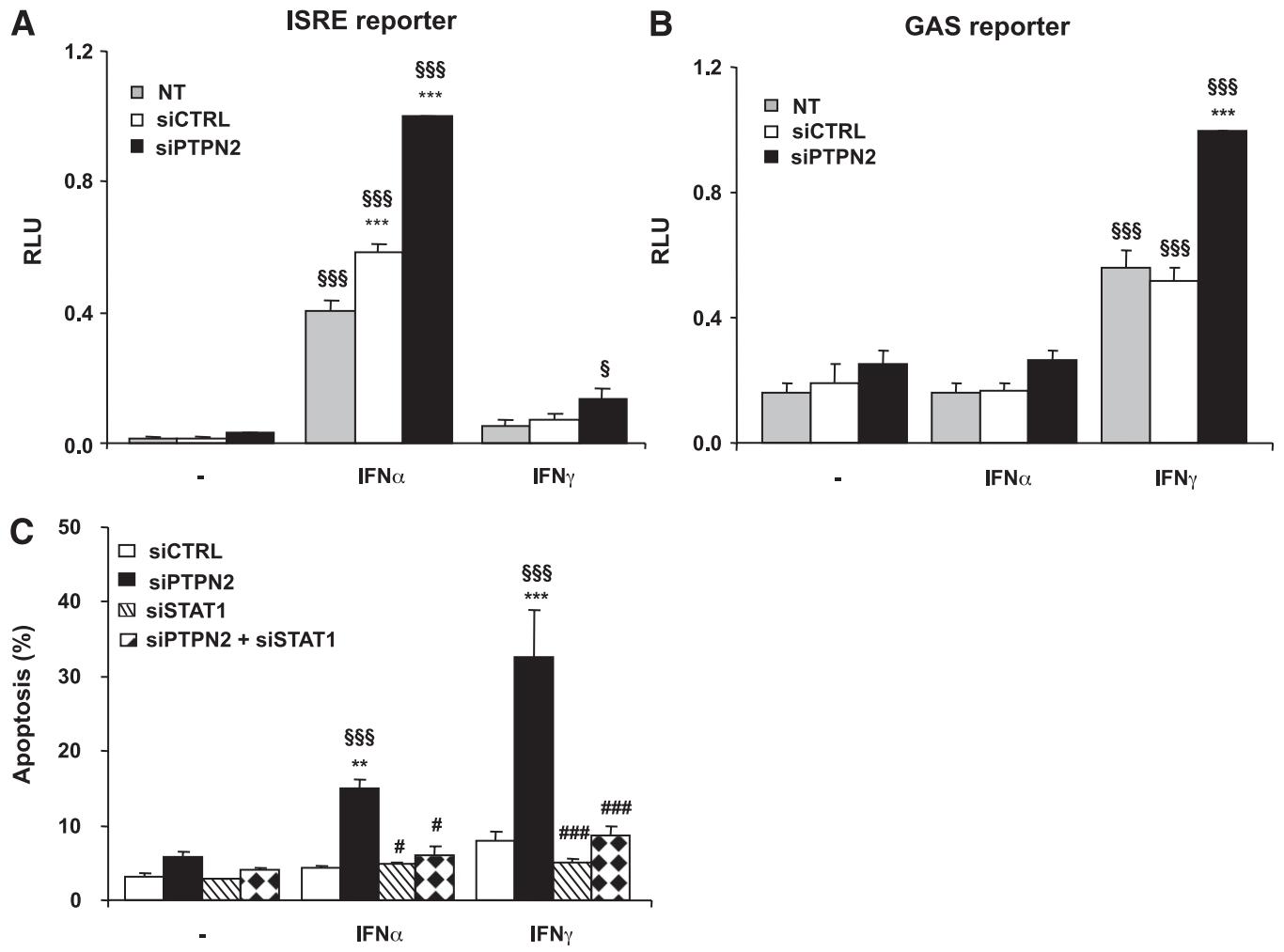

FIG. 2. Inhibition of PTPN2 increases ISRE and GAS reporter activity after IFNα or IFNγ treatment and favors apoptosis via STAT1 activation. Twelve hours after transfection with siCTRL or siPTPN2, cells were cotransfected with an ISRE (*A*) or GAS reporter construct (*B*) plus a pRL-CMV plasmid (used as internal control); cells were then treated with IFNα or IFNγ for 4 h, and luciferase activity was measured. Results are mean ± SEM of four to seven independent experiments shown as relative light units; §*P* < 0.05 and §§§*P* < 0.001 vs. untreated (i.e., not treated with cytokines) transfected with the same siRNA; ***P* < 0.001 vs. untransfected (NT) or siCTRL treated with the same cytokine; ANOVA. *C*: INS-1E cells were transfected with siCTRL, siPTPN2, siSTAT1, or the combination of siPTPN2 and siSTAT1. After 48 h of recovery, cells were left untreated or treated for 24 h with IFNα (1,000 units/mL) or IFNγ (100 units/mL) as indicated. Apoptosis was evaluated using Hoechst and propidium iodide staining. Results are means ± SEM of three to five independent experiments; §§§*P* < 0.001 vs. untreated (i.e., not treated with cytokines) siPTPN2; **P* < 0.01 and ***P* < 0.001 vs. siCTRL treated with the same cytokine; #*P* < 0.05 and ###*P* < 0.001 vs. siPTPN2 treated with the same cytokine; ANOVA. RLU, relative light units.

treatment induced death protein 5 and p53 upregulated modulator of apoptosis expressions over time in the treated cells, but there were no significant differences between siCTRL- and siPTPN2-transfected cells (Supplementary Fig. 3). On the other hand, cytokine-induced Bim mRNA upregulation was exacerbated in PTPN2-deficient INS-1E cells after 16–24 h of IFNα or IFNγ treatment compared with their respective controls (Fig. 5*A* and *B*). However, there was not a significant modulation of Bim protein expression in siCTRL and siPTPN2-transfected cells after treatment with IFNα (Fig. 5*C*) or IFNγ (Fig. 5*D*).

Bim activity may be controlled by posttranscriptional phosphorylation at multiple serine and threonine residues (33). PTPN2 inhibition increased Bim phosphorylation at residue 65 in untreated INS-1E cells, and this effect was prolonged until 4 h of treatment with IFNα or 8 h after treatment with IFNγ (Fig. 5*E*). Similar phosphorylation levels were observed in CTRL and PTPN2-deficient cells after 16 h of treatment of IFNα or IFNγ. These results suggest that Bim phosphorylation is increased in PTPN2-silenced cells, which may render these cells more sensitive to the proapoptotic effects of IFNs.

To assess whether Bim indeed contributes to type I and II IFN–induced β-cell death in PTPN2-inhibited cells, we silenced PTPN2 and Bim in a double-knockdown approach. As previously shown (Fig. 1), inhibition of PTPN2 significantly exacerbated INS-1E cell apoptosis after treatment with IFNα, IFNβ, or IFNγ (Fig. 6*A*). This effect was counteracted by Bim knockdown, which protected INS-1E cells against IFNα-, IFNβ-, and IFNγ-induced apoptosis in PTPN2-silenced cells by 58, 67, and 78%, respectively (Fig. 6*A*). These observations were confirmed in FACS-purified rat primary β-cells (Fig. 6*B*), in which Bim inhibition again reversed the exacerbating effect of PTPN2 knockdown, with a 59 and 63% reduction of cell apoptosis after IFNα and IFNγ treatment, respectively. Similar observations were made in dispersed human islet cells (Fig. 6*C*). Thus, PTPN2 knockdown exacerbated IFN-induced cell

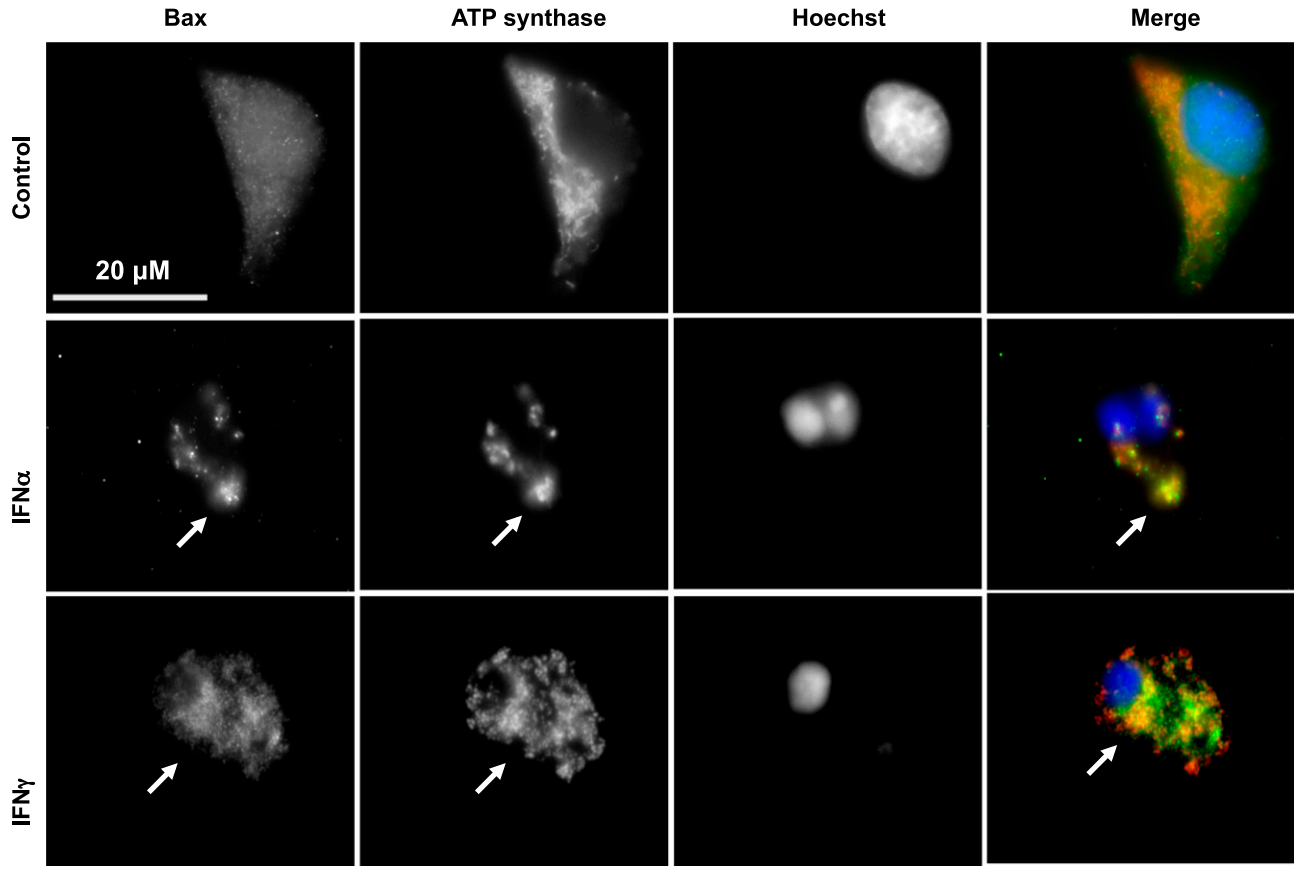

FIG. 3. PTPN2 inhibition increases IFN-induced β-cell death via BAX activation. Cells were transfected with an siPTPN2, and after 48 h of recovery they were left untreated (control) or treated for 16 h with IFNα or IFNγ. For immunofluorescence analysis, they were incubated with antibodies specific for BAX, used as a mitochondrial marker ATP synthase, and stained with HO for nuclear morphology evaluation. Arrows indicate mitochondrial localization of BAX. The figure is representative of three independent experiments. (A high-quality digital representation of this figure is available in the online issue.)

death and Bim knockdown reversed the deleterious effect of PTPN2 knockdown, with a reduction of cell death nearly to basal levels (Fig. 6*C*).

The protective effects of Bim knockdown were confirmed by Western blot analysis of caspases 9 and 3 activation in INS-1E cells. As shown in Figure 6*D–F*, PTPN2 knockdown exacerbated caspases 9 and 3 activation in IFNα-, IFNβ-, and IFNγ-treated INS-1E cells, whereas the concomitant inhibition of PTPN2 and Bim abrogated this deleterious effect (Fig. 6*E* and *F*). As a whole, these results suggest that Bim plays a central role in the exacerbation of IFN-induced β-cell apoptosis in PTPN2-deficient cells.

To assess whether the effect of PTPN2 on Bim phosphorylation was mediated by Jun NH_2-terminal kinase 1 (JNK1), we performed a double-knockdown approach, targeting PTPN2 and JNK1 and evaluating Bim phosphorylation by Western blot in INS-1E cells. As previously shown (Fig. 5*E*), PTPN2 knockdown increased Bim phosphorylation mainly in the nontreated condition (Fig. 7*A* and *B*), although this effect was also evident after 2 h of treatment with IFNα (Fig. 7*A*) or IFNγ (Fig. 7*B*). After 24 h of treatment with IFNα (Fig. 7*A*) or IFNγ (Fig. 7*B*), the phosphorylation level observed in CTRL and PTPN2-deficient cells was similar. After JNK1 knockdown or double knockdown of PTPN2 and JNK1, Bim phosphorylation was decreased in both the nontreated condition (Fig. 7*A* and *B*) and after 2 or 24 h of IFNα (Fig. 7*A*) or IFNγ (Fig. 7*B*) treatment. These results indicate that the protein kinase JNK1 is in part responsible for the hyperphosphorylation of Bim at serine 65 (Ser65) in PTPN2-silenced β-cells.

We next silenced PTPN2 and JNK1 in a double-knockdown approach to test whether the observed decrease in Bim phosphorylation inhibited IFN-induced apoptosis in PTPN2-silenced cells. As shown in Figure 7*C*, JNK1 knockdown counteracted the increase in cell death in PTPN2-silenced INS-1E cells after 24 h of treatment with IFNα or IFNγ. These results were confirmed in FACS-purified rat primary β-cells, in which double knockdown of PTPN2 and JNK1 partially prevented the increase in IFN-induced cell death in the presence of PTPN2 inhibition (Fig. 7*D*).

The protective effects of JNK1 knockdown were confirmed by Western blot analysis of cleaved caspase 3 in INS-1E cells (Fig. 7*A* and *B*): PTPN2 inhibition increased cleaved caspase 3 in IFNα- and IFNγ-treated cells, whereas the double knockdown of PTPN2 and JNK1 revoked this effect.

DISCUSSION

The current study shows that inhibition of the T1D candidate gene *PTPN2* sensitizes pancreatic β-cells to apoptosis induced by both type I and II IFNs. We further demonstrate that blocking IFNAR abolishes the exacerbation of apoptosis induced by the viral dsRNA analog PIC

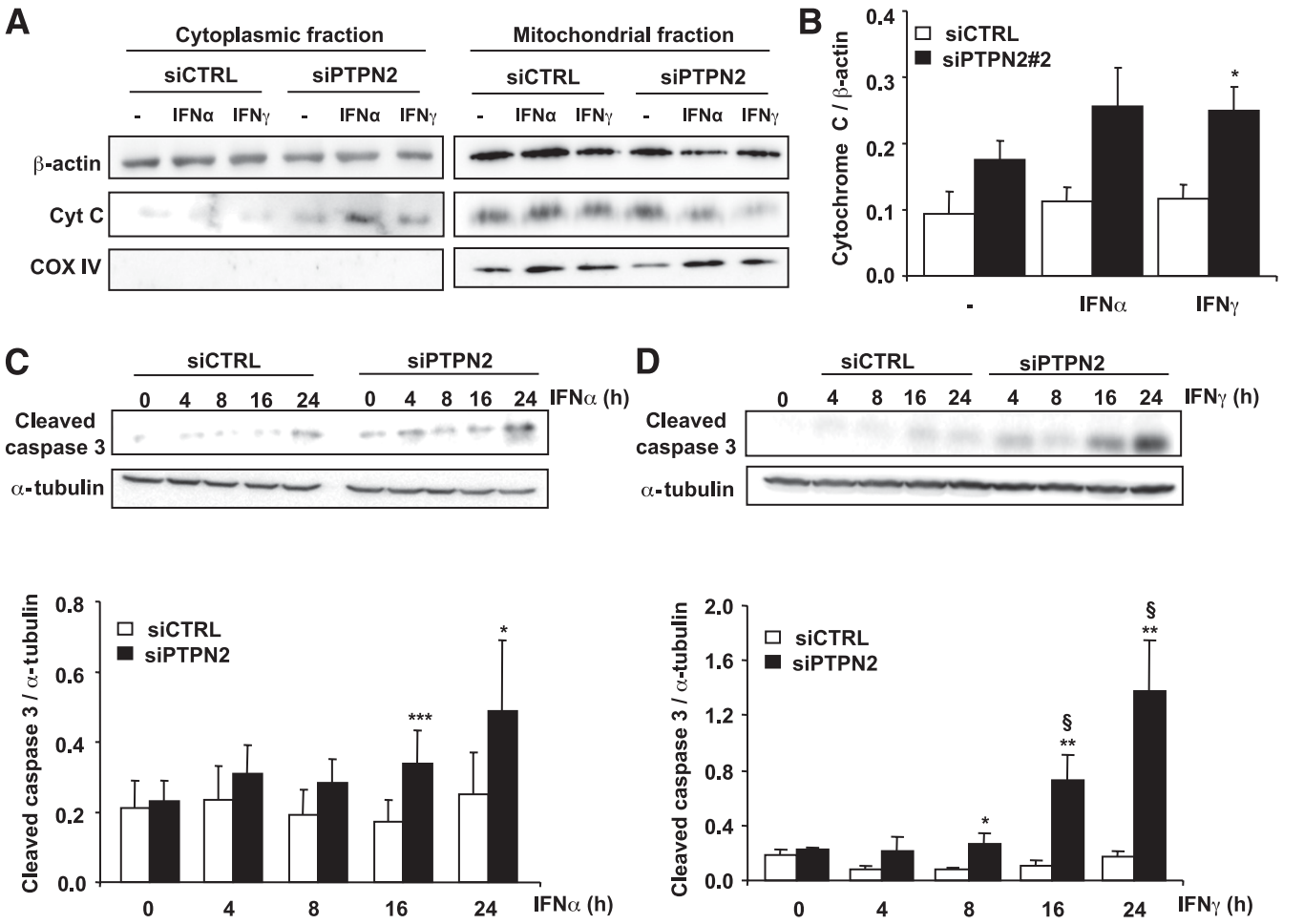

FIG. 4. Inhibition of PTPN2 in INS-1E cells induces cytochrome c release to the cytoplasm and activation of the effector caspase 3 after IFN stimulation. *A*: Cells were transfected with siCTRL or siPTPN2, and after 48 h of recovery they were exposed to IFNα or IFNγ. Cytochrome c release from the mitochondria to the cytoplasm was analyzed by Western blot. β-Actin was used as loading control, and cyclooxygenase IV was used as mitochondrial marker. *B*: Mean optical densitometry measurement of cytochrome c in the cytoplasmic fraction. Results are mean ± SEM of five independent experiments; **P* < 0.05 vs. siCTRL treated with the same cytokine; ANOVA. *C* and *D*: Cells transfected with siCTRL or siPTPN2 were exposed to IFNα (*C*) or IFNγ (*D*) for 4, 8, 16, or 24 h, and expression of cleaved caspase 3 protein was measured by Western blot. Cleaved caspase 3 values are corrected for protein loading by α-tubulin determination, and densitometry results are represented as the mean ± SEM of three to six independent experiments; §*P* < 0.05 vs. untreated (i.e., not treated with cytokines) transfected with the siRNA; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 vs. siCTRL treated at the same time point with IFNα or IFNγ; ANOVA.

in PTPN2-silenced cells. dsRNA is produced in the cytosol of infected cells as a by-product of viral replication. This suggests that type I IFNs released by β-cells in response to a viral insult play an important role in cell death. This is in line with previous observations suggesting that intracellular dsRNA induces type I IFN release by β-cells, which contributes to cell death (7,11).

Silencing of PTPN2 increases ISRE and GAS reporter activity after IFN treatment, and double knockdown of PTPN2 and STAT1 reverses the proapoptotic effect of PTPN2 inhibition. PTPN2 silencing induces BAX translocation to the mitochondria, cytochrome c release to the cytosol, and caspase 3 activation after exposure to type I and II IFNs, characterizing activation of the intrinsic mitochondrial pathway of cell death. This process might be secondary to hyperphosphorylation of Bim in PTPN2-silenced cells via modulation of the protein kinase JNK1. In line with this hypothesis, both Bim and JNK1 knockdown protects β-cells against IFN-induced apoptosis in PTPN2-silenced cells. The key role of Bim was confirmed in human islet cells. This suggests that local IFN production may interact with a genetic factor (*PTPN2*) to induce aberrant proapoptotic activity of the BH3-only protein Bim, resulting in increased β-cell apoptosis.

Viruses are one of the putative environmental factors associated with T1D triggering in genetically predisposed individuals; several epidemiologic studies in humans and in animal models support this association (34). Thus, it was recently reported that enterovirus-positive blood samples are more frequent among subjects with T1D than among healthy individuals (35), and histologic studies of pancreas from deceased patients with T1D indicate a higher prevalence of Coxsackievirus B4 than in pancreas from control individuals (9,36). Type I IFNs are important mediators of the immune response against viral infections, and several studies demonstrated expression of type I IFNs in the islets and blood of individuals with T1D (37,38) and their role in the initiation or the acceleration of the autoimmune process in NOD mice (39). The present results indicate a potential link between a candidate gene for T1D (*PTPN2*) and the effects of type I IFNs in the progressive β-cell loss initiated by viral infections in genetically susceptible individuals. Thus, we suggest that development of T1D in some individuals may require a

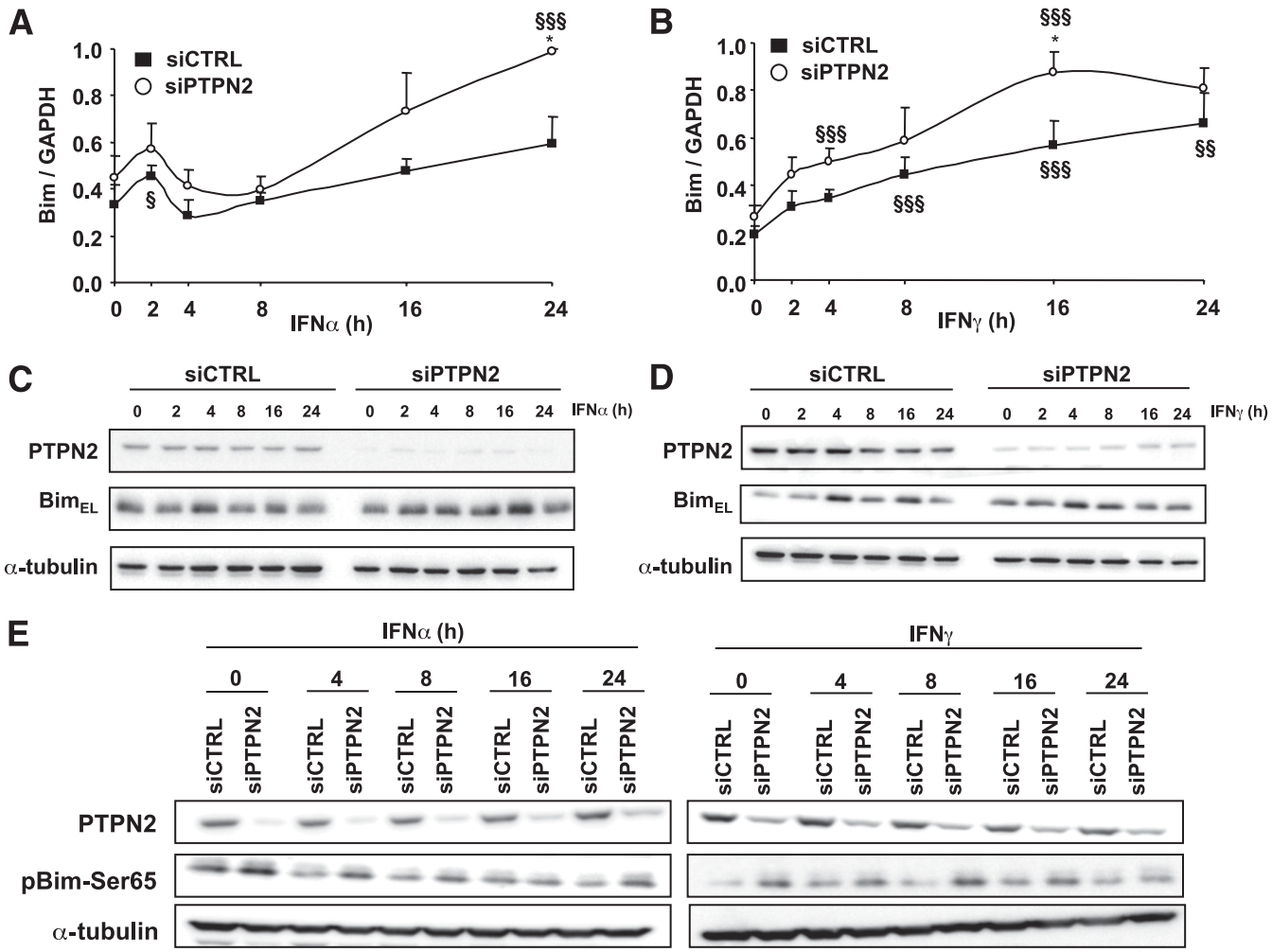

FIG. 5. Silencing of PTPN2 in INS-1E cells upregulates Bim mRNA (*A* and *B*) but not Bim protein expression (*C* and *D*) after IFNα or IFNγ treatment. PTPN2 inhibition in INS-1E cells induces Bim phosphorylation at Ser65 (*E*). Cells were transfected with siCTRL or siPTPN2, and after 48 h of recovery, they were left untreated or treated with IFNα (*A*) or IFNγ (*B*) for 2, 4, 8, 16, or 24 h. Bim mRNA expression was assayed by RT-PCR and normalized by the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Results are means ± SEM of five independent experiments; §$P < 0.05$, §§$P < 0.01$, and §§§$P < 0.001$ vs. untreated (i.e., not treated with cytokines) transfected with the same siRNA; *$P < 0.05$ vs. siCTRL treated at the same time point with IFNα or IFNγ; ANOVA. *C* and *D*: Cells were transfected with siCTRL or siPTPN2 and after 48 h of recovery were left untreated or treated with IFNα (*C*) or IFNγ (*D*) for 2, 4, 8, 16, or 24 h. Cells were lysed with Laemmli buffer, and expression of PTPN2 (for knockdown confirmation), Bim_{EL}, and α-tubulin (as protein loading control) was analyzed by Western blot. Results are representative of five independent experiments. *E*: Cells were transfected with siCTRL or siPTPN2; after 48 h of recovery, they were left untreated or exposed to IFNα or IFNγ for 4, 8, 16, or 24 h. Expression of PTPN2 (for knockdown confirmation), phosphorylated Bim (pBim)-Ser65, and α-tubulin (used as loading control) was analyzed by Western blot. Results are representative of five to eight experiments.

combination of infection by a potential diabetogenic virus (e.g., Coxsackievirus B4) (9), a vigorous and probably excessive local production of IFNs and other chemokines/cytokines, and the presence of particular polymorphisms in a candidate gene (i.e., *PTPN2*) that exacerbates IFN signaling (present data).

Type I and II IFN–induced β-cell apoptosis in PTPN2-silenced cells is accompanied by the exacerbation of the JAK-STAT signaling pathway (present data). On cytokine treatment, PTPN2 inhibition increases the activity of reporter vectors containing ISRE or GAS consensus sequences, and concomitant knockdown of STAT1 and PTPN2 significantly decreases the exacerbation of IFN-induced apoptosis. Because PTPN2 is a well-known negative regulator of STAT1 activation (40), our data suggest a key role for STAT1 in type I and II IFN–induced β-cell apoptosis. Aberrant activation of the JAK-STAT pathway is associated with both T1D and other autoimmune and inflammatory diseases (41,42). For example, STAT1 phosphorylation is increased in the intestinal mucosa of patients with celiac disease, suggesting that persistent STAT1 activation contributes to maintaining and expanding the local inflammatory response (41).

As mentioned above, the present results demonstrate that PTPN2 knockdown contributes to type I and II IFN–induced β-cell apoptosis via activation of the BH3-only protein Bim and consequent activation of the intrinsic mitochondrial pathway of cell death. This pathway leads to BAX translocation to the mitochondrial membrane, mitochondrial permeabilization, cytochrome c release to the cytosol, activation of caspase 9, subsequent activation of the effector caspase 3, and cell apoptosis. Mitochondrial integrity is controlled by interactions between pro- and antiapoptotic members of the B-cell lymphoma 2 (Bcl-2) protein family (20). Bim is a Bcl-2 member that mediates apoptosis by activating the proapoptotic Bcl-2 members Bax and Bak (43), and it contributes to β-cell apoptosis induced by chronic exposure to high glucose and the Fas-FasL

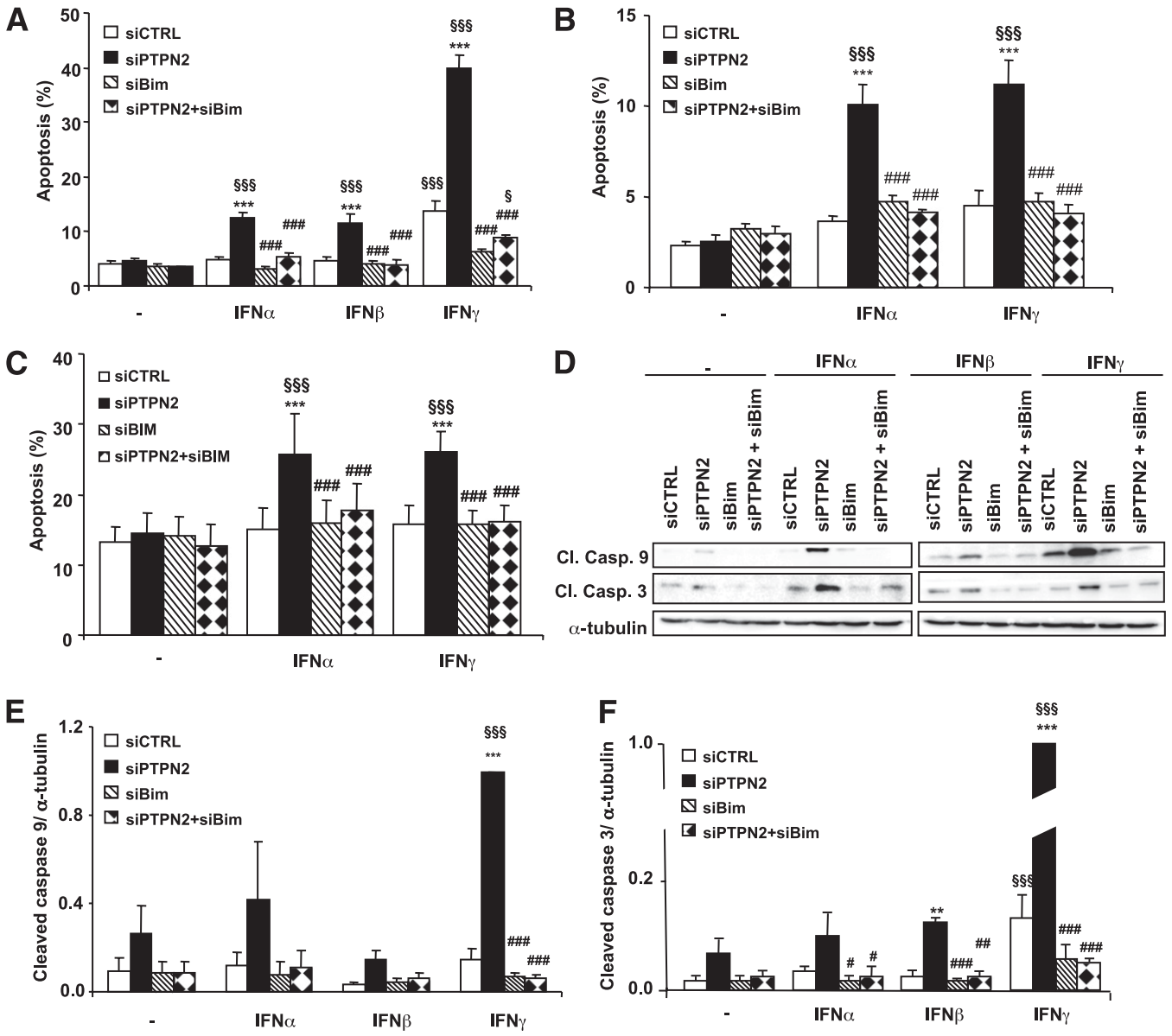

FIG. 6. Double knockdown of PTPN2 and Bim protects INS-1E cells, primary rat β-cells, and human islets cells from type I and II IFN–induced apoptosis. INS-1E cells, primary rat β-cells, and human islet cells were transfected with an siCTRL, siPTPN2, or siRNA targeting Bim, or double transfected with both siPTPN2 and siRNA targeting Bim. After 48 h of recovery, they were left untreated (-) or treated with IFNα, IFNβ, or IFNγ as indicated. Apoptosis was evaluated in INS-1E cells (*A*), rat primary β-cells (*B*), and dispersed human islet cells (*C*) by Hoechst and propidium iodide staining. Results are the mean ± SEM of three to four independent experiments; §§§*P* < 0.001 and §*P* < 0.05 vs. untreated (i.e., not treated with cytokines) transfected with the same siRNA; ***$P < 0.001$ vs. siCTRL treated with the same cytokine; ###$P < 0.001$ vs. siPTPN2 treated with the same cytokine; ANOVA. *D*: Expression of cleaved caspase 9, cleaved caspase 3, and α-tubulin was evaluated by Western blot in INS-1E cells. Mean optical densitometry measurements of cleaved caspases 9 (*E*) and 3 (*F*) (Western blots corrected by protein loading by α-tubulin). Results are means ± SEM of three independent experiments; §§§$P < 0.001$ vs. untreated (i.e., not treated with cytokines) transfected with the same siRNA; ***$P < 0.001$ and **$P < 0.01$ vs. siCTRL treated with the same cytokine; ###$P < 0.001$, ##$P < 0.01$, and #$P < 0.05$ vs. siPTPN2 treated with the same cytokine; ANOVA.

system (32). Bim-dependent apoptosis is regulated by both modulation of its expression and phosphorylation at specific serine or threonine residues (33). We presently show that PTPN2 inhibition increases Bim phosphorylation at Ser65 via JNK1 activation, and that Bim inhibition abrogates IFN-induced cell death in PTPN2-deficient cells. Our results are in line with previous reports showing that MAPK-p38- or JNK-induced phosphorylation of Bim at Ser65 increases its proapoptotic activity (44–46). It is interesting that PTPN2 silencing in β-cells (present data) induces hyperphosphorylation of Bim at residue Ser65 even in untreated cells, which is not sufficient to decrease cell viability. However, when this is combined with exposure to IFNs, cell death is triggered. This suggests that phosphorylation at Bim Ser65 is an apoptosis-sensitizing event in β-cells, leading to apoptosis only in the context of a wider proapoptotic environment prompted by local production of proinflammatory cytokines or viral infection.

SNPs in the *PTPN2* gene are implicated in susceptibility to several autoimmune disorders and inflammatory diseases, including T1D, Crohn's disease, and celiac disease (1,47,48). One of the SNPs (rs2542151) in the *PTPN2* gene is associated with an earlier onset of T1D, marking a genetic difference between patients with early and late onset of the disease (49). The intronic risk allele (rs1893217-C) in *PTPN2* correlates with decreased IL-2 receptor signaling in

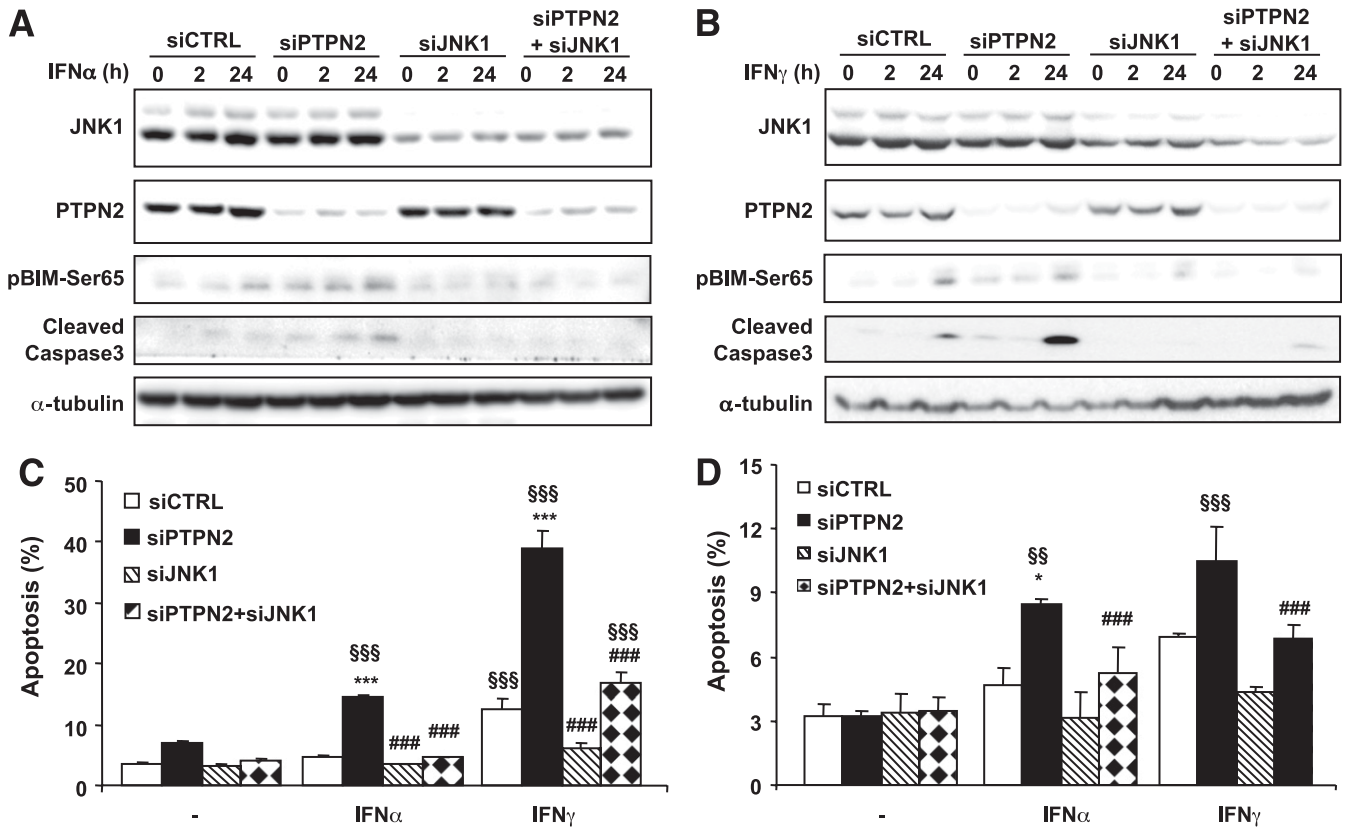

FIG. 7. Double knockdown of PTPN2 and JNK1 reduces phosphorylated Bim (pBim)-Ser65 phosphorylation and protects INS-1E cells and primary rat β-cells from type I and II IFN–induced apoptosis. INS-1E cells and primary rat β-cells were transfected with an siCTRL, siPTPN2, or siRNA targeting JNK1, or double transfected with both siPTPN2 and siRNA targeting JNK1. After 48 h of recovery, they were left untreated or treated with IFNα or IFNγ as indicated. Expression of pBim-Ser65, cleaved caspase 3, JNK1, PTPN2, and α-tubulin was evaluated by Western blot in INS-1E cells after IFNα (*A*) or IFNγ (*B*) treatment. Results are representative of five independent experiments. Apoptosis was evaluated in INS-1E cells (*C*) and primary rat β-cells (*D*) by Hoechst and propidium iodide staining. Results are the mean ± SEM of three to four independent experiments; §§§$P < 0.001$ and §§$P < 0.01$ vs. untreated (i.e., not treated with cytokines) transfected with the same siRNA; ***$P < 0.001$ and *$P < 0.05$ vs. siCTRL treated with the same cytokine; ###$P < 0.001$ vs. siPTPN2 treated with the same cytokine; ANOVA.

CD4$^+$ T cells, as well as with decreased *PTPN2* expression in CD4$^+$ CD45RO T cells (15). Our present data demonstrate that inhibition of PTPN2 activity in pancreatic β-cells increases IFN-induced cell death, suggesting that SNPs leading to a decreased expression of this gene may sensitize β-cells to apoptosis after a triggering event (e.g., type I IFNs produced by β-cells in response to a viral infection). In line with this possibility, PTPN2 knockdown in a model of Crohn's disease leads to increased claudin-2 (pore-forming protein) expression, further increasing IFNγ-induced intestinal epithelium permeability, which is an important feature of Crohn's disease (17). Moreover, PTPN2 knockout mice have abnormal production of IFNγ, tumor necrosis factor-α, and other cytokines, resulting in a systemic inflammatory disease (50). Further studies are now required to define how allelic differences in humans affect PTPN2 activity and function, and the impact of these differences in immune responses and β-cell apoptosis.

The current study suggests that environmental factors (e.g., viral infections and the resulting IFN production) may interact with a genetic predisposition factor (e.g., reduced PTPN2 activity) to induce aberrant proapoptotic activity of the BH3-only protein Bim, resulting in increased β-cell apoptosis via the intrinsic apoptotic pathway. Moreover, it indicates that PTPN2 modulates the apoptotic activity of Bim via regulation of the protein kinase JNK1. These findings provide the first indication of a direct interaction between a candidate gene for T1D and the activation of a specific downstream proapoptotic pathway in pancreatic β-cells. Further studies of the molecular interactions between predisposition genes and environmental triggers for autoimmune diseases should clarify the early triggering of autoimmunity and indicate novel avenues for their prevention.

ACKNOWLEDGMENTS

This work was supported by grants from the Communauté Francaise de Belgique–Actions de Recherche Concertées, Fonds National de la Recherche Scientifique, Belgium, the Belgium Program on Interuniversity Poles of Attraction initiated by the Belgium State (IUAP P6/40) and the European Union (project NAIMIT, in the Framework Programme 7 of the European Community). I.S. is the recipient of a postdoctoral fellowship from the Education Department of the Basque Country. F.M. is the recipient of a postdoctoral fellowship from Fonds National de la Recherche Scientifique, Belgium. E.N.G. is supported by an EMBO long-term fellowship.

No potential conflicts of interest relevant to this article were reported.

I.S. and F.M. contributed to the original idea and the design of the experiments, researched data, contributed to discussion, and wrote, revised, and edited the manuscript.

M.L.C. and E.N.G. researched data, contributed to discussion, and revised and edited the manuscript. L.M. and P.M. researched data. D.L.E. contributed to the original idea and the design of the experiments, contributed to discussion, and wrote, revised, and edited the manuscript.

The authors thank G. Vandenbroeck, R. Makhnas, A.M. Musuaya, S. Mertens, M.A. Neef, and M. Pangerl from the Laboratory of Experimental Medicine, Université Libre de Bruxelles, for excellent technical support.

REFERENCES

1. Todd JA, Walker NM, Cooper JD, et al.; Genetics of Type 1 Diabetes in Finland; Wellcome Trust Case Control Consortium. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857–864
2. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009;360:1646–1654
3. Peng H, Hagopian W. Environmental factors in the development of type 1 diabetes. Rev Endocr Metab Disord 2006;7:149–162
4. Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med 2009;360:1759–1768
5. Cadwell K, Patel KK, Maloney NS, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. Cell 2010;141:1135–1145
6. Lempainen J, Vaarala O, Mäkelä M, et al. Interplay between PTPN22 C1858T polymorphism and cow's milk formula exposure in type 1 diabetes. J Autoimmun 2009;33:155–164
7. Colli ML, Moore F, Gurzov EN, Ortis F, Eizirik DL. MDA5 and PTPN2, two candidate genes for type 1 diabetes, modify pancreatic β-cell responses to the viral by-product double-stranded RNA. Hum Mol Genet 2010;19:135–146
8. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulitis and β-cell loss in type 1 diabetes. Nat Rev Endocrinol 2009;5:219–226
9. Dotta F, Censini S, van Halteren AG, et al. Coxsackie B4 virus infection of β cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A 2007;104:5115–5120
10. Nair S, Leung KC, Rawlinson WD, Naing Z, Craig ME. Enterovirus infection induces cytokine and chemokine expression in insulin-producing cells. J Med Virol 2010;82:1950–1957
11. Rasschaert J, Ladrière L, Urbain M, et al. Toll-like receptor 3 and STAT-1 contribute to double-stranded RNA+ interferon-γ-induced apoptosis in primary pancreatic β-cells. J Biol Chem 2005;280:33984–33991
12. Devendra D, Jasinski J, Melanitou E, et al. Interferon-α as a mediator of polyinosinic:polycytidylic acid-induced type 1 diabetes. Diabetes 2005;54:2549–2556
13. Alba A, Puertas MC, Carrillo J, et al. IFN β accelerates autoimmune type 1 diabetes in nonobese diabetic mice and breaks the tolerance to β cells in nondiabetes-prone mice. J Immunol 2004;173:6667–6675
14. Smyth DJ, Cooper JD, Bailey R, et al. A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat Genet 2006;38:617–619
15. Long SA, Cerosaletti K, Wan JY, et al. An autoimmune-associated variant in PTPN2 reveals an impairment of IL-2R signaling in CD4(+) T cells. Genes Immun 2011;12:116–125
16. Doody KM, Bourdeau A, Tremblay ML. T-cell protein tyrosine phosphatase is a key regulator in immune cell signaling: lessons from the knockout mouse model and implications in human disease. Immunol Rev 2009;228:325–341
17. Scharl M, Paul G, Weber A, et al. Protection of epithelial barrier function by the Crohn's disease associated gene protein tyrosine phosphatase n2. Gastroenterology 2009;137:2030–2040
18. Scharl M, Hruz P, McCole DF. Protein tyrosine phosphatase non-receptor Type 2 regulates IFN-γ-induced cytokine signaling in THP-1 monocytes. Inflamm Bowel Dis 2010;16:2055–2064
19. Moore F, Colli ML, Cnop M, et al. PTPN2, a candidate gene for type 1 diabetes, modulates interferon-γ-induced pancreatic β-cell apoptosis. Diabetes 2009;58:1283–1291
20. Gurzov EN, Eizirik DL. Bcl-2 proteins in diabetes: mitochondrial pathways of β-cell death and dysfunction. Trends Cell Biol 2011;21:424–431
21. Lupi R, Dotta F, Marselli L, et al. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that β-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes 2002;51:1437–1442
22. Ortis F, Cardozo AK, Crispim D, Störling J, Mandrup-Poulsen T, Eizirik DL. Cytokine-induced proapoptotic gene expression in insulin-producing cells is related to rapid, sustained, and nonoscillatory nuclear factor-kappaB activation. Mol Endocrinol 2006;20:1867–1879
23. Moore F, Naamane N, Colli ML, et al. STAT1 is a master regulator of pancreatic β-cell apoptosis and islet inflammation. J Biol Chem 2011;286:929–941
24. Colli ML, Nogueira TC, Allagnat F, et al. Exposure to the viral by-product dsRNA or Coxsackievirus B5 triggers pancreatic β cell apoptosis via a Bim/Mcl-1 imbalance. PLoS Pathog 2011;7:e1002267
25. Gurzov EN, Ortis F, Cunha DA, et al. Signaling by IL-1β+IFN-γ and ER stress converge on DP5/Hrk activation: a novel mechanism for pancreatic β-cell apoptosis. Cell Death Differ 2009;16:1539–1550
26. Cunha DA, Ladrière L, Ortis F, et al. Glucagon-like peptide-1 agonists protect pancreatic β-cells from lipotoxic endoplasmic reticulum stress through upregulation of BiP and JunB. Diabetes 2009;58:2851–2862
27. Gurzov EN, Germano CM, Cunha DA, et al. p53 up-regulated modulator of apoptosis (PUMA) activation contributes to pancreatic β-cell apoptosis induced by proinflammatory cytokines and endoplasmic reticulum stress. J Biol Chem 2010;285:19910–19920
28. Ortis F, Pirot P, Naamane N, et al. Induction of nuclear factor-kappaB and its downstream genes by TNF-α and IL-1β has a pro-apoptotic role in pancreatic β cells. Diabetologia 2008;51:1213–1225
29. Ortis F, Naamane N, Flamez D, et al. Cytokines interleukin-1β and tumor necrosis factor-α regulate different transcriptional and alternative splicing networks in primary β-cells. Diabetes 2010;59:358–374
30. Wesoly J, Szweykowska-Kulinska Z, Bluyssen HA. STAT activation and differential complex formation dictate selectivity of interferon responses. Acta Biochim Pol 2007;54:27–38
31. Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. Endocr Rev 2008;29:42–61
32. McKenzie MD, Jamieson E, Jansen ES, et al. Glucose induces pancreatic islet cell apoptosis that requires the BH3-only proteins Bim and Puma and multi-BH domain protein Bax. Diabetes 2010;59:644–652
33. Hübner A, Barrett T, Flavell RA, Davis RJ. Multisite phosphorylation regulates Bim stability and apoptotic activity. Mol Cell 2008;30:415–425
34. Filippi CM, von Herrath MG. Viral trigger for type 1 diabetes: pros and cons. Diabetes 2008;57:2863–2871
35. Oikarinen S, Martiskainen M, Tauriainen S, et al. Enterovirus RNA in blood is linked to the development of type 1 diabetes. Diabetes 2011;60:276–279
36. Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. Diabetologia 2009;52:1143–1151
37. Huang X, Yuang J, Goddard A, et al. Interferon expression in the pancreases of patients with type I diabetes. Diabetes 1995;44:658–664
38. Chehadeh W, Weill J, Vantyghem MC, et al. Increased level of interferon-α in blood of patients with insulin-dependent diabetes mellitus: relationship with coxsackievirus B infection. J Infect Dis 2000;181:1929–1939
39. Li Q, Xu B, Michie SA, Rubins KH, Schreriber RD, McDevitt HO. Interferon-α initiates type 1 diabetes in nonobese diabetic mice. Proc Natl Acad Sci U S A 2008;105:12439–12444
40. Simoncic PD, McGlade CJ, Tremblay ML. PTP1B and TC-PTP: novel roles in immune-cell signaling. Can J Physiol Pharmacol 2006;84:667–675
41. Mazzarella G, MacDonald TT, Salvati VM, et al. Constitutive activation of the signal transducer and activator of transcription pathway in celiac disease lesions. Am J Pathol 2003;162:1845–1855
42. Avouac J, Fürnrohr BG, Tomcik M, et al. Inactivation of the transcription factor STAT-4 prevents inflammation-driven fibrosis in animal models of systemic sclerosis. Arthritis Rheum 2011;63:800–809
43. Strasser A. The role of BH3-only proteins in the immune system. Nat Rev Immunol 2005;5:189–200
44. Becker EB, Howell J, Kodama Y, Barker PA, Bonni A. Characterization of the c-Jun N-terminal kinase-BimEL signaling pathway in neuronal apoptosis. J Neurosci 2004;24:8762–8770
45. Putcha GV, Le S, Frank S, et al. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. Neuron 2003;38:899–914
46. Cai B, Chang SH, Becker EB, Bonni A, Xia Z. p38 MAP kinase mediates apoptosis through phosphorylation of BimEL at Ser-65. J Biol Chem 2006;281:25215–25222
47. Smyth DJ, Plagnol V, Walker NM, et al. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med 2008;359:2767–2777
48. Festen EA, Goyette P, Green T, et al. A meta-analysis of genome-wide association scans identifies IL18RAP, PTPN2, TAGAP, and PUS10 as shared risk loci for Crohn's disease and celiac disease. PLoS Genet 2011;7:e1001283
49. Espino-Paisan L, de la Calle H, Fernández-Arquero M, et al. A polymorphism in PTPN2 gene is associated with an earlier onset of type 1 diabetes. Immunogenetics 2011;63:255–258
50. Heinonen KM, Nestel FP, Newell EW, et al. T-cell protein tyrosine phosphatase deletion results in progressive systemic inflammatory disease. Blood 2004;103:3457–3464