The scaffold protein IB1/JIP-1 is a critical mediator of cytokine-induced apoptosis in pancreatic β cells

Jacques-Antoine Haefliger[1],[*],[‡], Thomas Tawadros[1],[*], Laure Meylan[1], Sabine Le Gurun[1], Marc-Estienne Roehrich[1], David Martin[1], Bernard Thorens[2] and Gérard Waeber[1]

[1]Department of Internal Medicine, CHUV-1011 Lausanne, Switzerland
[2]Institute of Pharmacology and Toxicology, University Hospital, CHUV-1011 Lausanne, Switzerland
*These authors contributed equally to this work
‡Author for correspondence (e-mail: jhaeflig@chuv.hospvd.ch)

Summary

In insulin-secreting cells, cytokines activate the c-Jun N-terminal kinase (JNK), which contributes to a cell signaling towards apoptosis. The JNK activation requires the presence of the murine scaffold protein JNK-interacting protein 1 (JIP-1) or human Islet-brain 1(IB1), which organizes MLK3, MKK7 and JNK for proper signaling specificity. Here, we used adenovirus-mediated gene transfer to modulate IB1/JIP-1 cellular content in order to investigate the contribution of IB1/JIP-1 to β-cell survival. Exposure of the insulin-producing cell line INS-1 or isolated rat pancreatic islets to cytokines (interferon-γ, tumor necrosis factor-α and interleukin-1β) induced a marked reduction of IB1/JIP-1 content and a concomitant increase in JNK activity and apoptosis rate. This JNK-induced pro-apoptotic program was prevented in INS-1 cells by overproducing IB1/JIP-1 and this effect was associated with inhibition of caspase-3 cleavage. Conversely, reducing IB1/JIP-1 content in INS-1 cells and isolated pancreatic islets induced a robust increase in basal and cytokine-stimulated apoptosis. In heterozygous mice carrying a selective disruption of the IB1/JIP-1 gene, the reduction in IB1/JIP-1 content in happloinsufficient isolated pancreatic islets was associated with an increased JNK activity and basal apoptosis. These data demonstrate that modulation of the IB1-JIP-1 content in β cells is a crucial regulator of JNK signaling pathway and of cytokine-induced apoptosis.

Key words: Type-I diabetes, β-Cell line, Islet-brain-1, IB1/JIP-1, INS-1, Pancreatic islets, Apoptosis, Adenovirus, JNK activity

Introduction

The c-Jun N-terminal kinase (JNK) is a mitogen-activated protein (MAP) kinase (MAPK) that is activated by cytokines and exposure to environmental stress (Davis, 2000; Dickens et al., 1997; Whitmarsh et al., 1998; Yang et al., 1997a; Yang et al., 1997b). Phosphorylation of the AP-1 transcription factor is a key target of JNK, which in turn regulates various cellular functions such as proliferation, differentiation and apoptosis (Davis, 2000). In pancreatic β cells, JNK activation might play a crucial role in the pro-apoptotic program observed during the onset of type-1 diabetes (Waeber et al., 2000). For proper JNK activation, the presence of the scaffold protein JNK-interacting protein 1 (JIP-1) or Islet-brain 1 (IB1) (Bonny et al., 1998; Dickens et al., 1997; Mooser et al., 1999) is required. This protein interacts with mixed-lineage protein kinases (MLKs), the MAPK kinase MKK7, JNK, the cytoskeleton-interacting protein p190^{RhoGEF}, kinesin, the amyloid precursor protein and the low-density-lipoprotein-receptor-related family members to allow proper signaling specificity (Davis, 2000; Gotthardt et al., 2000; Meyer et al., 1999; Scheinfeld et al., 2002; Stockinger et al., 2000; Verhey et al., 2001; Verhey and Rapoport, 2001). In pancreatic β cells, IB1/JIP-1 was found to be highly produced and to modulate JNK activity (Bonny et al., 1998; Bonny et al., 2000). Exposure of insulin-secreting cell lines to cytokines reduces the IB1 content, which is in turn associated with an increase phosphorylation of c-Jun and apoptosis rate (Bonny et al., 2000). In transient transfection studies of β-TC3, overproduction of IB1/JIP-1 was shown to prevent JNK-mediated activation of the transcription factors c-Jun, Elk1 and ATF2 (Bonny et al., 2000). The IB/JIP proteins contain several interacting domains including an SH3 domain, a JNK domain and a phosphotyrosine-interacting domain (Davis, 2000). Small-molecule inhibitors such as SP600125 or small interacting peptides (Barr et al., 2002; Bonny et al., 2001) were described to interfere with specific domains of IB1/JIP-1. In vitro, these molecules and peptides can inhibit JNK activity and could represent an useful tool for studying the JNK signaling pathway (Barr et al., 2002; Bonny et al., 2001).

The molecular basis of JNK activation and the functional role of IB1/JIP-1 were mostly assessed in the transformed mouse insulin-secreting cell line βTC3. Accordingly, we investigated the contribution of IB1/JIP-1 to rat β-cell survival in freshly isolated pancreatic islets and in the differentiated insulin-secreting β-cell line INS-1. Exposure of primary pancreatic islets and INS-1 cells to cytokines drastically reduced the IB1/JIP-1 content. Using adenovirus-mediated gene transfer of IB1/JIP-1 to increase or reduce the IB1/JIP-1 content, we could modulate the content of the scaffold protein in primary pancreatic islets and in INS-1 cells. Decreasing IB1/JIP-1 content induced an increase in JNK-activity and a

concomitant increase in cytokine-mediated apoptosis rate, whereas cells with higher levels of IB1/JIP-1 are protected against the cytokine-induced apoptosis. Last, we assessed the sensitivity of isolated pancreatic islets obtained from haploinsufficient mice (IB1/JIP-1$^{+/-}$) and found that, compared with wild-type pancreatic islets, the reduction of IB1/JIP-1 content is associated with an increase JNK activity and basal apoptosis.

Materials and Methods

Rodent islet isolation and cell lines

Our institutional review committee for animal experiments approved all the procedures for rat and mouse care, surgery and euthanasia. Adult rats weighing 250-350 g were anesthetized using Halothane (Arovet AG, Switzerland), sacrificed and immediately used for pancreas sampling. Rat islets were isolated from male Wistar rats by collagenase (NJ, USA) digestion. The pancreas were excised and placed in a 2 mg ml^{-1} collagenase solution (collagenase type IV; Worthington) in Hank's balanced salt solution containing 10 mmol l^{-1} Hepes, pH 7.4. Tissue was minced and incubated for 40 minutes at 37°C. After purification on a Ficoll gradient (Sigma, Switzerland), the isolated islets were washed twice in a phosphate-buffered saline (PBS) and the islets were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 10 mmol l^{-1} Hepes, 1 mmol l^{-1} sodium pyruvate and 50 µmol l^{-1} β-mercaptoethanol. The isolated islets were then exposed for 6-7 minutes at 37°C to the same medium supplemented with 0.16 mg ml^{-1} trypsin (1:250) (Gibco, Grand Island, NY). The incubation was stopped by the addition of 10 ml ice-cold Krebs-Ringer-bicarbonate buffer, supplemented with 0.5% bovine serum albumin (BSA), 2.8 mM glucose and 10 mM Hepes, pH 7.4 (KRB$^+$ buffer). The resulting suspension, which comprised mostly single cells, was centrifuged for 5 minutes at 130 $\boldsymbol{g}$. The pellet was resuspended in KRB$^+$ buffer to obtain a final concentration of 3×10^6 cells ml^{-1}.

For mouse islet isolation, pancreases were excised and placed in a 2 mg ml^{-1} collagenase solution (collagenase type IV; Worthington) in Hank's balanced salt solution containing 10 mmol l^{-1} Hepes, pH 7.4. Tissue was minced and incubated for 38 minutes at 37°C. After washing of the digested tissue, islets were hand picked under a stereomicroscope. Rodent islets were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 10 mmol l^{-1} Hepes, 1 mmol l^{-1} sodium pyruvate and 50 µmol l^{-1} β-mercaptoethanol (Guillam et al., 2000).

The INS-1 (Asfari et al., 1992) cells were cultured in RPMI-1640 medium supplemented with 11.1 mmol l^{-1} glucose, 10% fetal calf serum (heat-inactivated for INS-1), 2 mmol l^{-1} L-glutamine, 1 mmol l^{-1} sodium pyruvate, 50 mmol l^{-1} β-mercaptoethanol, 110 U ml^{-1} penicillin and 110 µg ml^{-1} streptomycin. INS-1 cells were kept at 37°C in a humidified incubator gassed with air and CO$_2$ to maintain medium pH at 7.4, fed at 3-day intervals and passed by trypsinization once a week. A combination of cytokines comprising interleukin 1β (IL-1β) (2×10^5 units µg^{-1}; Alexis) tumor necrosis factor-α (TNF-α) (10^5 units µg^{-1}; Alexis) and interferon γ (IFN-γ) (10 ng ml^{-1}; Alexis) was added for a period of 24 hours.

Hoechst-33342 staining

INS-1 cells and dispersed rat islets were grown on coverslips. After 2 days, the culture medium was changed and cytokines were added. Incubations were performed for 48 hours and cells were then fixed for 15 minutes with 1% paraformaldehyde. To evaluate the number of apoptotic cells, cells were incubated in the presence of Hoechst 33342 (dilution 1:1500) for 7 minutes and the nuclear morphology analyzed under a fluorescence microscope. The number of cells displaying a pycnotic (highly condensed) nucleus and/or a fragmented nucleus was evaluated in a blindly fashion. A minimum of 250 cells in four

separate experiments was counted for each condition (Bonny et al., 2000; Hoorens et al., 2001). Mouse islets were cultured for 48 hours and then fixed in 1% paraformaldehyde, stained with Hoechst 33342 and mounted in mowiol (Calbiochem) before counting the apoptotic nuclei.

Western blotting

Polyclonal IB1/JIP-1 antiserum was described previously (Bonny et al., 1998). These antibodies were affinity purified from crude serum using a Hitrap NHS-activated affinity Sepharose column (Amersham Pharmacia Biotech, Switzerand) coupled to the immunogenic protein. Polyclonal antibodies against cleaved caspase-3 (Asp175, CST) and monoclonal antibodies against α-tubulin (T5168, Sigma, Switzerland) were diluted 1:100 and 1:10,000, respectively. The cells were lysed in 5% SDS. Protein content was determined using the DC protein assay reagent kit (Bio-Rad Laboratories, Switzerland). Aliquots were fractionated by electrophoresis in a 10% polyacrylamide gel and immunoblotted onto Immobilon PVDF membranes (Millipore, MA) overnight, at a constant voltage of 30 V. The membranes were blocked for 3 hours at room temperature in PBS containing 3% bovine serum albumin and 0.1% Tween 20, and then incubated for 24 hours with antibodies diluted in blocking buffer. After repeated rinsing in PBS and PBS + 0.1% Tween20, immunoblots were incubated overnight at 4°C with an anti-mouse antibody coupled to alkaline phosphatase (Dako Diagnostic, Zug, Switzerland), diluted 1:5000. Specific antigen-antibody complexes were detected using the alkaline phosphatase development reagent kit (BCIP-NBT) method (AP development reagent, BioRad Laboratories, Glattburg, Switzerland). For the Phototope-HRP western blot detection system, membranes were incubated for 1 hour at room temperature in PBS containing 5% milk and 0.1% Tween 20 (blocking buffer) and then incubated for 2 hours at room temperature with antibodies directed against IB1/JIP-1. Specific antigen-antibody complexes were detected with the Phototope-HRP western blot detection system (Amersham, Switzerland).

Protein-kinase assays

JNK activity was measured using glutathione-S-transferase/c-Jun$_{(1-79)}$ bound to glutathione/Sepharose-4B (Bonny et al., 1998; Tawadros et al., 2002). INS-1 cells or rodent islets were lysed in 0.5% Nonidet P-40, 20 mM Tris-HCl, pH 7.6, 0.25 mM NaCl, 3 mM EDTA, 3 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM sodium vanadate, 20 µg ml^{-1} aprotinin and 5 µg ml^{-1} leupeptin. Lysates were centrifuged at 14,000 $\boldsymbol{g}$ for 10 minutes to remove nuclei, and supernatants (50 µg of protein) were mixed with 19 µl of glutathione-S-transferase/c-Jun$_{(1-79)}$). The mixture was rotated at 4°C for 1 hour, washed twice in lysis buffer and twice in kinase buffer (20 mM Hepes, pH 7.5, 10 mM MgCl$_2$, 20 mM β-glycerophosphate, 10 mM p-nitrophenylphosphate, 1 mM dithiothreitol, 50 µm sodium vanadate). Beads were suspended in 40 µl of kinase buffer with 10 µCi of [γ^{33}P] ATP and incubated at 30°C for 25 minutes. Samples were boiled in loading buffer and phosphorylated proteins were resolved on 10% SDS-polyacrylamide gels. To verify the selectivity of the JNK assay, cell lysates were fractionated by Mono-Q ion exchange chromatography and each fraction assayed as described above. Fractions were immunoblotted with rabbit antiserum recognizing JNK. Only fractions containing immunoreactive JNK phosphorylated the glutathione-S-transferase/c-Jun$_{(1-79)}$ protein.

DNA fragmentation assay

INS-1 cells or rat islets were washed in PBS and genomic DNA was isolated using the InViSorb Apoptosis Detection Kit following the manufacturer's procedure (InViTek, Germany). Using the ApoAlert ligation-mediated PCR Kit (LM-PCR, Clonteck, CA), 50 µg genomic
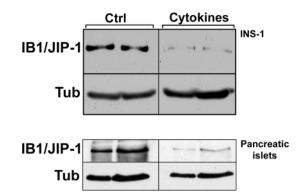
Fig. 1. Cytokines regulate the production of IB1/JIP-1. Western-blot analysis of INS-1 cellular extracts revealed that the levels of IB1/JIP-1 decreased by 80% in INS-1 cells and in freshly isolated rat islets treated with cytokines [IL-1β (2×10^5 units μg^{-1}) TNF-α (10^5 units μg^{-1}) and IFN-γ (10 ng ml^{-1})] during a period of 24 hours compared with controls (Ctrl). All lanes were loaded with 50 μg total proteins and normalized with tubulin (Tub).

DNA was PCR amplified and the resulting nucleosomal ladder was visualized on an agarose/ethidium-bromide gel.

Generation of recombinant adenoviruses

We generated recombinant adenoviruses comprising the complete cDNA of rat IB1/JIP-1 (Bonny et al., 1998; Tawadros et al., 2002) in the sense or antisense orientation to modulate IB1. Adenoviruses expressing green fluorescent protein (GFP) were used as a control. To generate the adenoviruses, the cDNAs were inserted into the plasmid pXC15 (Schaack et al., 1995) and adenoviruses were then generated by homologous recombination in 293 cells following co-transfection by the calcium-phosphate procedure of the plasmid pJM17 and pXC15 constructs. Viruses were further plaque-purified three times on HR911 cells (IntroGene, Leiden, The Netherlands). A large stock of viruses was purified by two rounds of CsCl centrifugation. After the second centrifugation, the virus band (1.5 ml) was collected and dialysed at 4°C against three changes (at least 200 volumes each) of 10 mM Hepes pH 8.0, 150 mM NaCl in a Slide-A-Lyzer (0.5-3.0 ml capacity) γ-irradiated 10K dialysis cassette (Pierce, Switzerland). The recombinant adenoviruses allowed expression of IB1/JIP-1, the GFP and/or the IB1/JIP-1 antisense RNA under the control of the strong immediate early cytomegalovirus (CMV) promoter.

Statistical analysis

Densitometric analysis of signals (autoradiograms) were performed using a Molecular Dynamics scanner (Sunnyvale, USA), which integrates areas and corrects for background. Data were expressed as means ± s.e.m. and were compared using Student's-*t* test and/or the Mann–Whitney *U* test. Statistical significance was defined at a value $P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***).

Results

Cytokines decrease the scaffold protein IB1/JIP-1 in β cells

To determine whether the JNK activators IL-1β, TNF-α and IFN-γ could modulate IB1/JIP-1 content, we performed western-blot analysis of INS-1 cells and freshly isolated rat pancreatic islets extracts treated with cytokines for two days (Fig. 1). Compared with controls, cytokines induced a drastic reduction of IB1/JIP-1 content (80%).
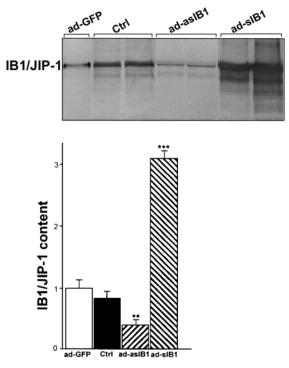

Fig. 2. Regulated production of IB1/JIP-1 by gene transfer approach. The IB1/JIP-1 content was measured from INS-1 cells infected with different adenovirus constructs. Western-blot analysis and quantitative assessment of these data showed that IB1/JIP-1 content is increased by 300% in cells overproducing IB1/JIP-1 (ad-sIB1), whereas decreased production was observed in cells infected with the Ad-asIB1 compared with controls (Ad-GFP or Ctrl). All lanes were loaded with 50 μg total proteins.
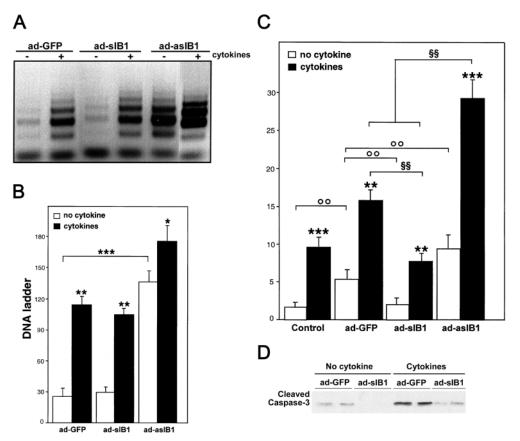
IB1/JIP-1 production is regulated by gene transfer in INS-1 cells

IB1/JIP-1 content was experimentally modulated in β cells using adenovirus gene transfer. We used recombinant adenoviruses containing the complete IB1/JIP-1 cDNA inserted in the sense or antisense orientation (Ad-sIB1 and Ad-asIB1, respectively). A control adenovirus encoding GFP was used as control. To study the efficiency of adenovirus-mediated transfer of IB1/JIP-1, western-blot analysis of INS-1 cells infected with Ad-GFP, Ad-sIB1/JIP-1 or Ad-asIB1/JIP-1 showed that the cells infected with the Ad-sIB1 produce high amounts of IB1/JIP-1 compared with β-cell extracts infected with Ad-GFP. By contrast, the IB1/JIP-1 content was decreased with the Ad-asIB1 (Fig. 2). Similar results were observed using freshly dispersed primary rat islets (data not shown). Quantitative assessment of IB1 content in β cells demonstrates a 50% decrease in IB1 content in cells infected with Ad-asIB1 and a 300% increase in IB1 content in cells overproducing IB1, whereas no change was observed in cells infected with Ad-GFP (Fig. 2).

Contribution of IB1/JIP-1 to control of the rate of apoptosis

Representative gel-electrophoresis images of DNA laddering analysis are shown in Fig. 3A. Cells grown in the absence of cytokines show a barely visible DNA laddering in control and

Fig. 3. Modulation of IB1/JIP-1 regulates apoptosis in INS-1 cells. (A) Reduced IB1/JIP-1 content in INS-1 cells is associated with DNA laddering. DNA laddering in INS-1 cells producing different levels of IB1/JIP-1 shows a large increase in nucleosomal band staining in cells exposed to cytokines. Cells producing lower amounts of IB1 show an increase in the intensity of nucleosomal bands in the absence and in the presence of cytokines (IL-1β, TNF-α and IFN-γ). (B) Quantitative assessment shows a 400% increase in the intensity of nucleosomal bands in the presence of cytokines, whereas no change was observed in cells overproducing IB1. By contrast, cells decreasing their IB1 content show an increase in the DNA laddering pattern in the presence (500%) and the absence (400%) of cytokines. (C) Apoptosis rate in INS-1 cells is dependent on the IB1/JIP-1 content. Cells infected or not with the different adenovirus constructs were treated with cytokines or not for 2 days. Cells were finally stained with Hoechst 33342 and propidium iodide, and counted. The ratio between apoptotic and normal cells is indicated. Columns represent mean ± s.e.m. of three independent experiments performed in triplicate. **, °° or §§ $P<0.01$; ***$P<0.001$. (D) To confirm the protective effect of IB1 in INS-1 cells, western blots of cleaved caspase were carried out. Cleaved caspase was increased in cells infected with control adenovirus (Ad-GFP) in the presence of cytokines. By contrast, overproduction of IB1 decreased the presence of caspase 3 cleavage.
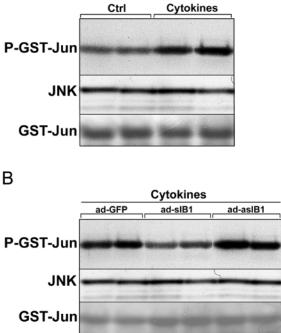

cells overproducing IB1. By contrast, cells whose IB1 content is reduced using antisense RNA showed a significant DNA laddering (Fig. 3B). Furthermore, cells incubated in presence of different cytokines show a drastic increase of DNA laddering. These data indicate that modulation of IB1/JIP-1 content contributes to DNA cleavage quantified by DNA laddering (Fig. 3A,B).

Apoptosis is characterized by morphological changes including the condensation of nuclear chromatin, cellular shrinkage, membrane blebbing and the formation of apoptotic bodies. Changes in nuclear morphology are recognized as a reliable criterion with which to assess apoptosis, and the DNA-binding dye Hoechst 33342 was used to visualize nuclear shape. The rate of apoptosis was evaluated by scoring blindly the number of cells displaying a fragmented nucleus. In INS-1, 48 hours incubation in presence of cytokines IL-1β, TNF-α and IFN-γ induced an increase in the rate of apoptosis from 2% to 9% (Fig. 3C). INS-1 cells infected with the control virus Ad-GFP increased their basal apoptosis rate threefold, whereas cells overproducing IB1 had a similar rate of apoptosis to non-infected cells. β Cells with reduced IB1 content had, without any stimuli, a 80% increase in the number of apoptotic cells. These data suggest that IB1/JIP-1 can protect cells from apoptosis caused by viral load. In the presence of cytokines, the increase in apoptosis rate in cells infected with a control adenovirus (Ad-GFP) was similar to that observed in non-

infected cells (16% versus 5% instead of 9% versus 2% for controls). This increase was abolished in cells overproducing IB1 and, moreover, cells overproducing IB1 have a lower rate of apoptosis (7.5%) than cells producing GFP (9.5%), indicating a protective effect of IB1 to apoptosis. By contrast, cells producing lower levels of IB1 have a twofold increase (29%) in apoptosis compared with cells infected with Ad-GFP (16%) (Fig. 3C). This protective effect of IB1/JIP-1 was confirmed using caspase-3 cleavage (Widmann et al., 1998) as a marker for apoptosis (Fig. 3D).

The JNK activity was then evaluated in INS-1 cells incubated in presence of cytokines. The presence of cytokines was associated with an increase JNK activity (Fig. 4A). By contrast, when the IB1/JIP-1 content is experimentally increased by Ad-sIB1, JNK activity is reduced compared with the controls (Ad-GFP) and, conversely, as expected, JNK activity was found to be increased in cells producing lower amounts of IB1 (Fig. 4B). Quantitative assessment demonstrated a twofold increase in c-Jun phosphorylation in INS-1 cells infected with the Ad-asIB1 and, conversely, a 50% decrease in cells infected with the Ad-sIB1 compared with controls (Ad-GFP).

In primary rat islet cells, a reduction of IB1/JIP-1 content increased the susceptibility of the pancreatic cells to cytokine-induced apoptosis (Fig. 5). A trend towards a reduced cytokine-induced apoptosis was seen in primary pancreatic cells

A

B

Fig. 4. Gene transfer of IB1/JIP-1 can prevent JNK activity in INS-1 cells. (A) JNK activity analysis in INS-1 revealed that cells incubated in presence of cytokines (IL-1β, TNF-α and IFN-γ) have a twofold increase in their JNK activity. The JNK content was evaluated by western blot and equal loading of substrate was confirmed by Coomassie-blue staining of the gel. (B) JNK activity analysis in INS-1 revealed that cells infected with Ad-asIB1 increased their JNK activity about twofold. By contrast, cells infected with ad-sIB1 showed a 50% decrease in their JNK activity.
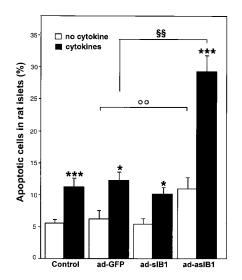

Fig. 5. Modulation of IB1/JIP-1 regulates apoptosis in isolated rat islets induced by cytokines. Apoptosis rate in dispersed rat islet cells infected with the different adenovirus constructs and treated or not with cytokines (IL-1β, TNF-α and IFN-γ). Columns represent mean ± s.e.m. of three independent experiments performed in duplicate. *$P<0.05$; **, °°or §§ $P<0.01$; ***$P<0.001$.
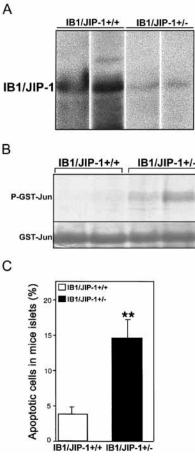
A

B

C

Fig. 6. The IB1/JIP-1 content is crucial to mediate JNK activity and apoptosis. (A,B) Western-blot analysis and solid-phase kinase assay using glutathione-*S*-transferase/Jun as substrate revealed a decrease of IB1/JIP-1 expression in isolated islets associated with an increase of JNK activity (P-GST/Jun) in mice islets from heterozygous (IB1/JIP-1$^{+/-}$) compared with wild-type mice (IB1/JIP-1$^{+/+}$). The JNK content was evaluated by western blotting. Equal loading of substrate was confirmed by Coomassie-blue staining of the gel (GST-Jun). (C) Apoptosis rate in whole mouse islets from heterozygous (IB1/JIP-1$^{+/-}$) compared with wild-type mice (IB1/JIP-1$^{+/+}$). Quantitative assessment of 12 measurements showed that apoptosis levels were increased approximately fourfold over control values. Columns represent mean ± s.e.m. **$P<0.01$

overproducing IB1/JIP-1 (Fig. 5). To evaluate the potential role of IB1/JIP-1 in the control of JNK activation in islets, we measured the JNK activity in the islets of mice in which the gene encoding IB1/JIP-1 had been disrupted. Because the homozygote IB1/JIP-1 (–/–) mice are associated with embryonic lethality (Tawadros et al., 2002; Thompson et al., 2001), we studied heterozygous IB1/JIP-1 (+/–) mice. These mice have a normal phenotype but the IB1/JIP-1 content is decreased (Fig. 6A) in their islets compared with their wild-type littermates (+/+). Concomitantly, the JNK activity was increased twofold (Fig. 6B). These data indicate that the JNK activity in mice islets is controlled by the IB1/JIP-1 content. The rate of apoptosis was further evaluated in whole islets by blindly scoring the number of cells displaying a fragmented nucleus (Fig. 6C). The basal apoptosis was increased ~3.7 times in islets from heterozygous mice compared with islets

from wild-type mice (14.9% in IB1/JIP-1$^{+/-}$ versus 4.0% in IB1/JIP-1$^{+/+}$).

Discussion

We evaluated the contribution of IB1/JIP-1 to the control of the cytokine-induced apoptosis in rodent isolated islets and in the differentiated insulin-secreting cell line INS-1 using recombinant adenovirus gene transfer. We previously reported that cytokines decrease IB1/JIP-1 content in βTC-3 cells, leading to an increase in cytokine-induced apoptosis in these cells (Bonny et al., 2000). We have now developed a gene-transfer approach to modulate the IB1/JIP-1 content in rat islets and in INS-1 cells. Cytokines reduced the content of IB1/JIP-1 in INS-1 cells and is associated with an increase in both JNK activity and apoptosis. Reducing the IB1/JIP-1 content using an antisense strategy increases the basal apoptosis rate, whereas increasing the IB1/JIP-1 content reduced basal apoptosis. JNK-activation induced by cytokines could be prevented in INS-1 cells by overproducing IB1/JIP-1. However, overproduction of IB1/JIP-1 in primary isolated pancreatic islets did not reduce the susceptibility of cells to apoptosis, whereas reduced levels of IB1/JIP-1 drastically increase the cytokine-induced apoptosis. Cell responses to extracellular stimuli included the activation of protein kinases that phosphorylate substrates such as transcription factors. MAPKs include extracellular-regulated kinases 1 and 2, p38 kinases, and JNKs. The JNK pathway is activated by different environmental stress including cytokines, heat shock or radiation. Phosphorylation of c-Jun by JNK causes increased transcription activity; the precise role of c-Jun in the response to JNK activation is likely to be modified by the activity of other transcription factors that interact with the complex AP-1 on the promoters of target genes (Davis, 2000; Davis, 1999; Ip and Davis, 1998).

IB1/JIP-1 binds to JNK, MKK7 and members of the MLK group of MAPK kinase kinases (Bonny et al., 1998; Bonny et al., 2000; Whitmarsh et al., 1998). The specificity and functions of JNK is controlled by the scaffold protein IB1/JIP-1. IB1/JIP-1 facilitates the signal transduction mediated by the interacting proteins. IB1/JIP-1 interacts with JNK through the JBD, a domain able to prevent apoptosis of pancreatic β-cell lines induced by IL-1β. Moreover, it has been demonstrated that producing the JBD in cortical neurons prevent apoptotic cell death induced by arsenite (Namgung and Xia, 2000) and is sufficient to prevent apoptotic death of nerve growth factor (NGF)-deprived superior cervical ganglion neurons by inhibiting the phosphorylation of c-Jun (Harding et al., 2000). Recently, it has been shown in sympathetic neurons undergoing apoptosis following NGF withdrawal, that the IB1/JIP-1 JBD inhibits c-Jun phosphorylation (Eilers et al., 1998).

We have recently investigated a rat model of complete bladder-outlet obstruction to study in vivo the activity of MAPKs (Tawadros et al., 2001; Tawadros et al., 2002). In this model, an increased voiding pressure enhances bladder wall stress. A drastic increase in the phosphorylated state of JNK was observed that was associated with a reduced IB1/JIP-1 content in the urothelium. We therefore demonstrated that IB1/JIP-1 is a crucial regulator of JNK activity in vivo and that increased levels of IB1/JIP-1 prevents the stress-induced activation of c-Jun.

We also generated mice carrying a targeted disruption of the *MAPK8IP1* gene, which encodes IB1/JIP-1 (Waeber et al., 2000). The null mutation resulted in early embryonic lethality. The JNK activation was found to be increased in freshly isolated mouse islets of heterozygous mice carrying a disruption of the *MAPK8IP1* gene exposed to cytokines. Similarly, we have recently shown that JNK activation was increased in unstressed urothelial cells of heterozygous mice (Tawadros et al., 2002) and these data further indicating a crucial role for IB1/JIP-1in islet homeostasis.

In conclusion, we show that IB1/JIP-1 content within β cells is crucial to protect cells from the stress-induced apoptosis. IB1 is therefore a regulator of survival in insulin-secreting cells and might represent an alternative therapeutic tool to prevent β-cell damage observed in diabetes or during β-cell transplantation.

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