Cell-Permeable Peptide Inhibitors of JNK

Novel Blockers of β-Cell Death

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Stress conditions and proinflammatory cytokines activate the c-Jun NH_2-terminal kinase (JNK), a member of the stress-activated group of mitogen-activated protein kinases (MAPKs). We recently demonstrated that inhibition of JNK signaling with the use of the islet-brain (IB) 1 and 2 proteins prevented interleukin (IL)-1β–induced pancreatic β-cell death. Bioactive cell-permeable peptide inhibitors of JNK were engineered by linking the minimal 20–amino acid inhibitory domains of the IB proteins to the 10–amino acid HIV-TAT sequence that rapidly translocates inside cells. Kinase assays indicate that the inhibitors block activation of the transcription factor c-Jun by JNK. Addition of the peptides to the insulin-secreting βTC-3 cell line results in a marked inhibition of IL-1β–induced *c-jun* and *c-fos* expression. The peptides protect βTC-3 cells against apoptosis induced by IL-1β. All-D retro-inverso peptides penetrate cells as efficiently as the L-enantiomers, decrease c-Jun activation by JNK, and remain highly stable inside cells. These latter peptides confer full protection against IL-1β–induced apoptosis for up to 2 weeks of continual treatment with IL-1β. These data establish these bioactive cell-permeable peptides as potent pharmacological compounds that decrease intracellular JNK signaling and confer long-term protection to pancreatic β-cells from IL-1β–induced apoptosis. *Diabetes* 50:77–82, 2001

A critical component of the cell response to extracellular stimuli is the activation of protein kinases that phosphorylate numerous substrates, including nuclear proteins. In yeasts and mammals, the mitogen-activated protein (MAP) signal transduction pathways converge to kinases that are directly able to activate transcription factors. Well-characterized MAP kinase (MAPK) pathways include extracellular-regulated kinases (ERKs)-1/2, p38 kinases, and c-Jun NH_2-terminal kinases (JNKs).

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ATF, activating transcription factor; BSA, bovine serum albumin; ERK, extracellular-regulated kinase; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; GST, glutathione S-transferase; IB, islet-brain; IL, interleukin; JBD, JNK-binding domain; JIP, JNK-interacting protein; JNK, c-Jun NH_2-terminal kinase; JNKI, JNK inhibitor; KRBH, Krebs-Ringer bicarbonate-HEPES; MAP, mitogen-activated protein; MAPK, MAP kinase; NF, nuclear factor; NO, nitric oxide; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PDX, pancreatic duodenal homeobox factor.

The JNK signal transduction pathway is preferentially activated in response to environmental stress and by the engagement of several classes of cell surface receptors, including cytokine receptors, serpentine receptors, and receptor tyrosine kinases (1). Targets of JNKs are mostly transcription factors, including c-Jun– (2), activating transcription factor (ATF) 2– (3), and ETS-containing factors such as Elk1 (4). Other targets having function regulated by JNK-mediated phosphorylation include insulin receptor substrate 1 (5) and Bcl-2 (6). In murine fibroblasts, the absence of JNK causes the failure to release cytochrome c (7).

In type 1 diabetes, we recently provided evidence that JNK plays a central role in the intracellular events that signal β-cell loss after exposure to the proinflammatory cytokine interleukin (IL)-1β (8,9). To address the specific role of JNK in pancreatic β-cell death, we used two different subclones of the pluripotent pancreatic endocrine stem cell clone (MSL). These cells were used to derive two lines, namely the glucagon-secreting AN-glu, and, after stable transfection with the transcription factor pancreatic duodenal homeobox factor (PDX)-1, the insulin-secreting AN-ins (10). The AN-ins cells were reported to be more susceptible to apoptosis elicited by IL-1β, an effect not accounted for by increased nitric oxide (NO) production (8). In contrast, the AN-ins cells showed an increased activation of JNK in response to IL-1β. In these cell systems, we demonstrated that the two MAPKs, p38 and ERK, were unnecessary to promote the apoptotic response. JNK activity, however, was essential because blocking JNK with the use of the dominant inhibitor JNK-binding domain (JBD) of the islet-brain (IB)-1/JNK-interacting protein (JIP)-1 (11,12) prevented apoptosis by >90%. JBD also prevented apoptosis in βTC-3, RINm5F, and INS-1 cells (8,9,13).

The IB-1/2 proteins are natural regulators of the JNK-signaling pathway and are highly expressed in pancreatic β-cells (9,11). A mutation in the *IB1* gene has recently been shown to be associated with a familial form of type 2 diabetes and to decrease the resistance of cells to proapoptotic stimuli (14). Decreased IB-1 levels in pancreatic β-cells sensitize cells to IL-1β–induced apoptosis (13). IB1 is an isoform of the JNK-interacting protein JIP-1 and interacts with JNK through JBD, a 280–amino acid domain. IB-2/JIP-2 has a similar domain of 240 amino acids (9,15). We have demonstrated that the JBD of both IB-1 and IB-2 is able to prevent apoptosis of pancreatic β-cell lines induced by IL-1β (8,9).

Here, we have used a sequence comparison to define the minimal conserved domains of IB-1 and IB-2 that block β-cell apoptosis. We show that peptides of 20 and 18 amino acids derived from IB-1 and IB-2, respectively, are sufficient to block activation (i.e., phosphorylation of the activation domains) of c-Jun by JNK. After covalent linkage of these

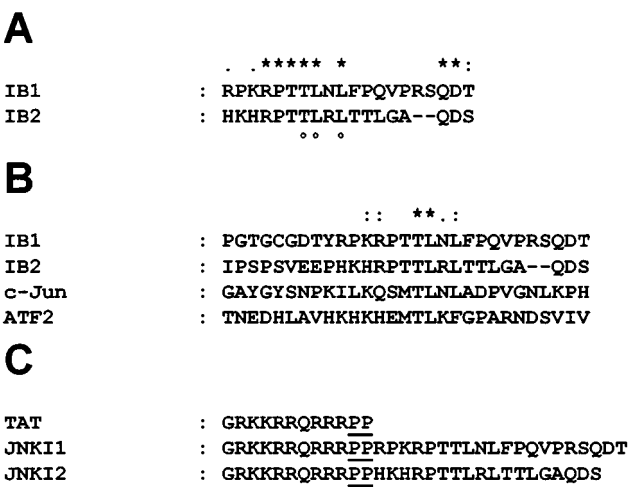

FIG. 1 *A*: Amino acid sequence alignment of the JBDs of IB-1 and IB-2 only. *B*: Amino acid sequence alignment showing the region of highest homology between the JBDs of IB1, IB2, c-Jun, and ATF2. *C*: Sequence of the different peptides used. The underlining indicates where proline was added between the TAT and IB sequences. *Fully conserved residues; °residues mutated into glycine in the GFP-JBD_{20Mut} vector, as shown in Fig. 2.

peptides to the 10–amino acid HIV-TAT sequence that directs cellular import in cells and animals (16), we obtained chemically synthesized cell-permeable JNK-ligands that block JNK-mediated activation of c-Jun, penetrate β-cells throughout the cytoplasm and the nucleus (17), and prevent IL-1β–induced apoptosis. Furthermore, we show that synthesis of the all-D retro-inverso form of these peptides produces molecules that conserve all of the essential biological properties of the L-enantiomers. However, their markedly expanded half-life in vivo allows for the continuous protection against IL-1β–induced apoptosis for several days to weeks. The elaboration of these tools will allow us to study the role of JNK in IL-1β–induced apoptosis in more sophisticated systems, including islet studies and animal models.

RESEARCH DESIGN AND METHODS

Plasmids and peptides. The human JNK1α1, JNK2α2, JNK3α1, ERK1, and p38 sequences were cloned into the expression vector pCDNA3.1 (Invitrogen). The vector expressing FLAG-JBD (amino acids 1–280 of IB-1 [11,12]) in the plasmid pBK (Stratagene) has been described previously (11). The pEGFP-N1 vector encoding the green fluorescent protein (GFP) was from Clontech. Oligonucleotides corresponding to the JBD_{20} and JBD_{20Mut} sequences were synthesized and directionally inserted, in frame with the GFP sequence, into the *Eco*RI and *Sal*I sites of this vector.

Peptides were purchased from Auspep (Australia). They were purified by high-performance liquid chromatography (HPLC) and analyzed by mass spectrometry. For the fluorescence studies, peptides were NH_2-terminally labeled with fluorescein isothiocyanate (FITC)-conjugated glycine. The COOH-termini of all peptides were amide groups.

Cell lines. The insulin-secreting cell line βTC-3 (18) was cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 µg/ml streptomycin, 100 U/ml penicillin, 1 mmol/l Na-pyruvate, and 2 mmol/l glutamine. TAT, JNK inhibitor (JNKI) 1, or JNKI2 peptides were added at a concentration of 1 µmol/l each 30 min before the addition of IL-1β (10 ng/ml) and again 24 h later. Apoptotic cells were counted 48 h after the addition of IL-1β by propidium iodide and Hoechst 33342 staining (13,19). The number of apoptotic cells in experiments involving transfected GFP constructs was evaluated using an inverted fluorescence microscope (Axiovert 25; Zeiss). Apoptotic cells were discriminated from normal cells by the characteristic blebbing of the cytoplasm, which was easily determined from the fluorescence emitted by the GFP. A minimum of 1,000 cells in duplicate was counted for each experiment.

Insulin secretion was quantified using a commercial radioimmunoassay (Linco). Cells (100,000/well) were first equilibrated in Krebs-Ringer bicarbonate-HEPES (KRBH) buffer (120 mmol/l NaCl, 4 mmol/l KH_2PO_4, 20 mmol/l HEPES, 1 mmol/l $MgCl_2$,1 mmol/l $CaCl_2$, and 5 mmol/l $NaHCO_3$) containing 2.8 mmol/l glucose for 1 h. Buffer was washed off and KRBH containing 16.7 mmol/l glucose was added. Insulin content in the buffer was then measured after 1 h of incubation at 16.7 mmol/l glucose.

Fluorescence studies. FITC-TAT, -JNKI1, or -JNKI2 peptides (1 µmol/l each) were added to cells in culture medium. Cells were then washed with phosphate-buffered saline (PBS) and fixed for 5 min in methanol-acetone (1:1) before being examined under the fluorescence microscope. FITC-labeled bovine serum albumin (BSA) (1 µmol/l of 12 mol/l FITC per mol/l BSA) (Sigma) was used as control.

Solid phase JNK assays. βTC-3 cells were activated with IL-1β for 1 h before being used for cell extract preparation. Cellular extracts were prepared by scraping control and activated cells in lysis buffer (20 mmol/l Tris-acetate, 1 mmol/l EGTA, 1% Triton X-100, 10 mmol/l p-nitrophenyl-phosphate, 5 mmol/l sodium pyrophosphate, 10 mmol/l β-glycerophosphate, and 1 mmol/l dithiothreitol). Debris was removed by centrifugation for 5 min at 15,000 rpm in a SS-34 rotor (Beckman). A sample of 100 µg extract was incubated for 1 h at room temperature with 1 µg glutathione S-transferase (GST)-Jun (amino acids 1–89) and 10 µl of glutathione-agarose beads (Sigma). After four washes with the scraping buffer, the beads were resuspended in the same buffer supplemented with TAT, JNKI1, or JNKI2 peptides for 20 min. Kinase reactions were then initiated by the addition of 10 mmol/l $MgCl_2$ and 5 µCi [γ-^{33}P]ATP and incubated for 30 min at 30°C. Reaction products were then separated by SDS-PAGE on a denaturing 10% polyacrylamide gel. The gels were dried and subsequently exposed to X-ray films (Kodak).

Recombinant JNKs, p38 kinases, and ERKs that were tagged with a FLAG epitope (Sigma) were produced using the transcription and translation rabbit reticulocyte lysate kit (Promega) and the specified plasmids. The kinases were then immunopurified with agarose beads covalently linked to the anti–FLAG M2 antibody and eluted with FLAG peptides as indicated by the manufacturer (Sigma). Beads were washed four times with 1 ml PBS solution and were then used in solid-phase kinase assays as described above. JNKI and control peptides were mixed with recombinant JNKs, p38 kinases, and ERKs in the kinase buffer 20 min before GST-Jun was added.

Reverse transcriptase–polymerase chain reaction analysis. RNA was extracted according to the guanidium isothiocyanate method of Chomczynski and Sacchi (20). IL-1β (10 ng/ml) was added for 30 min before RNA was prepared. Analyses were then performed using a commercial kit (PerkinElmer) according to the manufacturer's instructions, except that [α-^{33}P]dATP was added to the polymerase chain reaction (PCR). Aliquots of the reactions were then taken every three cycles, starting at cycle 10, during the amplification process and were analyzed by agarose gel electrophoresis. Photographs showed the lowest number of cycles that allowed visualization of the reactions. Control reactions in the absence of reverse-transcriptase gave no amplification products. Primer sequences were as follows: Jun forward, 5′-GTG CAG CAC CCG CGG CTG CA-3′; Jun reverse, 5′-TGC AAC TGC TGC GTT AGC ATG-3′; Fos forward, 5′-GAT ACA CTC CAA GCG GAG AC-3′; Fos reverse, 5′-CCA GTC TGC TGC ATA GAA GG-3′; MIF forward, 5′-AGT ACA TCG CRG TGC ACG TGG T-3′; MIF reverse, 5′-TCC GGG CTG ATG YGC AGG C-3′; Actin forward, 5′-AAC GGC TCC GGC ATG TGC AA-3′; and Actin reverse, 5′-ATT GTA GAA GGT GTG GTG CCA-3′. Fos and actin primer pairs span one intron. Quantitative real-time PCRs were performed with a LightCycler apparatus (Roche) using the same set of primers. Controls for absence of primer dimers were performed as recommended by the manufacturer.

Statistics. Distribution of data was controlled for normality. Data were analyzed with an unpaired Student's t test, and $P < 0.01$ was considered significant. Means ± SE were calculated.

RESULTS

A conserved minimal inhibitory domain of IB-1 prevents β-cell death. The 280– and 240–amino acid JBDs of IB-1 and IB-2 prevent pancreatic β-cell apoptosis in vitro (8,9). Alignment of the JBDs of IB-1 and IB-2 define two blocks of seven and three amino acids conserved between the two sequences (Fig. 1*A*). The two blocks span a peptide sequence of 20 (IB-1) and 18 (IB-2) amino acids within a segment that has been shown to be critical for interaction with JNK (12,15). Sequence comparison with the JBDs of c-Jun and ATF2 indicates that these two blocks are only minimally conserved in these two transcription factors (Fig. 1*B*).

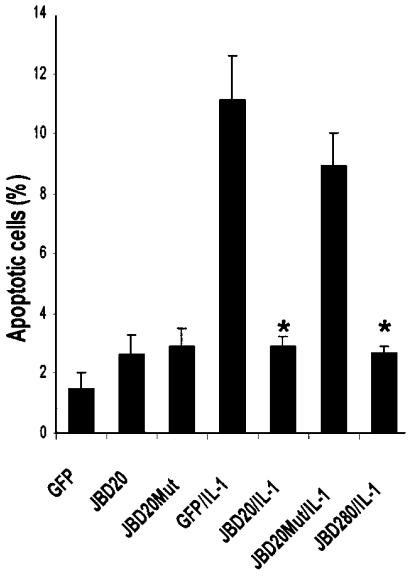

FIG. 2. Inhibition of β-cell death by the minimal 20–amino acid JBD of IB-1. Insulin-producing βTC-3 cells were transfected with the indicated vectors and IL-1β was added as indicated. Apoptotic cells were counted after 2 days. The GFP-expressing vector was used as the control. JBD_{20} represents a vector expressing a chimeric GFP linked to the 20–amino acid sequence from the JBD of IB-1. JBD_{20Mut} is the same vector as GFP-JBD_{20}, but with a JBD mutated at three conserved residues (Fig. 1*A*). JBD_{280} is a GFP vector linked to the entire JBD (amino acids 1–280). Data are means ± SE from three experiments. *$P < 0.01$ for JBD conditions relative to GFP/IL-1 and $JBD20_{Mut}$/IL-1.

The conserved 20–amino acid sequence of IB-1 was linked to the NH_2-terminal of the GFP (JBD_{20} construct). The JBD_{20}-expressing construct prevented IL-1β–induced pancreatic β-cell apoptosis as efficiently as the entire $JBD_{1–280}$ (Fig. 2). As controls, mutated sequences (Fig. 1*A*) at conserved residues did not show any protective activity (Fig. 2).

Synthesis of cell-permeable JNK-inhibitory peptides. To convert the minimal JBDs into bioactive cell-permeable compounds, two bipartite peptides were synthesized as follows: the COOH-terminal end was the 20– or 18–amino acid sequence derived from the JBD of IB1 or IB2 that was covalently linked to an NH_2-terminal 10–amino acid carrier peptide derived from the HIV-$TAT_{48–57}$ sequence (17) (Fig. 1*C*). Previous studies have shown that the $TAT_{48–57}$ peptide efficiently accumulated into a variety of cells and that it could be useful for delivering macromolecules (21,22), which includes efficient delivery to animal tissues (16). Two proline residues were inserted between the TAT and JBD sequences as spacer to allow for maximal flexibility. We named the bipartite peptides JNKI1 and JNKI2. Sequences of peptides are given in Fig. 1*C*.

To investigate whether the JNKI peptides translocated inside cells, we labeled the peptides at the NH_2-terminus by the addition of an FITC-glycin group. Labeled peptides (1 μmol/l) were then added to the cell medium. Fluorescein-labeled BSA was used as a control and its fluorescence after washing off the cells was shown to be negligible (data not shown). As shown in Fig. 3 (bottom), fluorescently labeled JNKI peptides efficiently and rapidly entered cells once added to the culture medium. A time-course study indicated that the fluorescent signal became extinguished after 24 h.

Similar cellular uptake and increased stability of all-D enantiomers allows prolonged transfection of cells. We synthesized an all-D retro-inverso peptide (23,24). Thereafter, this peptide was referred to as D-JNKI1 to discriminate it from its L-enantiomeric counterpart.

FITC-labeled D-JNKI1 or (L-)JNKI1 peptides were added at decreasing concentrations to βTC-3 cells and the fluorescent signal was recorded. Cellular uptake of the D-JNKI1 peptide was as efficient as that for JNKI1 (data not shown). The intensity of the fluorescent signal emitted by the D- isoform in βTC-3 cells at increasing time intervals indicates that D-JNKI1 appears stable for up to 2 weeks (Fig. 3).

JNK-inhibition in vitro. Effects of the peptides on JNK-mediated phosphorylation of the target transcription factor c-Jun were then investigated in vitro. Recombinant JNK1, JNK2, and JNK3 were produced in reticulocyte lysates and used with c-Jun as substrate. Kinase experiments indicated that JNKI peptides at the concentration of 25 μmol/l blocked JNK1, JNK2, and JNK3 phosphorylation of c-Jun (Fig. 4*A*). Dose-response studies indicated that JNK activity was reduced by 50% at concentrations of peptides of ~1 μmol/l (data not shown). Inhibition of ERK-1/2 or p38 activity was not observed in similar experiments, in agreement with the lack of effect of JBD on the activity of these MAPKs (12). D-JNKI1 inhibited phosphorylation of c-Jun, although at a level that is about 15- to 20-fold less than that of (L-) JNKI1 (Fig. 4*B*).

To characterize the effects of the JNKI peptides on JNK activated by stressful stimuli, we used GST-Jun to pull down JNK from IL-1β–activated cells. Control TAT and JNKI peptides were then added for 20 min, and kinase reactions were initiated by the addition of [γ-^{33}P]ATP. As shown in Fig. 5, JNKIs efficiently prevented phosphorylation of c-Jun by activated JNK.

Inhibition of *c-jun* and *c-fos* expression. To determine whether the cell-permeable peptides could interfere with JNK signaling in vivo, we measured their effects on the expression of the *c-jun* and *c-fos* genes. The transcriptional activity of these promoters is positively modulated by the two JNK-targets, the c-Jun and Elk1 transcription factors, respectively (25,26). Both the *c-jun* and *c-fos* genes had been shown to be induced by IL-1β in pancreatic β-cell lines (27), and constitu-

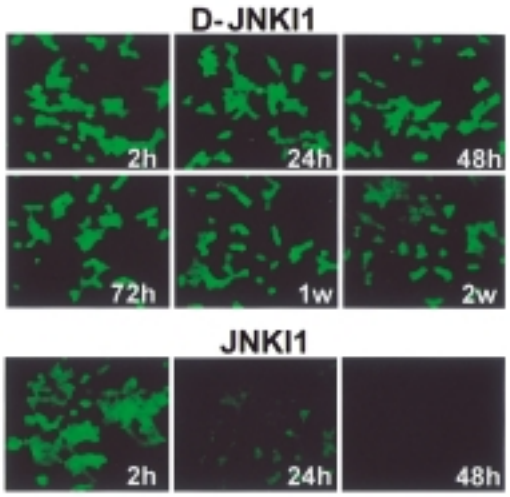

FIG. 3. Cellular import of the TAT-IB1 peptide. βTC-3 cells were incubated with the indicated FITC-labeled peptides (1 μmol/l) for the indicated times and were visualized under a fluorescence microscope.

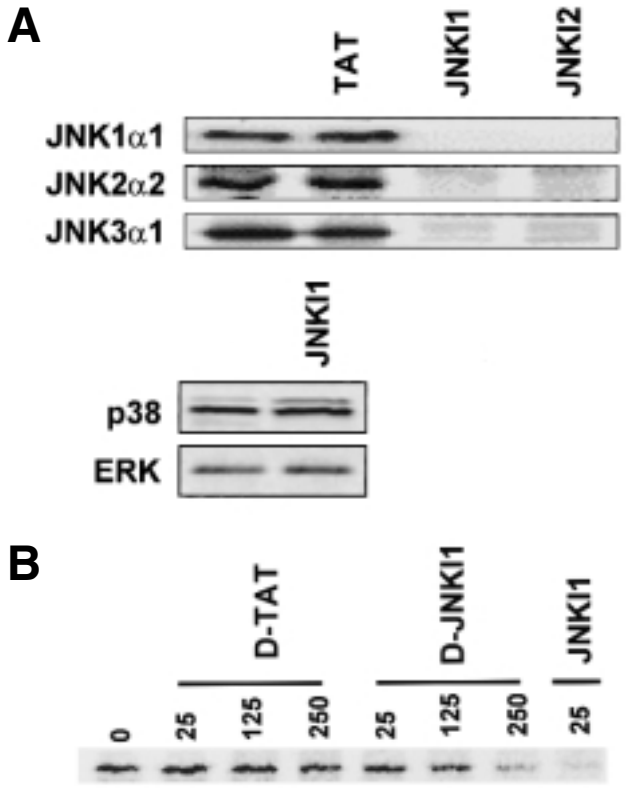

FIG. 4. *A*: Upper lanes: Inhibition of c-Jun phosphorylation by recombinant JNK in vitro. Recombinant JNK (JNK1α1, JNK2α2, and JNK3α1) were used in solid-phase kinase assays. Peptides (25 µmol/l) were added where indicated. Bottom lanes: similar experiments with recombinant p38 and ERK1 were performed. Phosphorylated substrates were resolved by SDS-PAGE analysis. *B*: Experiments similar to those in *A* (JNK1α1), but with D-JNKI1 compared with JNKI1. Values are expressed in micromoles per liter.

tive expression of the 280–amino acid JBD of IB-1 in βTC-3 cells decreases both *c-jun* and *c-fos* expression (A.O., C.B., unpublished observations). Addition of JNKI1 decreased the magnitude of the *c-jun* and *c-fos* response to IL-1β (Fig. 6). Quantification of these data and normalization to actin by real-time PCR (LightCycler) in three separate experiments indicated that *c-fos* expression in the presence of IL-1β is reduced 4.2 (± 0.3)-fold by JNKI1, and that *c-jun* expression induced by IL-1β is reduced 2.7 (± 0.2)-fold by JNKI1. These data indicate that both genes are at least partially under the control of JNK in pancreatic βTC-3 cells.

Inhibition of IL-1β–induced apoptosis. The above data indicated that the cell-permeable peptides might reduce the biological effects of activated JNK. Addition of the JNKI peptides inhibited IL-1β–induced apoptosis of the insulin-secreting βTC-3 cells (Fig. 7*A*). To achieve this level of protection, JNKI peptides (1 µmol/l) have to be added every day during the treatment period with IL-1β. No protection is observed after 2 days of incubation with one single addition of peptides (data not shown). In contrast, one single addition of D-JNKI1 (1 µmol/l) completely protected βTC-3 cells for up to 2 weeks of continual incubation with IL-1β (Fig. 7*B*).

To determine whether the peptides interfere with insulin secretion, βTC-3 cells were first equilibrated for 1 h in 2.8 mmol/l glucose in the presence and/or absence of JNKI1 and D-JNKI1. Secreted insulin was then measured after a 1-h incubation at

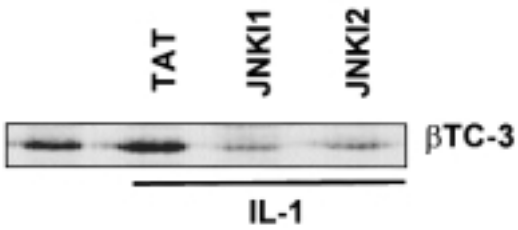

FIG. 5. Inhibition of c-Jun phosphorylation by activated JNK. Extracts were prepared from control or IL-1β–treated βTC-3 cells. JNK were then pulled down using GST-Jun and kinase assays were performed. Peptides (25 µmol/l) were added as indicated. Phosphorylated GST-Jun was resolved by SDS-PAGE analysis.

16.7 mmol/l glucose. No impairment of the total amount of secreted insulin was detected in these conditions (Fig. 8).

DISCUSSION

Small therapeutic molecules constitute the real target for pharmaceutical research. Our data establish that JNKI peptides are biologically active molecules that are able to decrease the effects of JNK signaling on pancreatic β-cell fate in vitro. The peptides might be used to study JNK signaling in different conditions (e.g., to establish the role of JNK in the apoptotic response of human islets or in animal models of diabetes). To this end, we indeed observed penetration of the peptides in the pancreatic islets of mice injected intraperitoneally with FITC-labeled JNKI1 (data not shown) (16).

JNK targets are mainly transcription factors including c-Jun, ATF2, Elk1, c-myc, or p53. Thus, JNK probably acts by modifying the expression of genes that play an important role in controlling cell death or survival. Therefore, the coordinate regulation of the genes controlled by JNK sensitizes β-cells to the proapoptotic action of IL-1β. It is expected that both upregulation of protective genes and downregulation of killer genes after JNK blockage will be observed. The identification and detailed characterization of the genetic targets of JNK is an important step for the understanding of the progression of type 1 diabetes. The production of JNKI peptides allows us to finally determine these genetic targets in β-cells from different sources, including isolated human islets.

In type 1 diabetes, β-cell loss appears essentially as an apoptotic process initiated by the coordinate secretions of the

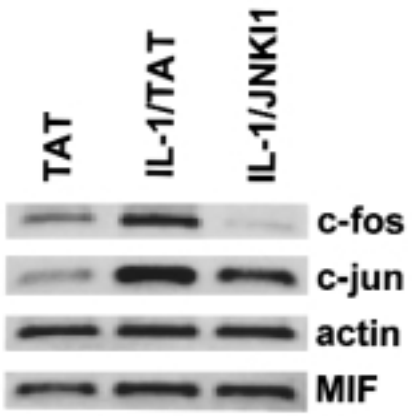

FIG. 6. Inhibition of c-jun and c-fos induction by JNKI1. Reverse transcriptase–PCR analyses were conducted with total RNAs extracted from βTC-3 cells that had been treated as indicated.

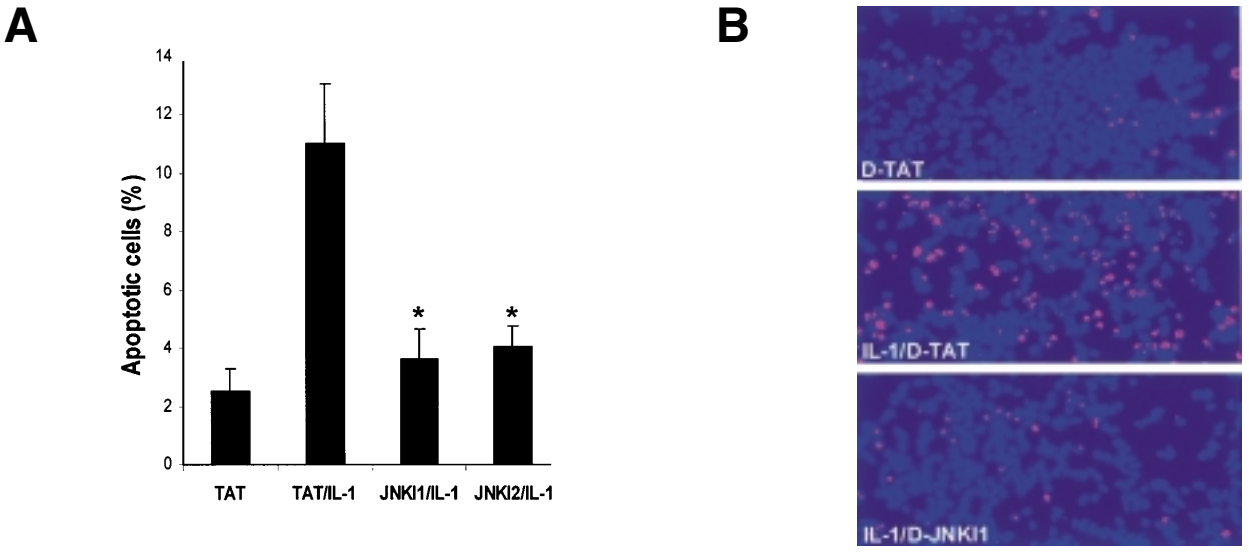

FIG. 7. *A*: Inhibition of IL-1β–induced βTC-3 cell death by JNKI peptides. Cells were incubated for 2 days with IL-1β (10 ng/ml) and the respective peptides (1 μmol/l added once per day) as described in RESEARCH DESIGN AND METHODS. Data are means ± SE from five experiments. *$P < 0.01$ for JNKI/IL-1 conditions relative to TAT/IL-1. *B*: Long-term inhibition of IL-1β–induced cell-death. βTC-3 cells were treated as in *A*, except that incubation with the D-peptides (1 μmol/l at one single addition) and IL-1β was pursued for 15 days; IL-1β was added every other day. Photographs show propidium iodide (red, dead cells) and Hoechst 33342 (blue, cells with intact plasma membrane) staining after 15 days of treatment. $n = 3$.

immune cells surrounding the inflamed islets (28). The extent to which the apoptotic response in vitro (~10% of the cells in presence of IL-1β) corresponds to the in vivo situation is not clear. Nevertheless, this rate of apoptosis (approximately fivefold the rate in the absence of cytokines after 48 h) may significantly contribute to the β-cell loss that develops during the postulated years of exposure of the pancreatic islets of type 1 diabetic patients to IL-1β and potentiating cytokines.

Accumulating evidence indicates that the regulatory intracellular signaling network engaged by the binding of IL-1β and potentiating cytokines (e.g., tumor necrosis factor-α and γ-interferon) to their receptors represents a potential target for the development of novel therapeutic approaches (29–33). Among the most promising tools for the prevention of β-cell loss are a number of large proteins (e.g., Bcl-2 [30]; inhibitors of cytokine signaling such as supressor of cytokine signaling [SOCS] proteins [34]; and the dominant negative versions of MyD88, TNF receptor–associated factor [TRAF], fas-associated death domain protein [FADD], Tollip, or IL-1 receptor–associated kinase [IRAK] [35–37]). These large molecules await their conversion into a form that would allow for their efficient delivery into pancreatic β-cells in vivo.

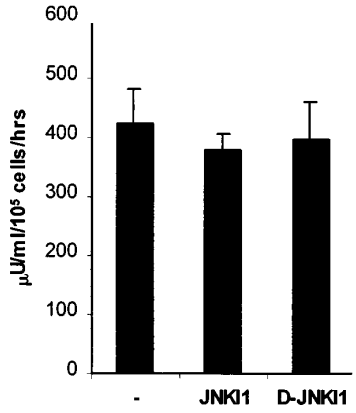

FIG. 8. Insulin secretion of βTC-3 cells at 16.7 mmol/l glucose in the presence and/or absence of peptides as indicated. Cells were first equilibrated in 2.8 mmol/l glucose for 1 h, then the total amount of insulin released for 1 h in a medium containing 16.7 mmol/l glucose was measured. Data are means ± SE for four experiments.

Toward this end, selected recent examples indicated that the conversion of large proteins into small bioactive compounds is amenable to success (38). For example, p16INK4a peptides linked to TAT inhibited hypophosphorylation of the retinoblastoma protein and cell-cycle progression (39). The covalent linkage of a short cell-permeable peptide to a seven–amino acid sequence that contains the nuclear localization signal of the transcription factor nuclear factor (NF)-κB has lead to the production of a cell-permeable peptide (SN50) that blocked translocation of NF-κB after activation by external stimuli (40). Blocking NF-κB protects β-cells from IL-1β–induced apoptosis (29). Similar approaches have been successfully used for blocking activating protein 1 (AP-1), nuclear factor of activated T-cells (NFAT), and signal tranducer and activator of transcription (STAT) 1 nuclear import (41). Biological activity of some of these peptides in animal models has been reported (42). All of these recent successes relied on the observation that the association between signaling molecules might be disrupted intracellularly by an excess of defined peptides derived from the contact domains of the interacting partners. Here we have followed the same approach to convert the 280–amino acid JNKI JBD into a small chemically synthesized and cell-permeable peptide that prevents activation of c-Jun by JNK and blocks apoptosis of the pancreatic β-cell line βTC-3. This new class of biological response modifiers that are involved in cytokine signaling may be applicable to preserve β-cells from autoimmune destruction.

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REFERENCES

1. Whitmarsh AJ, Davis RJ: Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med* 74:589–607, 1996
2. Hibi M, Lin A, Smeal T, Minden A, Karin M: Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7:2135–2148, 1993
3. Gupta S, Campbell D, Derijard B, Davis RJ: Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science* 267:389–393, 1995
4. Whitmarsh AJ, Shore P, Sharrocks AD, Davis RJ: Integration of MAP kinase signal transduction pathways at the serum response element. *Science* 269:403–407, 1995
5. Aguirre V, Uchida T, Yenush L, Davis R, White MF: The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275:9047–9054, 2000
6. Gross A, McDonnell JM, Korsmeyer SJ: BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 13:1899–1911, 1999
7. Tournier C, Hess P, Yang DD, Xu J, Turner TK, Nimnual A, Bar-Sagi D, Jones SN, Flavell RA, Davis RJ: Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* 288:870–874, 2000
8. Ammendrup A, Oberson A, Nielsen K, Andersen N, Serup P, Madsen O, Mandrup-Poulsen T, Bonny C: The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic β-cells. *Diabetes* 49:1468–1476, 2000
9. Negri S, Oberson A, Steinmann M, Nicod P, Waeber G, Schorderet DF, Bonny C: cDNA cloning and mapping of a novel islet-brain/JNK interacting protein. *Genomics* 64:324–330, 2000
10. Serup P, Jensen J, Andersen FG, Jorgensen MC, Blume N, Holst JJ, Madsen OD: Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1. *Proc Natl Acad Sci U S A* 93:9015–9020, 1996
11. Bonny C, Nicod P, Waeber G: IB1, a JIP-1-related nuclear protein present in insulin-secreting cells. *J Biol Chem* 273:1843–1846, 1998
12. Dickens M, Rogers JS, Cavanagh J, Raitano A, Xia Z, Halpern JR, Greenberg ME, Sawyers CL, Davis RJ: A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* 277:693–696, 1997
13. Bonny C, Oberson A, Steinmann M, Schorderet DF, Nicod P, Waeber G: IB1 reduces cytokine-induced apoptosis of insulin-secreting cells. *J Biol Chem* 275:16466–16472, 2000
14. Waeber G, Delplanque J, Bonny C, Mooser V, Steinmann M, Widmann C, Maillard A, Miklossy J, Dina C, Hani EH, Vionnet N, Nicod P, Boutin P, Froguel P: The gene encoding *MAPK8IP1*, encoding islet-brain 1, is a candidate for type 2 diabetes. *Nat Genet* 24:291–295, 2000
15. Yasuda J, Whitmarsh AJ, Cavanagh J, Sharma M, Davis RJ: The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol Cell Biol* 19:7245–7254, 1999
16. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF: In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* 285: 1573–1576, 1999
17. Vives E, Brodin P, Lebleu B: A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *J Biol Chem* 272:16010–16017, 1997
18. Efrat S, Linde S, Kofod H, Spector D, Delannoy M, Grant S, Hanahan D, Baekkeskov S: Beta-cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogene. *Proc Natl Acad Sci U S A* 85:9037–9041, 1988
19. Hoorens A, Van de Casteele M, Kloppel G, Pipeleers D: Glucose promotes survival of rat pancreatic β-cells by activating synthesis of proteins which suppress a constitutive apoptotic program. *J Clin Invest* 98:1568–1574, 1996
20. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
21. Nagahara H, Vocero-Akbani AM, Snyder EL, Ho A, Latham DG, Lissy NA, Becker-Hapak M, Ezhevsky SA, Dowdy SF: Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration. *Nat Med* 4:1449–1452, 1998
22. Vocero-Akbani AM, Heyden NV, Lissy NA, Ratner L, Dowdy SF: Killing HIV-infected cells by transduction with an HIV protease-activated caspase-3 protein. *Nat Med* 5:29–33, 1999
23. Herve M, Maillere B, Mourier G, Texier C, Leroy S, Menez A: On the immunogenic properties of retro-inverso peptides: total retro-inversion of T-cell epitopes causes a loss of binding to MHC II molecules. *Mol Immunol* 34:157–163, 1997
24. Brugidou J, Legrand C, Mery J, Rabie A: The retro-inverso form of a homeobox-derived short peptide is rapidly internalised by cultured neurones: a new basis for an efficient intracellular delivery system. *Biochem Biophys Res Commun* 214:685–693, 1995
25. Angel P, Hattori K, Smeal T, Karin M: The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. *Cell* 55:875–885, 1988
26. Cavigelli M, Dolfi F, Claret FX, Karin M: Induction of c-fos expression through JNK-mediated TCF/Elk-1 phosphorylation. *EMBO J* 14:5957–5964, 1995
27. Kwon G, Corbett JA, McDaniel ML: Interleukin 1-induced Fos and Jun do not regulate inducible nitric oxide synthase in rat islets of Langerhans and RINm5F cells. *Endocrinology* 137:825–830, 1996
28. Mauricio D, Mandrup-Poulsen T: Apoptosis and the pathogenesis of IDDM: a question of life and death. *Diabetes* 47:1537–1543, 1998
29. Stephens LA, Thomas HE, Kay TW: Protection of NIT-1 pancreatic β-cells from immune attack by inhibition of NF-κB. *J Autoimmun* 10:293–298, 1997
30. Rabinovitch A, Suarez-Pinzon W, Strynadka K, Ju Q, Edelstein D, Brownlee M, Korbutt GS, Rajotte RV: Transfection of human pancreatic islets with an anti-apoptotic gene (bcl-2) protects β-cells from cytokine-induced destruction. *Diabetes* 48:1223–1229, 1999
31. Bleich D, Chen S, Zipser B, Sun D, Funk CD, Nadler JL: Resistance to type 1 diabetes induction in 12-lipoxygenase knockout mice. *J Clin Invest* 103: 1431–1436, 1999
32. Welsh M, Christmansson L, Karlsson T, Sandler S, Welsh N: Transgenic mice expressing Shb adaptor protein under the control of rat insulin promoter exhibit altered viability of pancreatic islet cells. *Mol Med* 5:169–180, 1999
33. Chen G, Hohmeier HE, Gasa R, Tran VV, Newgard CB: Selection of insulinoma cell lines with resistance to interleukin-1β- and γ-interferon-induced cytotoxicity. *Diabetes* 49:562–570, 2000
34. Starr R, Hilton DJ: SOCS: suppressors of cytokine signalling. *Int J Biochem Cell Biol* 30:1081–1085, 1998
35. Burns K, Martinon F, Esslinger C, Pahl H, Schneider P, Bodmer JL, Di Marco F, French L, Tschopp J: MyD88, an adapter protein involved in interleukin-1 signaling. *J Biol Chem* 273:12203–12209, 1998
36. Stephens LA, Thomas HE, Ming L, Grell M, Darwiche R, Volodin L, Kay TW: Tumor necrosis factor-α-activated cell death pathways in NIT-1 insulinoma cells and primary pancreatic β-cells. *Endocrinology* 140:3219–3227, 1999
37. Burns K, Clatworthy J, Martin L, Martinon F, Plumpton C, Maschera B, Lewis A, Ray K, Tschopp J, Volpe F: Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. *Nat Cell Biol* 2:346–351, 2000
38. Hawiger J: Noninvasive intracellular delivery of functional peptides and proteins. *Curr Opin Chem Biol* 3:89–94, 1999
39. Gius DR, Ezhevsky SA, Becker-Hapak M, Nagahara H, Wei MC, Dowdy SF: Transduced p16INK4a peptides inhibit hypophosphorylation of the retinoblastoma protein and cell cycle progression prior to activation of Cdk2 complexes in late G1. *Cancer Res* 59:2577–2580, 1999
40. Lin Y-Z, Yao S, Veach RA, Torgerson TR, Hawiger J: Inhibition of nuclear translocation of transcription factor NF-κB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *J Biol Chem* 270:14255–14258, 1995
41. Torgerson TR, Colosia AD, Donahue JP, Lin YZ, Hawiger J: Regulation of NF-kappa B, AP-1, NFAT, and STAT1 nuclear import in T lymphocytes by noninvasive delivery of peptide carrying the nuclear localization sequence of NF-kappa B p50. *J Immunol* 161:6084–6092, 1998
42. Liu XY, Robinson D, Veach RA, Liu D, Timmons S, Collins RD, Hawiger J: Peptide-directed suppression of a pro-inflammatory cytokine response. *J Biol Chem* 275:16774–16778, 2000