Tumor Necrosis Factor Alpha Signaling Pathway and Apoptosis in Pancreatic β Cells

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Cytokines induce apoptosis in pancreatic β cells, but the exact mechanisms and sequence of events are not clear. Here, we investigate a role for tumor necrosis factor alpha (TNF-α) in the apoptosis of β cells. Using the ribonuclease (RNase) protection assay and the reverse transcriptase–polymerase chain reaction (RT-PCR) method, we confirmed that TNF receptor 1 (TNFR1), TNFR1-associated death domain protein (TRADD), Fas receptor–associated intracellular protein with death domain (FADD), and FADD-like interleukin-1β–converting enzyme (FLICE) were expressed in the pancreatic β cell line, MIN6 cells. Fluorescent microscopic examination using Hoechst 33342 dye (Sigma, St Louis, MO) demonstrated that TNF-α induced time- and dose-dependent apoptotic nuclear changes in these β cells. In situ end-labeling (ISEL) DNA analysis revealed that 10 nmol/L TNF-α generated new 3′-OH DNA strand breaks. Moreover, qualitative assessment of the induced DNA damage on agarose gels showed that 10 nmol/L TNF-α produced characteristic apoptotic patterns of DNA fragments formed by internucleosomal hydrolysis of static chromatin. In addition, C2-ceramides and natural ceramides dispersed in a solvent mixture of ethanol and dodecane induced characteristic features of apoptosis in MIN6 cells, mimicking TNF-induced DNA damage. We also determined endosomal ceramide production after TNF-α (10 nmol/L) treatment in MIN6 cells using the diacylglycerol kinase assay. These results suggest that TNF-α can cause apoptosis in pancreatic β cells through TNFR1-linked apoptotic factors, TRADD, FADD, and FLICE, and TNF-induced ceramide production may be involved in the pathways.

TYPE 1 DIABETES is a chronic disorder that results from destruction of pancreatic β cells. Although the component of the immune system that is responsible for the destruction of β cells has not been identified, expression of cytokines by cells within inflamed islets may have an important pathogenic function.[1] A combination of cytokines, including interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), has been shown to induce internucleosomal DNA fragmentation and islet cell death.[2] Internucleosomal DNA fragmentation is an early event in cytokine-induced β-cell destruction, but the mechanisms by which cytokines induce DNA damage in β cells remain to be elucidated.

TNF is a proinflammatory cytokine whose pleiotropic biologic properties are mediated through two distinct cell-surface receptors, TNFR1 and TNFR2.[3] TNFR1 can induce apoptosis through a death domain in its cytoplasmic region.[4] Recent studies have demonstrated three receptor-associated intracellular proteins, TNFR1-associated death domain protein (TRADD), Fas-associating protein with death domain (FADD), and receptor-interacting protein (RIP), that all contain death domains and interact with the death domain of TNFR1 and Fas.[5-7] The binding of TNF to its cell-surface receptor activates a complex signal transduction pathway, although the precise biochemical events leading to TNF-elicited cell death are poorly understood. Also, sphingomyelin hydrolysis and ceramide generation have been implicated in a signal transduction pathway that mediates the effects of TNF-α.[8-11] To clarify the mechanisms of TNF-α–induced apoptosis in pancreatic β cells, we examined whether TNFR1, FADD, and TRADD are expressed in the cells and then assessed whether ceramide functions as an intracellular mediator of this process.

MATERIALS AND METHODS

Cell Culture

The MIN6 cell line was established from an insulinoma developed in a transgenic mouse harboring the human insulin promoter gene fused to the simian virus 40 T-antigen gene.[12] The regulation of glucose-induced insulin and amylin secretion in this cell line resembles that of normal β cells.[13] MIN6 cells were maintained in culture in Dulbecco's modified Eagle's medium (DMEM) containing 25 mmol/L glucose supplemented with 15% heat-inactivated fetal bovine serum (GIBCO Oriental, Tokyo, Japan) and 1% penicillin-streptomycin (GIBCO). The cells were incubated in humidified 5% CO_2/95% air at 37°C.

Cytokines and Chemicals

Recombinant murine TNF-α was purchased from R&D Systems (Minneapolis, MN). Natural ceramide type III (from bovine brain sphingomyelin) and type IV (from cerebroside) were purchased from Sigma (St Louis, MO). C2-ceramide was obtained from Molecular Probes (Eugene, OR) and dodecane from Wako (Osaka, Japan). All ceramides were dissolved in ethanol or in a solvent mixture of ethanol:dodecane (98:2 vol/vol). The solution was added to culture medium with final ethanol and dodecane concentrations of 0.49% and 0.01%, respectively.[14]

Reverse Transcriptase–Polymerase Chain Reaction Amplification and Sequencing of TNFR1, TRADD, and FADD

RNA was isolated from MIN6 cells using the acid-guanidinium thiocyanate phenol-chloroform procedure. cDNA was prepared using 5 μg total RNA and 80 pmol $pd(N)_6$ primer (Takara, Otsu, Siga, Japan) in a 20-μL solution containing 50 mmol/L Tris hydrochloride, pH 8.3, 75 mmol/L KCl, 3 mmol/L $MgCl_2$, 10 mmol/L dithiothreitol, 0.125 mmol/L of each dNTP, 40 U RNasin (Promega, Madison, WI), and 200

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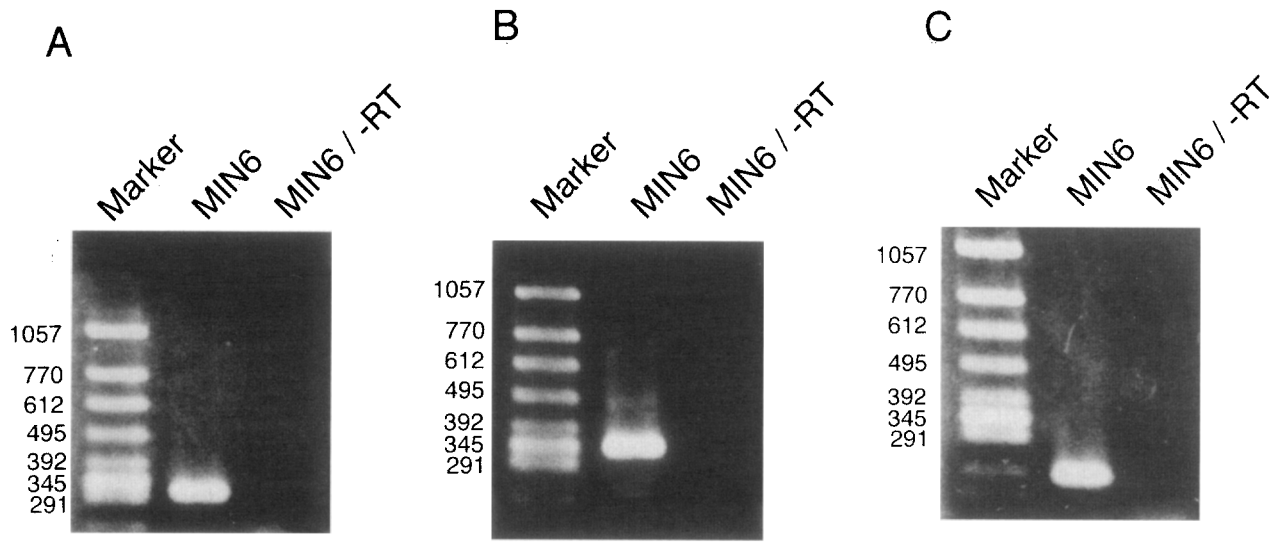

Fig 1. Detection of TNFR1, TRADD, and FADD transcripts in the pancreatic β-cell line MIN6 by RT-PCR. cDNA prepared using total RNA isolated from MIN6 cells was amplified with the mouse TNFR1, TRADD, and FADD primers. The TNFR1 PCR product (A), TRADD PCR product (B), and FADD PCR product (C) were isolated and cloned, and 2 independently isolated clones were sequenced. No PCR products were amplified in the absence of RT (-RT). Marker, ϕX174/*Hin*cII digest.

U Moloney murine leukemia virus reverse transcriptase (RT) (GIBCO). After incubation for 1 hour at 37°C, the reaction mixture was incubated at 65°C for 5 minutes to inactivate the RT. cDNA was amplified with mouse TNFR1 primers (GenBank accession no. M60468) TNFR1-F, 5′-TGTTCAGAAATGGGAAGACTC-3′ (1208 to 1228), and TNFR1-R, 5′-CTCAGAGCCTCGAGGATATTC-3′ (1499 to 1519); mouse TRADD primers (no. AA013629) TRADD-F, 5′-TAGGCCAGGCCGCCATCC-GGCTC-3′ (167 to 189), and TRADD-R, 5′-CCAGACTTTTCTGTTC-CACGG-3′ (489 to 509); and FADD primers (no. U43184) FADD-F, 5′-GAGAGACTGGAAAAGACTGGC-3′ (327 to 347), and FADD-R, 5′-CACCAGGTCAGCCACCAGATTCA-3′ (509 to 531). Polymerase chain reaction (PCR) was performed in the Gene Amp PCR system 9600 (Perkin Elmer, Norwalk, CT) in a 50-μL vol containing cDNA, dNTP, 10 pmol of the primer, 50 mmol/L KCl, 10 mmol/L Tris hydrochloride, pH 8.3, 1.5 mmol/L $MgCl_2$, and 1 U *Taq* polymerase. The PCR conditions were an initial denaturation at 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension step at 72°C for 10 minutes. Because RNA samples prepared using the acid-guanidinium thiocyanate phenol-chloroform procedure are usually contaminated by a small amount of genomic DNA and the target genes TNFR1 and FADD have no intron, we performed a RT-PCR control experiment without using RT to discriminate PCR products from those of contaminated genomic DNA. The PCR products were cloned into pGEM-T (Promega), and two independently isolated clones were sequenced using the Sequenase Version 2.0 sequencing kit (US Biochemical, Cleveland, OH).

Ribonuclease Protection Assay

Protection assays were performed using the RiboQuant multiprobe ribonuclease (RNase) protection assay system, mouse cytokine/chemokine receptor sets (mCR4), and a mouse apoptosis set (mAPO3; PharMingen, San Diego, CA). Riboprobes were synthesized and labeled using these templates, [α-^{32}P]UTP, and T7 polymerase according to the manufacturer's instructions. Five micrograms of total RNA isolated from MIN6 cells was resuspended in 8 μL hybridization buffer to which 2 μL of the labeled probe (300,000 to 400,000 cpm/μL) was added. The hybridization mixture was heated to 90°C for 2 minutes and incubated at 45°C for 16 hours. After hybridization, free probes and other single-strand RNAs were digested with RNase digestion cocktail.

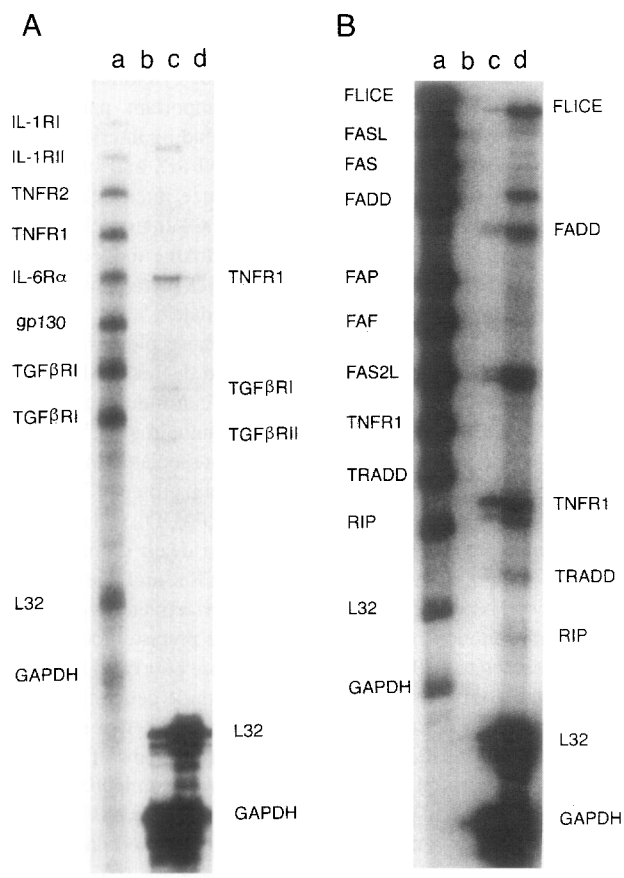

Fig 2. Detection of TNFR1, TRADD, FADD, RIP, and other transcripts in MIN6 cells by RNase protection assay. Protection assays were performed using the RiboQuant multiprobe RNase protection assay system, mouse cytokine/chemokine receptor set (mCR4) (A), and mouse apoptosis set (mAPO3) (B). Five micrograms of yeast total RNA (PharMingen) (lane b), total RNA isolated from MIN6 cells (lane c), and mouse control RNA (PharMingen) (lane d) were used in the assays. The probe set was diluted (3,000-4,000 cpm/lane) and loaded (lane a).

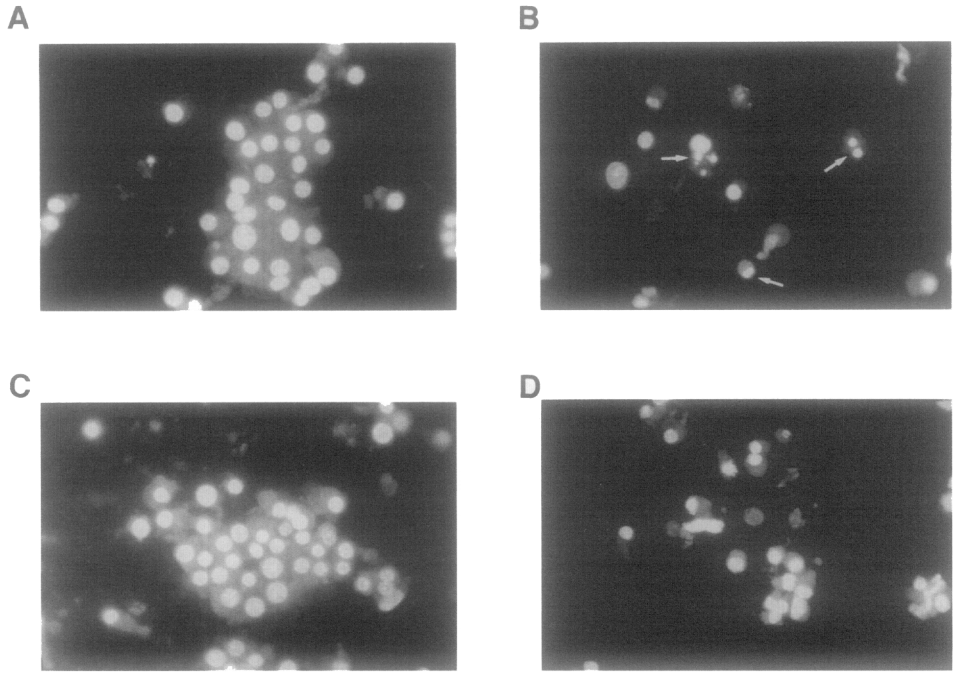

Fig 3. Apoptotic nuclear changes (arrows) in MIN6 cells detected by fluorescent microscopic examination. MIN6 cells were treated with DMEM (A), TNF-α 10 nmol/L (B), ethanol and dodecane (C), or natural ceramide type IV 200 nmol/L (D). MIN6 cells were incubated for 12 hours and then stained with Hoechst 33342 dye.

The remaining RNase protected probes were purified and resolved on a 5% polyacrylamide/8-mol/L urea denaturing gel. The gel was run at 50 W constant power with 0.5× TBE (1× TBE is 89 mmol/L Tris, 89 mmol/L boric acid, and 2 mmol/L EDTA), dried, and exposed to Kodak X-Omat AR film (Kodak, Rochester, NY) or a Fuji imaging plate (BAS-III; Fujifilm, Tokyo, Japan) that was analyzed by a BAS-2000II (Fujifilm).

Fluorescent Microscopic Examination

Apoptotic nuclear changes were assessed by fluorescent microscopic examination using Hoechst 33342 dye (Sigma).[15,16] MIN6 cells (10^5/0.5 mL) were seeded in a 24-well plate for 48 hours before experimentation and treated with ceramides or TNF-α. The cells were scraped from the plates and then stained with Hoechst 33342 dye. Cells were viewed by an inverted fluorescence microscope. Apoptotic cells were evaluated by nuclear shrinkage, fragmentation, and chromatic condensation. At least 800 cells were assessed in 30 contiguous fields. The percentage of apoptotic cells was assessed in each field. Two independent experiments were performed, and thus the sample number was 60. The data were tested by the Mann-Whitney *U* test.

In Situ End-Labeling of Fragmented DNA

New 3′-OH DNA ends generated by DNA fragmentation and typically localized in morphologically identifiable nuclei and apoptotic bodies were detected by an Apop Tag Kit (Oncor, Gaithersburg, MD).[17-19] The 3′-OH DNA strand breaks were catalytically labeled with digoxigenin-nucleotide via terminal digoxynucleotidyl transferase (TdT) and subsequently exposed to peroxidase-conjugated anti-digoxigenin antibody. Staining was developed in diaminobenzidine-H_2O_2; and specimens were counterstained with methyl green.

DNA Extraction and Gel Electrophoresis

The cells (5×10^6) were treated with 10 nmol/L TNF-α and 200 nmol/L ceramide type IV, washed with cold phosphate-buffered saline, suspended in a lysis buffer (5 mmol/L Tris hydrochloride, 20 mmol/L EDTA, 0.5% Triton X-100, and 1% sodium dodecyl sulfate, pH 8.0) containing proteinase K (Boehringer Mannheim, Tokyo, Japan), and incubated overnight at 37°C. DNA was extracted with phenol: chloroform:isoamyl alcohol, precipitated with ethanol and 0.3 mol/L NaCl, resuspended in 10 mol/L Tris hydrochloride, and 1 mmol/L EDTA, pH 7.5, and treated with 0.6 mg/mL DNase-free RNase A (Wako) at 37°C for 30 minutes. The DNA solution was electrophoresed on 2% agarose gel and visualized with ethidium bromide.

Ceramide Determination

Ceramide concentrations were measured in MIN6 cells by a modification of the diacylglycerol kinase assay.[20-22] The cells (3×10^6) were washed twice, and lipids were extracted by the method of Bligh and Dyer. The dried lipids were solubilized in 20 μL 7.5% (wt/vol) octyl-β-D-glucopyranoside (Calbiochem, San Diego, CA) and 25 mmol/L L-α-phosphatidyl-DL-glycerol dioleoyl (Avanti polar lipids, Alabaster, AL). After vortexing, 50 μL reaction mixture (100 mmol/L imidazole, pH 6.6, 100 mmol/L LiCl, 25 mmol/L $MgCl_2$, and 2 mmol/L EGTA, pH 6.6), 2 μL 100-mmol/L dithiothreitol, 1.7 μL diacylglycerol kinase (Calbiochem), 16.3 μL dilution buffer (50 mmol/L imidazole, pH 6.6, and 1 mmol/L diethylenetriaminepentaacetic acid) and 10 μL 2-mmol/L 4-μCi [γ-^{32}P]ATP were added to initiate the reaction. The reaction was continued for 30 minutes at room temperature. Lipids were extracted again, dried under N_2 gas, and resuspended in 20 μL chloroform. A 10-μL aliquot was spotted onto a Silica Gel TLC plate (Merck, Darmstadt, Germany) and developed with chloroform:acetone: methanol:acetic acid:water (10:4:3:2:1). The TLC plate was dried and exposed to Kodak X-Omat AR film. Radioactive spots corresponding to ceramide-1-phosphate were quantified using the NIH Images program.

Statistical Analysis

All data were compared using the Mann-Whitney *U* test and are expressed as the mean ± SE.

RESULTS

Using cDNA isolated from MIN6 cells, approximately 200- to 340-bp PCR products were amplified using TNFR1 (Fig 1A), TRADD (Fig 1B), and FADD primers (Fig 1C), respectively. PCR products were not detected in RT-PCR control experiments without using RT. Sequence analysis of the two PCR products

demonstrated that they were partial mouse TNFR1, TRADD, and mouse FADD,[23,24] indicating that the TNFR1, TRADD, and FADD genes are transcribed in MIN6 cells.

Because these target genes have no intron and the expression level of these genes may be low, RNase protection assays with greater sensitivity than Northern blot analysis were performed to detect and qualify the mRNA of these genes. In these assays, the protective bands corresponding to TNFR1 (Fig 2A and B), FADD, TRADD, and RIP (Fig 2B) were observed, suggesting the expression of these mRNAs in MIN6 cells. In addition, transforming growth factor beta (TGF-β) receptor and FADD-like ICE protease (FLICE) mRNAs were confirmed to be expressed in MIN6 cells.

Characteristic apoptotic nuclear changes such as shrinkage, fragmentation, and chromatic condensation were assessed by fluorescent microscopic examination in MIN6 cells treated with TNF-α (10 nmol/L; Fig 3B), C2-ceramide (10 μmol/L), ceramide type III (200 and 800 nmol/L), and ceramide type IV (200 nmol/L; Fig 3D) after 12 hours. All ceramides were dissolved in ethanol or in a mixture of ethanol and dodecane.

Apoptotic changes in the nuclei were significantly increased by the addition of 2 nmol/L TNF-α for 12 hours ($P < .0001$; Fig 4A) and were dependent on the dose increase from concentrations of 2 to 10 nmol/L. TNF-α (10 nmol/L) induced significant apoptotic changes after a 6-hour incubation, and these changes increased with the time of incubation ($P < .0001$; Fig 4B). C2-ceramide (10 μmol/L) induced apoptosis when dissolved in a mixture of ethanol and dodecane but not when dissolved in ethanol alone (Fig 5A). Additionally, both ceramide type III (800 nmol/L) and type IV (200 nmol/L) dissolved in a mixture of ethanol and dodecane induced the characteristic apoptotic nuclear changes after a 12-hour incubation ($P < .0001$; Fig 5B), but did not induce nuclear changes when dissolved in ethanol. At a concentration of 200 nmol/L, ceramide type IV was more effective than type III for inducing apoptosis. Apoptotic changes in MIN6 cells treated with 800 nmol/L ceramide type III or 200 nmol/L ceramide type IV were detected after a 6-hour incubation and increased with time (Fig 5C).

In situ end-labeling (ISEL) of fragmented DNA was used to detect new 3′-OH DNA ends generated by DNA fragmentation typically localized in morphologically identifiable nuclei and apoptotic bodies.[17,18] 3′-OH DNA strand breaks were observed in MIN6 cells treated with 10 nmol/L TNF-α (Fig 6B) and 200 nmol/L ceramide type IV (Fig 6D). Qualitative analysis of DNA damage was assessed by the electrophoretic pattern of DNA fragmentation on agarose gels as previously described.[25] The characteristic pattern of internucleosomal DNA fragmentation was observed in cells treated with 10 nmol/L TNF-α (Fig 7A) and 200 nmol/L ceramide type IV (Fig 7B).

To determine whether TNF-α induced ceramide production, MIN6 cells were treated with TNF-α and the level of endogenous ceramide was measured (Fig 8A and B). Ceramide levels increased to 466% ± 57% ($P = .0062$ *v* time 0) of the control level 1 hour after treatment and returned to 209% ± 11% ($P = .0201$ *v* time 0) of the control level by 4 hours (Fig 8B).

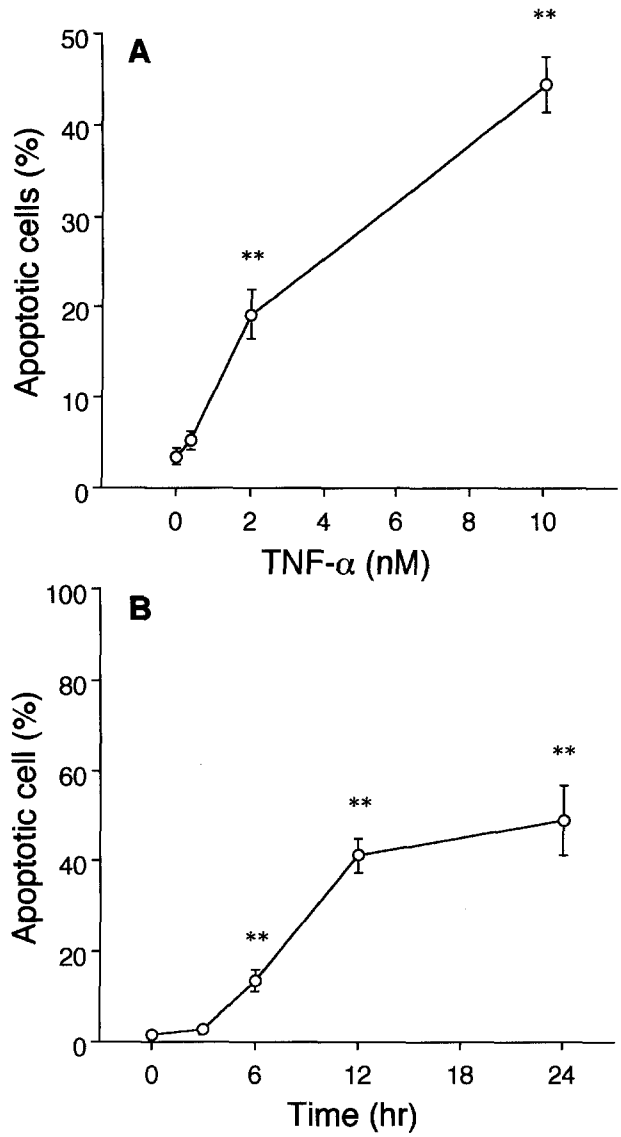

Fig 4. Dose-dependent effect of TNF-α on apoptotic nuclear changes in MIN6 cells following a 12-hour incubation (A). Time-dependent effect of TNF-α (10 nmol/L, 170 ng/mL) on apoptotic nuclear changes in MIN6 cells (B). Apoptotic nuclear changes were quantified by fluorescent microscopic examination. The percentage of apoptotic cells was determined by assessing at least 800 cell nuclei in 2 separate experiments. Results are expressed as the mean ± SE. **$P < .0001$ *v* apoptotic effects in the absence of TNF-α (A) and at time 0 (B).

DISCUSSION

We determined that TNFR1, TRADD, FADD, and FLICE were expressed in the pancreatic β cell line MIN6. TNF-α induced internucleosomal DNA fragmentation and characteristic apoptotic nuclear changes in the cells, as well as C2- and natural ceramides dispersed in a solvent mixture of ethanol and dodecane. We also demonstrated that exposure of MIN6 cells to TNF-α caused ceramide production. These findings suggest that TNF-α induces apoptotic death in pancreatic β cells through TNFR1-linked apoptotic factors, TRADD, FADD, and FLICE, and ceramide may be involved in the pathway.

TNF-α alone generated new 3′-OH DNA strand breaks and induced internucleosomal DNA fragmentation and characteristic apoptotic nuclear changes in MIN6 cells. Delaney et al[26]

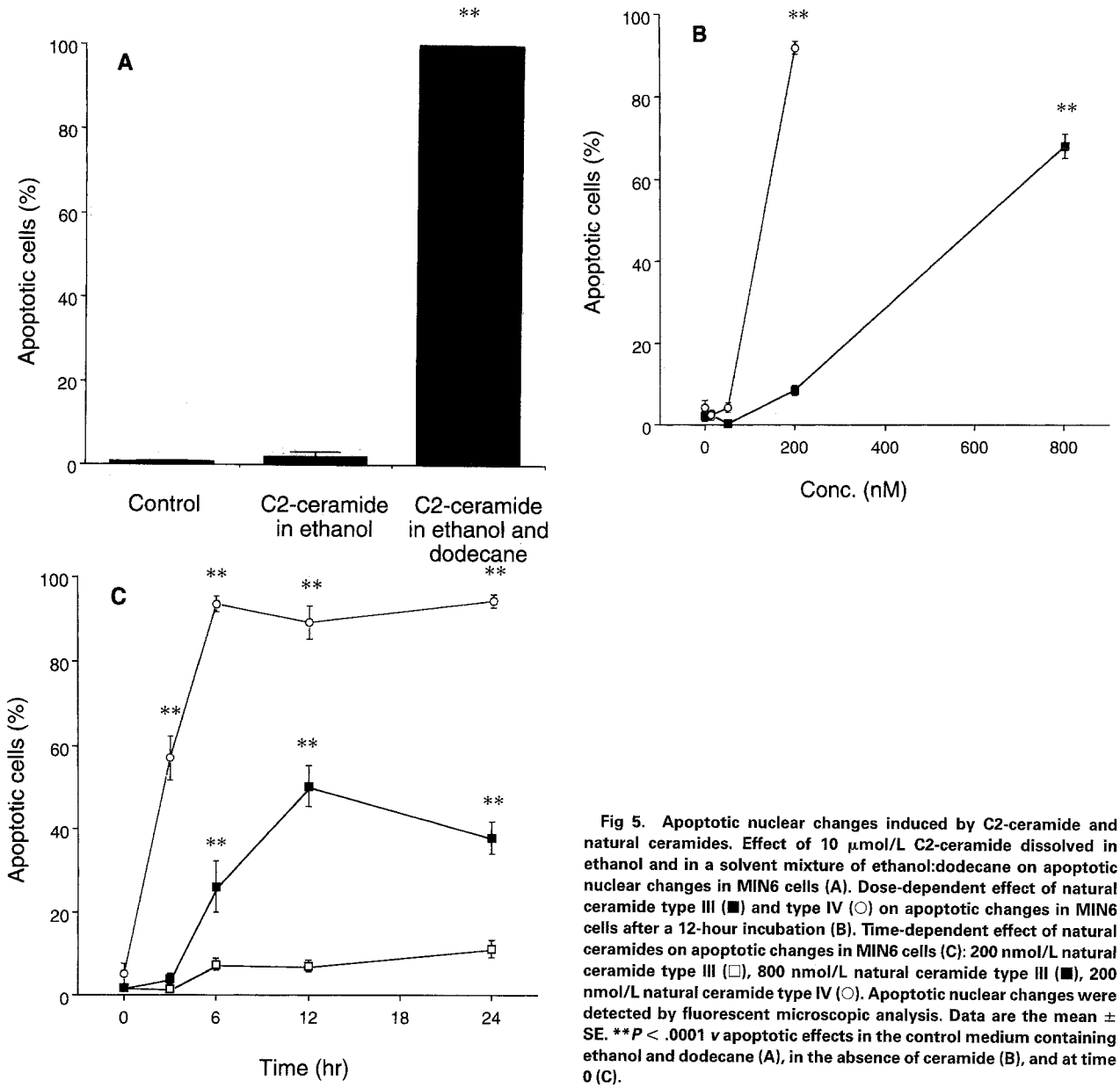

Fig 5. Apoptotic nuclear changes induced by C2-ceramide and natural ceramides. Effect of 10 μmol/L C2-ceramide dissolved in ethanol and in a solvent mixture of ethanol:dodecane on apoptotic nuclear changes in MIN6 cells (A). Dose-dependent effect of natural ceramide type III (■) and type IV (○) on apoptotic changes in MIN6 cells after a 12-hour incubation (B). Time-dependent effect of natural ceramides on apoptotic changes in MIN6 cells (C): 200 nmol/L natural ceramide type III (□), 800 nmol/L natural ceramide type III (■), 200 nmol/L natural ceramide type IV (○). Apoptotic nuclear changes were detected by fluorescent microscopic analysis. Data are the mean ± SE. **$P < .0001$ *v* apoptotic effects in the control medium containing ethanol and dodecane (A), in the absence of ceramide (B), and at time 0 (C).

demonstrated that IL-1β at a concentration of 0.1 nmol/L caused DNA damage in rat islets and a hamster insulinoma cell line. In contrast, Rabinovitch et al[2] reported that a combination of IL-1β, TNF-α, and IFN-γ induced DNA fragmentation and islet cell death; however, if used individually, these cytokines failed to induce DNA damage in RINm5F cells and NIT-1 cells. Although the differences in these studies may be related to the sensitivity of the assay used to assess DNA damage, they may also be related to differences in the various cell types. As such, although it has been demonstrated that TNF-α can induce apoptosis in monoblastic leukemia cells and other cell lines,[27] there is a wide variability in the susceptibility of cells to TNF-α–induced cell death.[28] The sensitivity of cells to TNF-α has been proposed to be regulated by certain oncogenes.[29-31] Previous studies[2,26] and this study assessing apoptosis in β cells used various transformed cell lines, and it is therefore possible that in some of these cell lines there are defects in TNF-α signaling.

TNFR1 is expressed on most cell types.[32] However, the expression of TNFR1 has not been previously examined in transformed pancreatic β cells.[2,26] This study is the first demonstration that TNFR1, TRADD, FADD, and FLICE are expressed in transformed pancreatic β cells. Apoptosis processes mediated by TNFR1 and Fas are similar in that both signaling cascades are initiated by death domains and activate interleukin-converting enzyme (ICE)-like proteases.[33] TRADD interacts specifically with the death domain of TNFR1,[5] and FADD interacts with the death domain of Fas.[6] TRADD also interacts with FADD and recruits it to TNFR1.[24] A dominant negative derivative of FADD has been shown to block TNF-induced apoptosis,[34] suggesting that FADD is involved in TNFR1-TRADD–mediated apoptosis. FLICE (also called

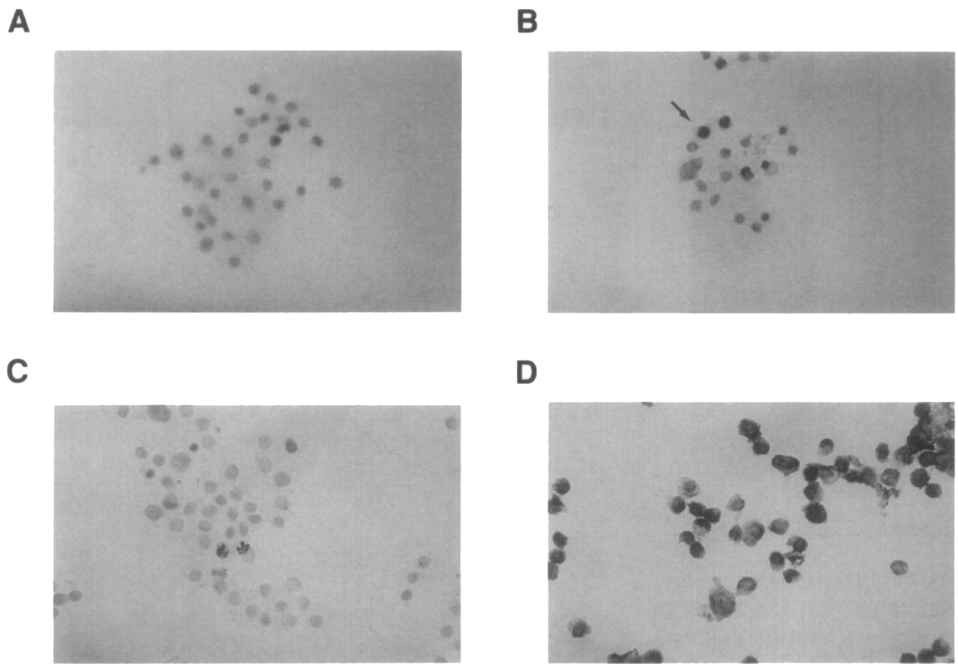

Fig 6. Generation of 3′-OH DNA strand breaks (arrows) in MIN6 cells treated with DMEM (A), TNF-α 10 nmol/L (B), ethanol and dodecane (C), and natural ceramide type IV 200 nmol/L (D). Cells were stained with the Apop Tag kit after a 12-hour incubation.

MACH), a member of the ICE/CED-3 family, binds FADD and activates an apoptotic cascade.[23,35] Thus, TRADD, FADD, and FLICE are likely important factors for TNFR1-mediated apoptosis in pancreatic β cells.

Obeid et al[10] demonstrated that C2-ceramide, a synthetic cell-permeable ceramide analog, dispersed in ethanol induced DNA fragmentation in the U937 monoblastic leukemia cells in which TNF-α causes apoptosis. However, C2-ceramide dispersed in ethanol failed to induce DNA damage and apoptotic nuclear changes in MIN6 cells. Because the fatty acid chain length of C2-ceramide is shorter than that of natural ceramide, the affinity of this synthetic agent for its targets may be lower than that of natural ceramide. Alternatively, C2-ceramide may not be delivered into MIN6 cells sufficiently to induce DNA damage. Ji et al[14] reported that a solvent mixture of ethanol and dodecane dispersed natural ceramide into aqueous solution. Thus, we dissolved C2- and natural ceramides in this solvent mixture of ethanol and dodecane and found that they all induced time- and dose-dependent apoptotic nuclear changes in MIN6 β cells. These results indicate that ceramides, when fully dispersed into an aqueous solution, can induce apoptotic changes in MIN6 cells.

Exogenous ceramides induce internucleosomal DNA fragmentation in leukemia cells[10,36] and in rat pheochromocytoma PC12 cells.[37] Our study demonstrates that C2- and natural ceramides generated new 3′-OH DNA strand breaks and induced inter-

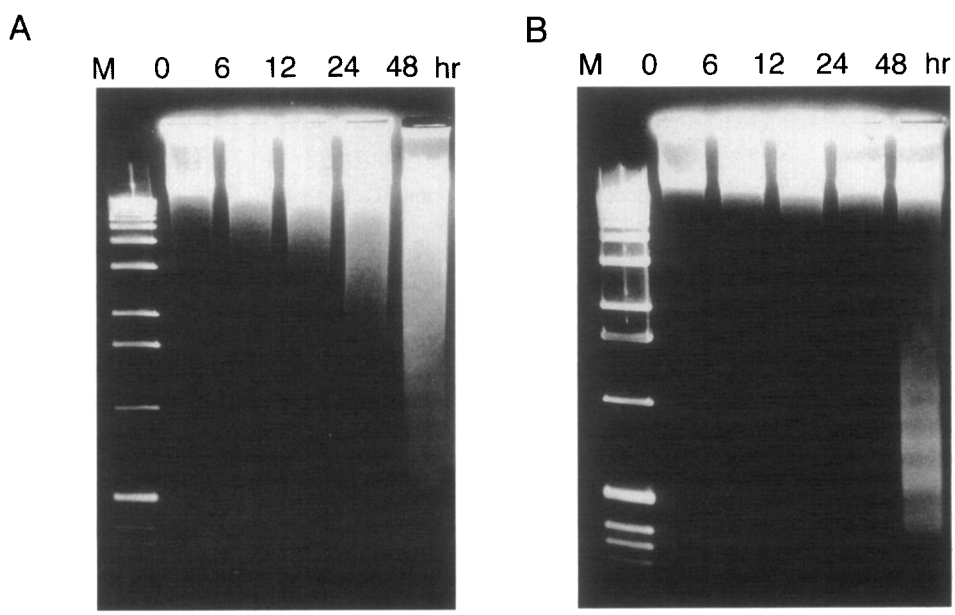

Fig 7. Characteristic electrophoretic patterns of DNA fragments formed by internucleosomal hydrolysis of static chromatin in MIN6 cells treated with TNF-α (A) and ceramide type IV (B). DNA extracted from 5 × 10⁶ MIN6 cells treated with 10 nmol/L TNF-α and 200 nmol/L ceramide type IV for 6, 12, 24, and 48 hours was electrophoresed on a 2% agarose gel and visualized with ethidium bromide.

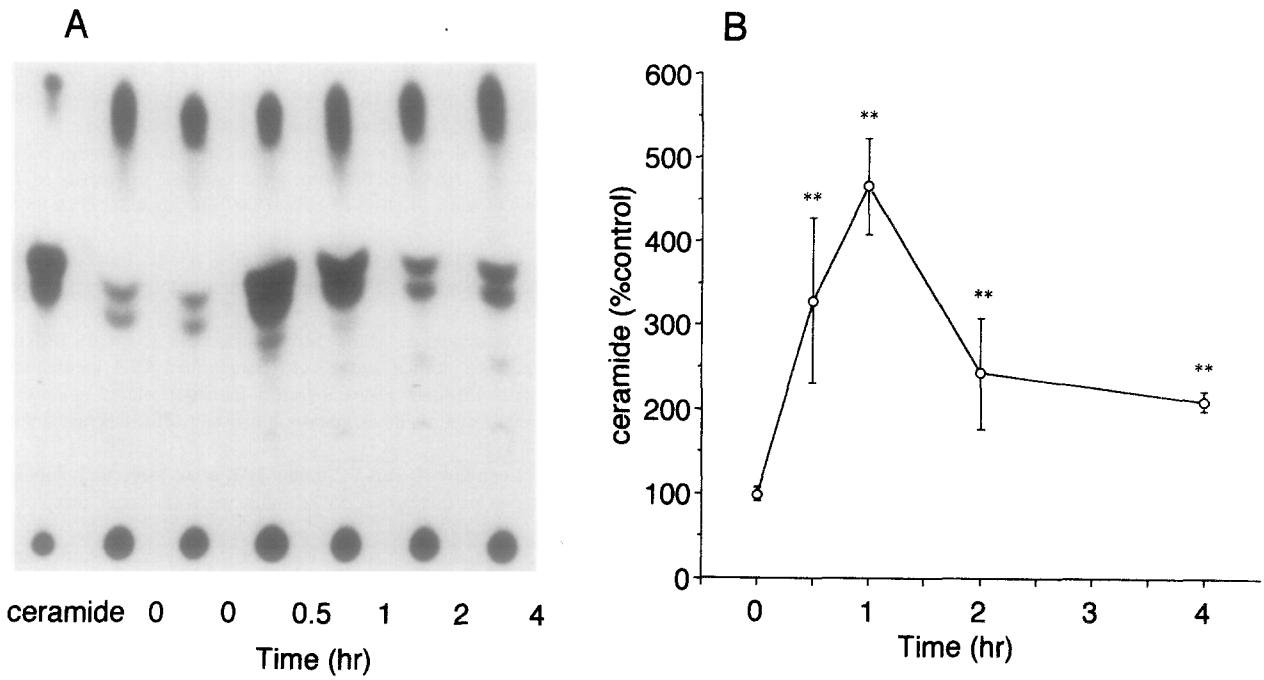

Fig 8. Ceramide production induced by TNF-α (10 nmol/L, 170 ng/mL) in MIN6 cells detected by diacylglycerol kinase assay (A). Lipids were extracted from MIN6 cells treated with 10 nmol/L TNF-α for 30 minutes and 1, 2, and 4 hours. Ceramide concentrations were measured by a modification of the diacylglycerol kinase assay. Arrows indicate radioactive spots corresponding to ceramide-1-phosphate. Ceramide, natural ceramide type III (425 pmol/L). Time-dependent effect of TNF-α 10 nmol/L on endogenous ceramide in MIN6 cells (B). Values are the mean ± SE of 5 experiments. **$P < .05$ *v* time 0 (B).

nucleosomal DNA fragmentation in MIN6 cells, mimicking TNF-α–induced DNA damage. TNF-α activates membrane sphingomyelinase, which produces ceramide from sphingomyelin.[38] This process likely occurs through TNFR1, which triggers a Mg^{2+}-dependent membrane neutral sphingomyelinase.[39-41] We have determined endosomal ceramide production after TNF-α treatment in the pancreatic β cell line MIN6. Thus, TNF-α may induce sphingomyelinase hydrolysis and ceramide production through TNFR1 in MIN6 cells. Ceramide activates proteases of the ICE family.[42] Therefore, ceramide is involved in DNA damage induced by TNF-α in the pancreatic β cells.

In conclusion, we have demonstrated that TNF-α–linked apoptosis factors are expressed in pancreatic β cells, and ceramide production is one of the important factors responsible for TNF-α–induced apoptosis in pancreatic β cells.

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