# Variations in IB1/JIP1 Expression Regulate Susceptibility of β-Cells to Cytokine-Induced Apoptosis Irrespective of C-Jun $NH_2$-Terminal Kinase Signaling

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**We previously reported that interleukin-1β (IL-1β) alone does not cause apoptosis of β-cells, whereas when combined with γ-interferon (IFN-γ) and tumor necrosis factor-α (TNF-α), it exerts a distinct apoptotic effect. Studies in β-cell lines indicated that IL-1β reduced expression of islet brain (IB)-1/JNK interacting protein (JIP)-1, a JNK scaffold protein with antiapoptotic action. We examined whether variations in IB1/JIP-1 expression in purified primary β-cells affect their susceptibility to cytokine-induced apoptosis. Exposure to IL-1β for 24 h decreased cellular IB1/JIP-1 content by 66 ± 17%; this IL-1β effect was maintained in the presence of TNF-α + IFN-γ, which did not influence IB1/JIP-1 levels by themselves. Addition of IL-1β to TNF-α + IFN-γ increased apoptosis from 20 ± 2% to 59 ± 5%. A similar increase in TNF-α + IFN-γ–induced apoptosis was produced by adenoviral expression of antisense IB1/JIP-1 and was not further enhanced by addition of IL-1β, indicating that IL-1β–mediated suppression of IB1/JIP-1 in β-cells increases their susceptibility to cytokine-induced apoptosis. However, adenovirally mediated overexpression of IB1/JIP-1 also potentiated TNF-α + IFN-γ–induced apoptosis, suggesting that the antiapoptotic effect of IB1/JIP-1 depends on well-defined cellular levels. We conclude that the IB1/JIP-1 level in β-cells can control their susceptibility to apoptosis independent of JNK signaling. *Diabetes* 52: 2497–2502, 2003**

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ATF2, acting transcription factor 2; IB, islet brain; IBMX, 3-isobutyl-1-methylxanthine; IFN-γ, γ-interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; JBD, JNK-binding domain; JIP, JNK interacting protein; NMA, $N^G$-methyl-L-arginine; MOI, multiplicity of infection; PI, propidium iodide; TNF-α, tumor necrosis factor-α.



In rodents with type 1 diabetes, destruction of β-cells has been associated with increased local production of proinflammatory type 1 cytokines (1). Combinations of these cytokines are cytotoxic in isolated rodent and human islets and in β-cell preparations, but single cytokines are not cytotoxic (1–4), suggesting that multiple signals from different cytokines are required to trigger the cell death program. Interleukin-1β (IL-1β) is known to alter the protein expression pattern of β-cells (4–8). We previously showed that an IL-1β–induced alteration in β-cell phenotype is associated with reduction in cellular sensitivity to conditions that cause necrosis but not to cytokine-induced apoptosis (4). This IL-1β–induced variation in β-cell susceptibility to damaging conditions may result from an altered expression of pro- or antiapoptotic proteins (8). Recent studies in insulin-producing cell lines have indicated that IL-1β reduces expression of islet brain (IB)-1/JNK interacting protein (JIP)-1 (9). IB1/JIP-1 is a scaffold protein that interacts with JNK and its upstream activating kinases MLK3 and MKK7 (10). These interactions can either stimulate or inhibit JNK signaling, depending on the level of scaffold protein and kinases (10,11). JNK binds to IB1/JIP-1 with greater affinity than its transcription factor substrates c-Jun, acting transcription factor-2 (ATF2), and ELK1 (10,11). Overexpression of IB1/JIP-1 in β-cell lines prevented JNK-mediated activation of c-Jun, ATF2, and ELK1 and decreased IL-1β–induced apoptosis, suggesting that IB1/JIP-1 plays an antiapoptotic role in insulin-producing cells by controlling the activity of the JNK signaling pathway (9). A missense mutation located in the gene MAPK8IP1 encoding IB1/JIP-1 was identified in one family with type 2 diabetes, demonstrating that IB1/JIP-1 is a regulator of β-cell function (12). The present study examined whether variations in IB1/JIP-1 expression can influence cytokine-induced apoptosis in purified primary rat β-cells. Recombinant adenoviruses were used to suppress or increase IB1/JIP-1 expression.

## RESEARCH DESIGN AND METHODS

**Preparation of β-cells.** Pancreatic islets were isolated from adult male Wistar rats as described previously (13). Rats were bred according to Belgian regulations of animal welfare. Islets were dissociated in a calcium-free medium containing trypsin and DNase, and single β-cells were purified by autofluorescence-activated cell sorting using cellular light-scatter and FAD(H)-autofluorescence as discriminating parameters (13).

**Culture and adenoviral infection.** The cytotoxicity assay consists of counting the percentage of dead cells and therefore requires the use of single

cells. Purified single rat β-cells ($3 \times 10^3$ cells/condition) were distributed over polylysine-coated microtiter plates and precultured for 24 h in Ham's F10 medium (Gibco, Strathclyde, U.K.) containing 6 mmol/l glucose and supplemented with 0.075 mg/ml penicillin, 0.1 mg/ml streptomycin, 2 mmol/l L-glutamine, 0.5% (wt/vol) BSA pretreated with charcoal (fraction V; Sigma, St. Louis, MO), and 50 μmol/l 3-isobutyl-1-methylxanthine (IBMX; Janssen Chimica, Beerse, Belgium) (4). Addition of IBMX is needed for survival of single purified β-cells in serum-free media (14). For analyzing protein expression in conditions used in the cytotoxicity assay, purified β-cells were cultured in suspension in the same medium after reaggregation of the cells during a shaking incubation (15).

After overnight culture, β-cells were infected at multiplicity of infection (MOI) 1–100 for 2 h with adenovirus expressing IB1/JIP-1 antisense and GFP genes (AdIB1as) (16), or the IB1 gene (AdIB1s) (16), or with control virus expressing only GFP (AdGFP) (16). Cells were then washed and cultured in the same medium. At 2 days postinfection, recombinant human IL-1β (30 units/ml, 19 units/ng; a gift of McKesson HBOC C&B Services), TNF-α (1,000 units/ml, 200 units/ng; Genzyme, Cambridge, MA), and IFN-γ (1,000 units/ml, 10 units/ng; Genzyme) were added alone or in combination, with or without 1 mmol/l $N^G$-methyl-L-arginine (NMA; Sigma). Cells were exposed to cytokines for 1–24 h (protein analysis) or 8 days (viability).

**Assessment of viability.** The viable, necrotic, and apoptotic cells were determined by fluorescence microscopy using propidium iodide (PI; Sigma) and Hoechst 3342 (HO342; Sigma) (17). Viable cells were identified by their intact nuclei with blue fluorescence (HO342), necrotic cells by their intact nuclei with yellow-red fluorescence (HO342+PI), and apoptotic cells by their fragmented nuclei with either blue (HO342, early apoptosis) or yellow-red fluorescence (HO342+PI, late apoptosis).

**Analysis of protein expression.** Protein expression was analyzed by Western blot as previously described (4). Antibodies against inducible nitric oxide (NO) synthase (iNOS; Transduction Laboratories, Lexington, KY) were used at 1:2000 dilution, anti–phospho-JNK (New England Biolabs) at 1:500, anti–phospho-c-Jun (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:1,000, anti–IB1/JIP-1 (18) at 1:500, and anti-actin (Santa Cruz Biotechnology) at 1:1,000. The intensity of bands of interest was quantified by Scion Image software and normalized for the intensity of actin from the same blot.

**Data analysis.** The apoptotic index (X) was calculated from the percentage apoptotic (%A) counted in condition x and in the corresponding control c (19):

$$X = \frac{\%Ax - \%Ac}{100 - \%Ac} \times 100$$

Data are shown as means ± SE. Statistical significance of differences was calculated by ANOVA.

## RESULTS

**IL-1β reduces IB1/JIP-1 expression in β-cells.** Exposure of rat β-cells to IL-1β (30 units/ml) for 24 h decreased their IB1/JIP-1 content by 66 ± 17% (Fig. 1). IL-1β also downregulated IB1/JIP-1 in the presence of IFN-γ (1,000 units/ml) and/or TNF-α (1,000 units/ml), neither of which influenced the IB1/JIP-1 content when tested alone or in combination (Fig. 1). This effect of IL-1β was associated with induction of iNOS expression and was NO dependent: addition of the iNOS blocker NMA (1 mmol/l) prevented the IL-1β–induced suppression of IB1/JIP-1 (Fig. 1).

**Contribution of IL-1β to cytokine-induced apoptosis.** When tested alone, IL-1β (30 units/ml), IFN-γ (1,000 units/ml), or TNF-α (1,000 units/ml) did not cause apoptosis of single β-cells during culture periods of up to 8 days. A combination of the three cytokines induced 60% apoptotic cell death (Table 1). The apoptotic effect of the combination seemed primarily dependent on the presence of IL-1β: without this cytokine, the two others induced only a minor degree of apoptosis (20%; Table 1); addition of IL-1β to IFN-γ or to TNF-α resulted in markedly increased apoptosis (Table 1). This apoptosis-promoting effect of IL-1β was prevented by addition of NMA (Table 2). These results indicate that IL-1β sensitizes β-cells to apoptotic effects of other cytokines, an effect that is NO dependent.

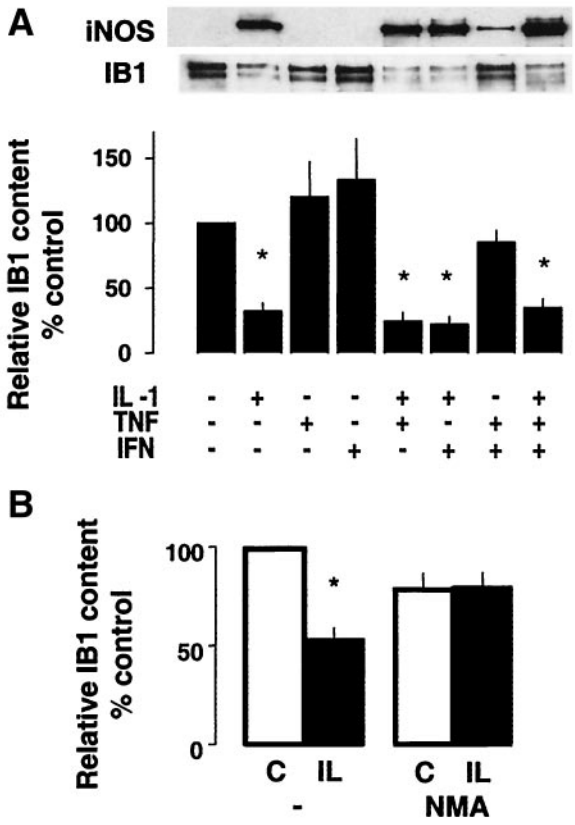


**FIG. 1. Effect of cytokines on IB1/JIP-1 expression in rat β-cells. Cells were cultured for 24 h with different cytokines (IL-1β 30 units/ml, IFN-γ 1,000 units/ml, TNF-α 1,000 units/ml) (*A*) or with IL-1β (30 units/ml) in the presence or absence of iNOS inhibitor NMA 1 mmol/l (*B*). The band intensity of IB1/JIP-1 was normalized to the corresponding actin intensity and expressed as percentage of control (no cytokine). Data represent means ± SE of three to eight independent experiments. Statistical significance of differences with control (C): *$P < 0.05$.**

**IB1/JIP-1 suppression potentiates cytokine-induced apoptosis.** To determine whether the NO-dependent IL-1β–mediated suppression of IB1/JIP-1 expression contributes to apoptosis induced by various cytokine combinations, we examined whether antisense suppression of IB1/JIP-1 potentiates cytokine-induced apoptosis. β-Cells

TABLE 1
Effect of variation in IB1/JIP-1 expression on cytokine-induced apoptosis

| Transfection | — | AdGFP | AdIB1as | AdIB1s |
|---|---|---|---|---|
| — | 0 | 2 ± 1 | 12 ± 3 | 5 ± 5 |
| IL-1β | 1 ± 1 | 2 ± 2 | 12 ± 4 | 3 ± 3 |
| IFN-γ | 2 ± 2 | 5 ± 1 | 17 ± 1 | 9 ± 6 |
| TNF-α | 5 ± 3 | 1 ± 1 | 20 ± 3 | 9 ± 4 |
| IL-1β/IFN-γ | 27 ± 7* | 30 ± 2* | 60 ± 1†‡ | 49 ± 4†‡ |
| IL-1β/TNF-α | 14 ± 5 | 18 ± 4 | 39 ± 1†‡ | 24 ± 3 |
| IFN-γ/TNF-α | 20 ± 2 | 19 ± 3 | 54 ± 4†‡ | 50 ± 4†‡ |
| IL-1β/IFN-γ/TNF-α | 60 ± 5† | 59 ± 1† | 61 ± 3† | 59 ± 2† |

Noninfected cells and AdGFP-, AdIB1as-, or AdIB1s-infected cells were cultured for 8 days with different cytokines (IL-1β 30 units/ml, IFN-γ 1,000 units/ml, TNF-α 1,000 units/ml) before apoptotic cells were counted. Data are expressed as apoptotic index (see RESEARCH DESIGN AND METHODS) and represent means ± SE of four independent experiments. Statistical significance of differences vs. non–cytokine-treated cells: *$P < 0.01$; †$P < 0.001$. Differences vs. noninfected cells: ‡$P < 0.001$.

TABLE 2
NO dependence of AdIB1as- and cytokine-induced apoptosis

| | AdGFP | | AdIB1as | |
|---|---|---|---|---|
| | — | NMA | — | NMA |
| Control | 0 ± 1 | 0 ± 1 | 9 ± 1 | 10 ± 2 |
| IL-1β/IFN-γ | 20 ± 3 | 7 ± 2* | 44 ± 4 | 29 ± 5* |
| IL-1β/TNF-α | 11 ± 1 | 2 ± 1* | 23 ± 2 | 11 ± 2* |
| IFN-γ/TNF-α | 11 ± 2 | 15 ± 2 | 42 ± 3 | 40 ± 3 |

AdGFP- and AdIB1as-infected cells were cultured for 8 days with cytokines (IL-1β 30 units/ml, IFN-γ 1,000 units/ml, TNF-α 1,000 units/ml) in the presence or absence of 1 mmol/l NMA. Data are expressed as apoptotic index (see RESEARCH DESIGN AND METHODS) and represent means ± SE of four independent experiments. Statistical significance of differences between condition with and without NMA: *$P < 0.05$.

were infected with adenovirus expressing either IB1 in antisense and GFP (AdIB1as) or GFP alone (AdGFP). The percentage of GFP-positive cells increased with adenoviral dose in both cases (10–20% at MOI 1; 50–70% at MOI 10; >90% at MOI 100). Infected cells remained GFP-positive for at least 10 days. After 6 days of culture, an infection at MOI 10 was not cytotoxic, but an MOI of 100 induced a slight degree of apoptosis (20%); we therefore used the MOI 10 condition.

β-Cells infected with AdIB1as expressed less IB1/JIP-1 (54 ± 5% of control; $n = 4$; Fig. 2) and underwent more apoptosis (12%) than AdGFP-infected or uninfected cells (Table 1). They were also more susceptible to apoptosis induction by the two-cytokine combinations IFN-γ + TNF-α, IFN-γ + IL-1β, and IL-1β + TNF-α (Table 1). This sensitizing effect of AdIB1as was most pronounced for the combination IFN-γ + TNF-α, thus in absence of IL-1β (Table 1); in fact, addition of IL-1β to IFN-γ + TNF-α did not result in a higher rate of apoptosis (Table 1). A tendency toward more apoptosis was also seen in AdIB1as cells that were exposed to single cytokines IFN-γ or TNF-α, but the differences are not statistically significant (Table 1). These results indicate an antiapoptotic role of IB1 expression in β-cells and suggest that downregulation of IB1/JIP-1 mediates, at least in part, the proapoptotic effect of IL-1β. The presence of NMA did not reduce the degree of apoptosis in AdIB1as-infected cells in the absence of IL-1β but partially prevented apoptosis in the presence of IL-1β in combination with either IFN-γ or TNF-α (Table 2), which is compatible with NO's role in suppressing IB1 expression but not in the subsequent signaling toward apoptosis. When the three cytokines (IL-1β, IFN-γ, and TNF-α) were present in the culture medium, NMA still reduced apoptosis in AdGFP cells (37 ± 8 vs. 59 ± 4, $n = 4$) but not in AdIB1as cells (71 ± 5 vs. 69 ± 5, $n = 4$).

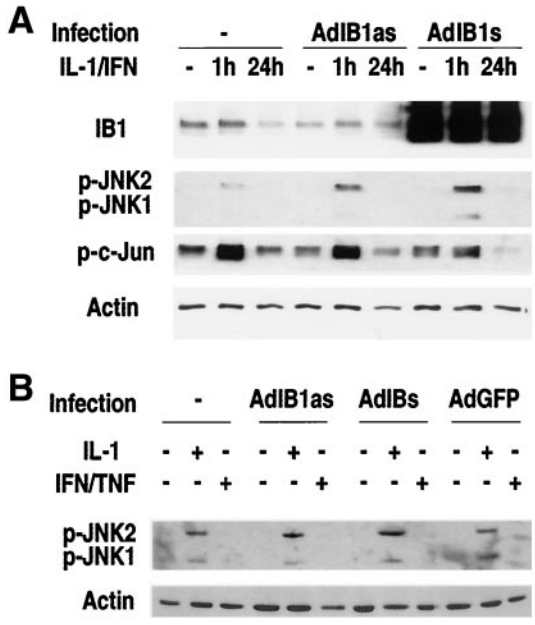


**FIG. 2. Effect of variation in IB1/JIP-1 expression on cytokine-induced phosphorylation of JNK and c-Jun. Noninfected cells (control) and AdIB1s- or AdIB1as-infected cells were exposed for 1 or 24 h to IL-1β (30 units/ml) plus IFN-γ (1,000 units/ml) (*A*) or for 1 h to IL-1β (30 units/ml) or TNF-α (1,000 units/ml) plus IFN-γ (1,000 units/ml) (*B*). Data shown are representative of two or three independent experiments.**

**IB1/JIP-1 overexpression potentiates cytokine-induced apoptosis.** We next examined whether cytokine-induced apoptosis is prevented by overexpression of IB1/JIP-1. Infection of β-cells with AdIB1s dose-dependently (MOI 1–100) increased the number of IB1-expressing cells and their IB1 protein content (Fig. 3*A* and *B*). At MOI 10 and 100, respectively, >70% and >90% of β-cells exhibited IB1/JIP-1 overexpression. AdIB1s did not by itself affect β-cell survival and did not prevent cytokine-induced apoptosis (Fig. 3, Table 1). On the contrary, an increased apoptosis was observed after exposure to the combinations of IL-1β + IFN-γ, IL-1β + TNF-α and IFN-γ + TNF-α, as compared with AdGFP-infected or uninfected cells (Fig. 3, Table 1).

**Variations in IB1/JIP-1 expression potentiate cytokine-induced apoptosis independent of JNK signaling.** It has previously been shown that JNK signaling contributes to cytokine-induced apoptosis (9); IB1/JIP-1 overexpression was suggested to prevent apoptosis by blocking JNK-mediated activation of c-Jun (9). To exclude that overexpressed IB1/JIP-1 lost its capacity to prevent c-Jun activation and thereby its antiapoptotic potential, we first examined IL-1β + IFN-γ–induced phosphorylation of JNK and c-Jun in AdIB1s- and AdIB1as-infected cells. In noninfected cells, phosphorylation of JNK and c-Jun occurred within 1 h of exposure to cytokines and reduced to basal levels after 24 h of exposure (Fig. 2*A*). AdGFP, AdIB1s, or AdIB1as infection did not induce phosphorylation of JNK and c-Jun in the absence of cytokines (Fig. 2). However, cytokine-induced phosphorylation of JNK-2 was increased in both AdIB1s (2- to 10-fold, $n = 3$) and AdIB1as cells (1.5- to 5-fold, $n = 3$; Fig. 2*A*), whereas phosphorylation of c-Jun was not increased in AdIB1as cells and reduced by 74 ± 7% in AdIB1s cells, as compared with uninfected cells (Fig. 2*A*). In both AdIB1s and AdIB1as cells, this was associated with a greater sensitivity to IL-1β + IFN-γ–induced apoptosis (Table 1, Fig. 3). These results indicate that IB1 indeed functioned as an inhibitor of JNK-mediated c-Jun activation in β-cells. Thus, the increased sensitivity of IB1-overexpressing (or IB1-suppressed) β-cells to IL-1β + IFN-γ–induced apoptosis was not mediated by increased JNK signaling via c-Jun, and also in IB1-suppressed cells the increased apoptosis did not correlate with increased c-Jun activation. We then

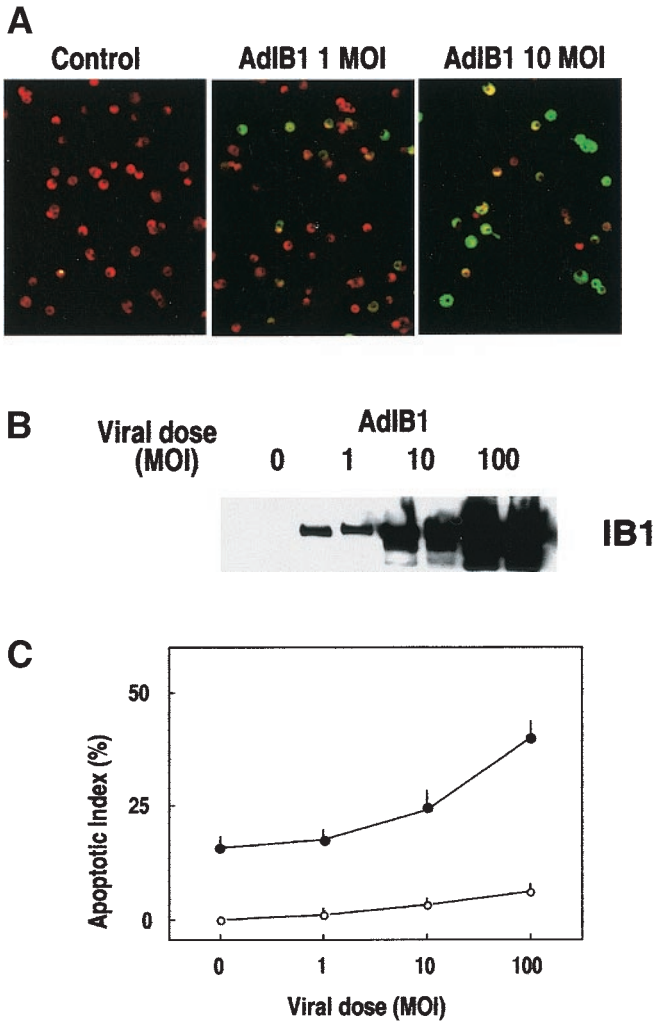


**FIG. 3. Overexpression of IB1/JIP-1 on cytokine-induced apoptotic cell death. β-Cells were cultured for 2 days after infection with AdIB1 using different viral concentrations (MOI 1–100). IB1 expression was analyzed by immunocytochemistry (green, IB1; red insulin) (*A*) or Western blot (*B*). Infected and noninfected cells were cultured for 8 days with IL-1β (30 units/ml) and IFN-γ (1,000 units/ml, ●) or without cytokines (●) before apoptotic cells were determined (*C*). *A* and *B*: Three independent experiments. *C*: Means ± SE of five independent experiments.**

examined whether the IFN-γ + TNF-α–induced JNK signaling was affected in AdIB1s- and AdIB1as-infected cells. However, exposure to IFN-γ + TNF-α did not induce phosphorylation of JNK in uninfected cells or in AdIB1s- and AdIB1as-infected cells, whereas IL-1β–induced phosphorylation of JNK-2, serving as a positive control in the same experiment, was slightly increased in AdIBs cells (50% higher than that in noninfected cells and AdGFP cells; $n = 2$; Fig. 2*B*). This result shows that variations in IB1/JIP-1 expression potentiate the IFN-γ + TNF-α–induced apoptosis in the absence of JNK activation.

## DISCUSSION

Previous studies showed that exposure to IL-1β reduced the expression of IB1/JIP-1 in βTC-3 cells (9). The present data show that this is also the case in purified rat β-cells. This downregulation was NO dependent and could not be reproduced by cytokines IFN-γ and/or TNF-α, suggesting that suppression of IB1/JIP-1 represents a specific effect of IL-1β. It has been proposed that IB1/JIP-1 plays an antiapoptotic role in insulin-producing cell lines (9). It thus was conceivable that the IL-1β–induced suppression of IB1/JIP-1 in β-cells would increase their sensitivity to apoptotic signals, for example, those generated by other cytokines. Our study demonstrates that this is indeed the case. First, the combination of IL-1β with IFN-γ and/or TNF-α induces apoptosis, whereas none of these single cytokines was toxic (3) (present study). Second, downregulation of IB1/JIP-1 expression by adenoviral antisense approach induced apoptosis in the absence of cytokines, demonstrating that IB1/JIP-1 has antiapoptotic properties. This result is similar to the recent report by Haefliger et al. (16) in which AdIB1as could induce apoptosis in unpurified rat islet cells. Third, IB1/JIP-1 suppression in β-cells increased β-cells' sensitivity to apoptotic signals from IFN-γ and TNF-α, in particular when these cytokines were used in combination, whereas IB1/JIP-1 suppression could not further potentiate apoptosis induced by the combination IL-1β + IFN-γ + TNF-α. This indicates that adenovirally mediated suppression of IB1 in β-cells reproduces proapoptotic effects of IL-1β. It was previously suggested that survival of β-cells depends on proteins whose continuous synthesis is required to suppress an endogenous suicide program (17). Our present observations further support this notion and indicate that IB1/JIP-1 may be such antiapoptotic protein; its antisense suppression was indeed sufficient to trigger apoptosis in cultured β-cells. The antiapoptotic function of IB1/JIP-1 is further supported by the recent observation that in heterozygous mice carrying a selective disruption of the IB1/JIP-1 gene, the reduction in IB1/JIP-1 content was associated with increased apoptosis (16).

In view of the antiapoptotic properties of IB1/JIP-1, overexpression of IB1/JIP-1 was expected to reduce or prevent cytokine-induced apoptosis. However, overexpression of full-length IB1 protein potentiated cytokine-induced apoptosis in β-cells. These results are in contrast with previously reported ones, which suggested that overexpression of IB1/JIP-1 could reduce cytokine-induced apoptosis in insulin-producing cell lines (9,12,20). Several factors may have contributed to this discrepancy: *1*) primary β-cells and βTC-3 or INS-1 cell lines may differ in their respective responses to cytokines, in their endogenous IB1/JIP-1 content, and in their expression of IB1/JIP-1 interacting proteins; *2*) the number of IB1-overexpressing cells and the intracellular recombinant IB1 protein level may vary depending on the strategy of gene transfer (adenovirus versus plasmid vector); and *3*) antiapoptotic effects and reduced JNK signaling have been described using overexpression of the JNK-binding domain (JBD) of IB1/JIP-1 (9,12,20,21), which functionally differs from the full-length protein used in the present work. Because JBD does not function as a scaffold protein, it cannot facilitate signal transduction between JNK and its upstream kinases but inhibits JNK activation by sequestering JNK. In addition, JBD may lack structural and functional elements crucial to JNK-independent regulation of apoptosis by IB1/JIP-1. Our observations clearly indicate that overexpression of full-length IB1/JIP-1 sensitizes β-cells to apoptosis. We recently demonstrated that adenoviral overexpression of IB1/JIP-1 increases AICA-riboside–

induced JNK activation and apoptosis in insulin-producing MIN6 cells (22). Use of both AdIB1s and AdIB1as thus has revealed proapoptotic and antiapoptotic roles of IB1 in β-cells. Moreover, these experiments have identified at least one fundamental difference between pro- and antiapoptotic properties of IB1/JIP-1: whereas IB1/JIP-1 is intrinsically antiapoptotic (IB1/JIP-1 downregulation sufficed to trigger apoptosis), this protein seems not to bear any intrinsic proapoptotic activities (its overexpression did not trigger apoptosis). On the basis of our results, IB1/JIP-1 overexpression can nevertheless potentiate apoptotic effects of extrinsic cell death stimuli.

The mechanisms by which IB1/JIP-1 controls apoptosis in β-cells should be further investigated. It is known that IB1/JIP-1 may influence JNK signaling positively or negatively, depending on the relative amounts of IB1/JIP-1 and its interacting protein kinases (10,11). Previous studies have suggested that the antiapoptotic function of IB1/JIP-1 is related to its role in controlling the JNK signaling pathway, in particular by preventing activation of downstream targets c-Jun and ATF2 (9). Our results show that, although overexpression of IB1/JIP-1 prevented the IL-1β + IFN-γ–induced c-Jun phosphorylation, this was associated with increased JNK activation and apoptosis, suggesting that c-Jun activation was no mediator of the cytokine-induced apoptosis or of the IB1 dosage effects in this process. This suggests that, at least in some cases, JNK signaling is dissociated from the effects of IB1/JIP-1 on apoptosis, although the present study did not exclude the possibility that variation in IB1/JIP-1 expression increases IL-1β + IFN-γ– or IL-1β + TNF-α–induced apoptosis through JNK-mediated activation of other transcription factors (ATF2, ELK-1). The strongest argument for the existence of a mechanism(s) in which IB1/JIP-1 expression influences β-cell survival independent of JNK signaling is our observation that either overexpression or downregulation of IB1/JIP-1 resulted in a markedly elevated IFN-γ + TNF-α–triggered apoptosis, whereas this occurred in complete absence of JNK activation. So far, most studies on IB1/JIP-1 have been focused on its role in JNK signaling, as little is known about other functions of this protein. In rat islets, IB1/JIP-1 also functions as a transactivator of the GLUT2 gene (18). However, under the present experimental conditions, GLUT2 expression at both mRNA and protein levels in AdIB1s and AdIB1as cells was not significantly different from that in noninfected cells (our unpublished observations), indicating that GLUT2 is probably not involved in IB1as-induced apoptosis. A recent study showed that JIP-1 can directly modulate amyloid precursor protein metabolism by interacting with the amyloid precursor protein cytoplasmic domain, independent of its regulation of the JNK signaling cascade (23). It thus is possible that IB1/JIP-1 also possess novel pro- and antiapoptotic features that are unrelated to its effects on JNK signal transduction.

That single cytokines could not induce apoptosis while combinations of cytokines exert an apoptotic effect suggests that multiple distinct signals are required to activate the β-cell suicide program. IFN-γ and TNF-α induce distinct JNK-independent proapoptotic signals that cooperatively trigger an apoptotic process that is, as our antisense experiments suggest, inhibited by the endogenous IB1/JIP-1 expression. IL-1β probably plays a dual role in promoting this process *1*) by inducing a proapoptotic signal that can substitute for signals from either IFN-γ or TNF-α and *2*) by suppressing the expression of antiapoptotic protein IB1/JIP-1 to potentiate apoptotic signals. That IL-1β–induced suppression of IB1/JIP-1 may result from its effect on JNK activation and iNOS expression is now supported by several findings: *1*) overexpression of constitutive upstream JNK activator MEKK1 reduced the IB1/JIP-1 content in a β-cell line (9); *2*) IL-1β–induced suppression of IB1/JIP-1 is NO dependent (present study); *3*) IL-1β induced the activation of JNK (within 60 min; present study) and the production of NO (within 6 h; our unpublished data) before it noticeably suppressed IB1/JIP-1 expression (24 h; present study); and *4*) IL-1β + IFN-γ + TNF-α–induced apoptosis is, at least in part, NO dependent (8) (present study) and may be related to JNK activation (24), whereas apoptosis by AdIB1as + IFN-γ + TNF-α is NO independent and does not require JNK activation (present study). Thus, in pancreatic β-cells, JNK and NO play important roles in IL-1β–mediated apoptosis but not in apoptosis induced by IFN-γ and TNF-α.

In conclusion, our data demonstrate that variation in IB1/JIP-1 expression in rat β-cells increases their sensitivity to cytokine-induced apoptosis. IL-1β may play an important role in cytokine-induced apoptosis by downregulating IB1/JIP-1 expression, whereas additional signals induced by other cytokines are clearly required to activate the apoptotic process. Finally, the IB1/JIP-1 protein itself exerts pro- and antiapoptotic effects that do not depend on JNK signaling in β-cells.

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