Essential Role of Caspase-3 in Apoptosis of Mouse β-Cells Transfected With Human Fas

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Several recent studies have indicated that the Fas-Fas ligand system may be critical for pancreatic β-cell destruction in type 1 diabetes. Although the fundamental roles of caspases in the mammalian apoptotic machinery have been elucidated, it is not known which caspase or caspases play a major role in Fas-mediated apoptosis of β-cells. In this study, we transfected human Fas cDNA into a mouse β-cell line (βTC1) and established a β-cell clone expressing human Fas. This clone, designated hFas/βTC1, underwent apoptosis when exposed to anti-Fas, showing hallmarks of apoptosis (chromatin condensation, nucleolar disintegration, internucleosomal DNA fragmentation, and annexin V staining), indicating that the mouse β-cell line has the intact machinery of Fas-mediated apoptosis. The cross-linking of Fas by anti-Fas resulted in the elevation of caspase-3–like, but not caspase-1–like, protease activity 2–12 h after the addition of the anti-Fas. A caspase-3 inhibitor, Z-Asp-Glu-Val-Asp-fluoromethyl ketone, attenuated the Fas-mediated β-cell apoptosis, while a caspase-1 inhibitor, acetyl-Tyr-Val-Ala-Asp-chloromethylketone, failed to suppress the apoptosis. Thus the Fas-induced death signal apparently bypassed caspase-1 in the cells. Furthermore, an antisense caspase-3 construct blocked caspase-3 activation and substantially suppressed Fas-triggered apoptosis of hFas/βTC1 cells. These observations suggest the essential role of caspase-3 in Fas-mediated apoptosis of the β-cell line. *Diabetes* 48:478–483, 1999

Type 1 diabetes is a cellular autoimmune disease resulting from a selective destruction of pancreatic β-cells. T-cell cytotoxicity is mediated by two complementary lytic pathways: the delivery of perforin-containing granules on target cells and the engagement of Fas (Apo-1/CD95) on target cells by membrane-bound or soluble Fas ligand (1–3). Fas is also involved in cytotoxicity by natural killer cells (4,5). Recent studies have suggested that the Fas-Fas ligand system may be critical for β-cell destruction in autoimmune insulitis (6–9). Although mouse islet cells do not constitutively express Fas, treatment with interleukin (IL)-1 induces Fas gene transcription and Fas expression by islet cells (6). IL-1–treated islet cells undergo apoptosis when exposed to anti-Fas antibody (6). It is difficult to detect autoimmune β-cell death in vivo, probably because of the slow kinetics of the inflammatory process and rapid clearance of dead cells by macrophages. However, β-cell apoptosis could be observed histologically in accelerated insulitis of NOD scid mice transferred with islet cell–reactive $CD4^+$ T-cells (7). The expression of Fas by β-cells in vivo was induced by the transfer of islet cell–specific $CD8^+$ T-cells, probably through cytokine release by infiltrated cells (8). In NOD mice, spontaneous β-cell destruction could be prevented by introduction of the Fas-disrupting *lpr* mutation (8,9). The most direct evidence that Fas is involved in the effector phase of autoimmune β-cell destruction is the demonstration that NOD-*lpr/lpr* mice were resistant to the transfer of islet cell–specific $CD8^+$ T-cells (8) or splenocytes from diabetic NOD mice (9).

Accumulating evidence has revealed that an essential component in the apoptosis-inducing machinery is the caspases, a family of cysteine proteases (10,11). Cross-linking of the Fas receptor with Fas ligand or anti-Fas antibody results in oligomerization of the receptors and formation of a death-inducing signaling complex consisting of FADD/MORT1 and pro–caspase-8 (MACH/FLICE/Mch5) (12). The activation of caspase-8 causes sequential proteolytic activation of other caspases. Each caspase family protease becomes active when the precursor is cleaved into a large subunit with a molecular mass of ~20 kDa and a small subunit with a molecular mass of ~10 kDa and forms a tetramer consisting of two large and two small subunits (13). The prototype subfamily of caspases are the caspase-1 (IL-1β–converting enzyme)-like proteases. Evidence for the involvement of caspase-1 in Fas-mediated apoptosis was derived from caspase-1–deficient mice, which developed normally but showed a marked reduction in Fas-mediated apoptosis of thymocytes (14). Studies with peptide inhibitors or antisense caspase-1 constructs have shown that caspase-1 or caspase-1–like proteases are involved in Fas-mediated cell death (15,16). Caspase-3 (CPP32/yama/apopain) is another protease that has been implicated in mediating apoptosis (17,18). Caspase-3–deficient mice had profoundly affected brains and died at 1–3 weeks of age (19). It has been shown that caspase-3 inhibitors blocked apoptosis induced by various stimuli (18,19). Pro–caspase-3 is proteolytically activated by caspase-1, indicating that caspase-3 may lie downstream of caspase-1 in the apoptotic program (20). In some cells, however, Fas-transmitted signals activate caspase-3 without detectable activation of caspase-1 (21,22). There is marked tissue and stimulus specificity in the requirement of caspases in Fas-mediated apoptosis (23,24).

In a previous study, we used IL-1 to induce Fas expression by mouse islet cells (6). However, IL-1 may modulate Fas sig-

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FITC, fluorescein isothiocyanate; IL, interleukin; nt, nucleotide; PI, propidium iodide; RT-PCR, reverse transcription–polymerase chain reaction.

nal transduction, because incubation of islet cells with IL-1 alone at higher concentrations induces apoptosis (25). In this study, we performed stable transfection of a human Fas construct into a mouse β-cell line to investigate the roles of caspases in Fas-mediated β-cell death without possible interference by cytokine-induced signals.

RESEARCH DESIGN AND METHODS

Transfection and functional characterization of the cloned cDNA. βTC1 cells (26), an insulin-positive, glucagon-negative mouse insulinoma cell line, were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 20 µg/ml gentamicin sulfate at 37°C in 5% CO_2, 95% air. Human Fas cDNA was synthesized by reverse transcription–polymerase chain reaction (RT-PCR) using primers 5′-CGGTTTACGAGTGACTTGGC-3′ (nucleotide [nt] 25–44) and 5′-GAGTGGGGTTAGCCTGTGGA-3′ (nt 1,547–1,566). The 1.5-kb human Fas cDNA containing the entire 335–amino acid coding region was ligated into eukaryotic expression vector pCR3.1 (Invitrogen, Carlsbad, CA) in the sense orientation. The sequence of the cloned cDNA was confirmed by the dichlororhodamine dye terminator method (Applied Biosystems, Foster City, CA), and found to be identical to that published (27). βTC1 cells were transfected with the plasmid by the cationic lipid method (Tfx-50; Promega, Madison, WI) and selected with 600 µg/ml G418 (Geneticin; Invitrogen). After 3-week culture, clonal cell lines were obtained using cloning cylinders and purified by limiting dilution. Control clones were obtained by transfection of empty vectors alone.

The expression level of human Fas was determined by flow cytometry. Clonal cells were incubated in 100 µl RPMI 1640 medium containing 1% bovine serum albumin and 10 µg/ml noncytolytic anti-Fas monoclonal antibody (UB2; MBL, Nagoya, Japan) for 2 h at 4°C, washed two times with cold RPMI 1640, and incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin G (Dako, Carpinteria, CA) for 30 min at 4°C. After washing, fluorescence intensity was analyzed by flow cytometry (Ortho, Tokyo).

The 347-bp cDNA encoding mouse caspase-3 was isolated by RT-PCR from βTC1 cell–derived mRNA using primers 5′-AGTGACCATGGAGAACAACA-3′ (nt 34–53) and 5′-AGCTGCTCCTTTTGCTATGA-3′ (nt 361–380) and cloned into a TA cloning vector, pCR2.1 (Invitrogen). An antisense construct was generated by cloning the insert as a *Bam*HI/*Apa*I fragment into a eukaryotic expression vector, pcDNA3.1 zeo (+) (Invitrogen), and sequenced. hFas/βTC1 cells were transfected with the antisense caspase-3 construct by the cationic lipid method and selected with 800 µg/ml zeocin (Invitrogen). As a control, a sense-oriented fragment was also ligated in the expression vector and transfected into hFas/βTC1 cells. Stable expressions of antisense and sense caspase-3 mRNAs were determined by RT-PCR using a T7 promoter primer, 5′-TAATACGACTCACTATAGGG-3′, and the forward PCR primer 5′-AGTGACCATGGAGAACAACA-3′ or the reverse PCR primer 5′-AGCTGCTCCTTTTGCTATGA-3′, respectively.

Determination of cell death and DNA cleavage. Cytotoxicity was assessed by propidium iodide (PI) staining. After exposure to 0.5 µg/ml anti-Fas antibody (CH11; MBL), floating cells and briefly trypsinized cells were harvested and incubated for 15 min with 5 µg/ml PI, which interacts with nuclear DNA of dead cells, producing a red fluorescence. Cells were examined in an inverted fluorescence microscope with excitation at 360 nm. In each experimental condition, at least 400 cells were counted. Apoptosis was also measured by the annexin V method using FITC-conjugated annexin V (Clontech, Palo Alto, CA). Briefly, cells were resuspended with trypsin, washed with RPMI 1640 medium supplemented with 10% calf serum, and incubated with 0.5 µg/ml annexin V-FITC for 10 min at 25°C in the dark. Then the cells were analyzed by flow cytometry (Ortho). A cell-permeable caspase-1 inhibitor, acetyl-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD-cmk; Peptide Institute, Osaka, Japan), and a cell-permeable caspase-3 inhibitor, Z-Asp-Glu-Val-Asp-fluoromethyl ketone (Z-DEVD-fmk; Enzyme System Products, Livermore, CA), were used at levels of 5–25 µmol/l in an experiment to assess the roles of the caspases in apoptosis induction.

DNA fragmentation was analyzed as previously described (6). Briefly, cells were incubated in 100 µl lysis solution containing 50 µg proteinase K, 50 µg RNase A, and 1 mg SDS at 37°C for 30 min. The lysates were added with 150 µl of solution containing 6 mol/l NaI, 13 mmol/l EDTA, 0.5% sodium-*N*-lauryl sarcosinate, and 26 mmol/l Tris-HCl, pH 8.0, and incubated at 60°C for 15 min. DNA was precipitated with isopropanol and washed with 50 and 100% isopropanol. DNA samples were analyzed by electrophoresis on 2% agarose.

Determination of proteolytic activities of caspases. After incubation with anti-Fas, cells were washed two times with cold phosphate-buffered saline, and the cell pellets were lysed for 60 min in 50 mmol/l HEPES buffer (pH 7.5) containing 0.2% Triton X-100 and 10% sucrose. After centrifugation at 10,000*g* for 10 min, protein concentration of the supernatant was measured by the DC protein assay (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard. The cell extracts were activated by the addition of dithiothreitol (10 mmol/l) and incubated with 100 µmol/l acetyl-Tyr-Val-Ala-asp-4-methyl-coumaryl-7-amide (Ac-YVAD-mca; Peptide Institute) or acetyl-Asp-Glu-Val-Asp-4-methyl-coumaryl-7-amide (Ac-DEVD-mca; Peptide Institute) for 30 min at 37°C to measure caspase-1 and caspase-3 activities, respectively. The reactions were halted by the addition of 10% SDS. The fluorescence of released 7-amino 4-methyl coumarin was measured at 380 nm for excitation and at 440 nm for emission.

Statistical analysis. All data are presented as means ± SD. Student's unpaired *t* test was used to compare the means. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

We introduced human Fas cDNA into βTC1 cells and established a β-cell clone that expresses human Fas on cell membrane by selection with G418 and limiting dilution. The β-cell clone, designated hFas/βTC1, showed a single peak of surface human Fas in the flow cytometric analysis (Fig. 1). To assess whether the β-cell line has the signal transduction system of Fas-triggered apoptosis and whether the transfected human Fas can mediate the death signal, hFas/βTC1 cells were exposed to the agonistic anti-human Fas antibody (CH11). The antibody at the concentration of 0.5 µg/ml successfully induced apoptosis of the transformed cells. Figure 2 shows the time course of cell lysis evaluated by PI staining. While original βTC1 cells and empty vector–transfected cells were resistant to the anti-Fas, the lysis of hFas/βTC1 cells was observed 4 h after the addition of CH11, and the ratio of dead cells was progressively increased up to 83 ± 5% at 24 h. The cells showed hallmarks of apoptotic cell death, that is, chromatin condensation, nucleolar disintegration, and apoptotic body formation. Nuclear DNA was analyzed by electrophoresis on agarose gels after 4- to 12-h incubation with the agonistic anti-Fas. Ethidium bromide staining showed internucleosomal fragmentation of DNA, confirming that the cell lysis was apoptosis (Fig. 3).

We then examined the enzymatic activities of caspase-1 and caspase-3 in the lysates from anti-Fas–stimulated hFas/βTC1

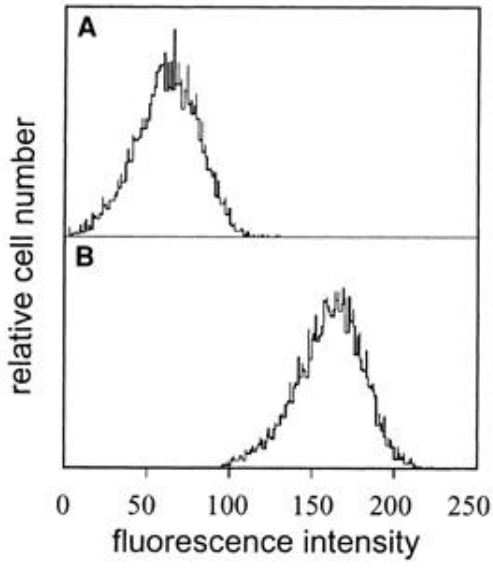

FIG. 1. Expression of human Fas by mouse β-cells transfected with human Fas cDNA. Original βTC1 cells (*A*) and transformed βTC1 cells (*B*) were incubated with noncytolytic anti-human Fas (UB2) for 2 h at 4°C, washed three times, and stained by FITC-conjugated anti-mouse immunoglobulin G.

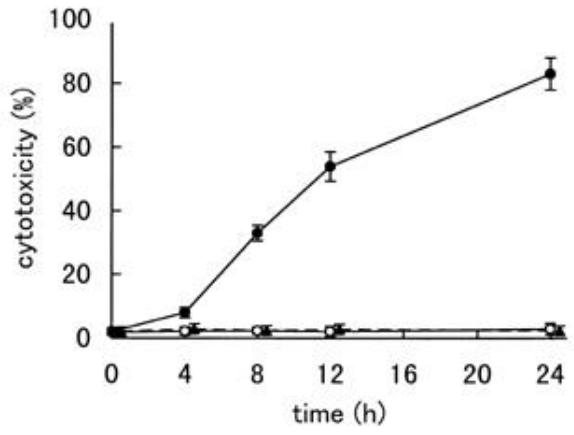

FIG. 2. Cytotoxicity of anti-Fas antibody to original βTC1 cells (○), human Fas-transfected βTC1 cells (●), and empty vector–transfected βTC1 cells (▲). Cells were exposed to 0.5 μg/ml cytolytic anti-Fas (CH11), and cell death was measured by PI staining. Data are means ± SD (n = 5).

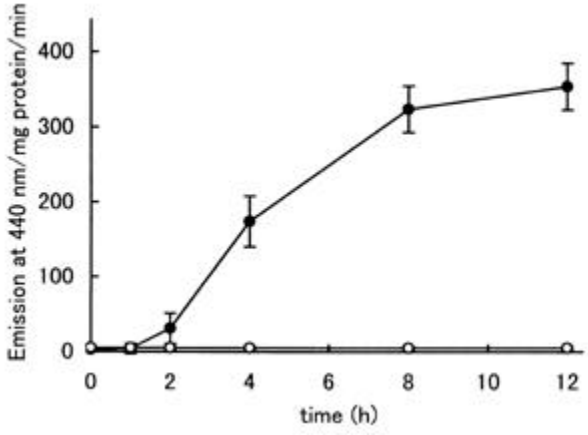

FIG. 4. Caspase-1 (○) and caspase-3 (●) activities in lysate from human Fas-transfected βTC1 cells exposed to 0.5 μg/ml CH11. Caspase-1– and caspase-3–like activities were assayed with fluorogenic tetrapeptide substrates after the addition of cytolytic anti-human Fas. Data are means ± SD (n = 5).

cells using fluorogenic peptide substrates Ac-YVAD-mca and Ac-DEVD-mca, respectively. Caspase-1 activity was detected in only a trace amount in unstimulated cells: 6 ± 3, 7 ± 2, and 7 ± 3 units in βTC1, hFas/βTC1, and βTC1 transfected with empty pCR3.1 vector, respectively (a unit is defined as emission at 440 nm · mg^{-1} protein · min^{-1}). Caspase-3 activity was not detectable in any of the cells at basal unstimulated state. However, marked elevation in caspase-3 activity was observed in hFas/βTC1 cells 2–12 h after the addition of anti-Fas (Fig. 4). In contrast, no increase in caspase-1 activity was detected in the lysates from anti-Fas–treated hFas/βTC1 cells. Neither caspase-1 nor caspase-3 activation was detected in original βTC1 cells or empty vector–transfected βTC1 cells during 12-h incubation with 0.5 μg/ml anti-Fas.

In the experiment to assess the role of caspases in β-cell apoptosis, hFas/βTC1 cells were preincubated for 3 h with the tetrapeptide caspase-1 inhibitor Ac-YVAD-cmk or the caspase-3 inhibitor Z-DEVD-fmk before the addition of anti-Fas. While Ac-YVAD-cmk did not inhibit Fas-induced apoptosis at concentrations up to 25 μmol/l, 25 μmol/l Z-DEVD-fmk significantly attenuated the cytotoxicity induced by anti-Fas (P = 0.003), providing evidence that one or more caspase-3–like proteases was required for Fas-induced apoptosis of hFas/βTC1 cells (Table 1). Next we assessed surface appearance of phosphatidylserine, an early event of apoptosis, using annexin V-FITC. Annexin V binding to hFas/βTC1 cells was detected 2 and 4 h after the addition of anti-Fas (Figs. 5 and 6). Z-DEVD-fmk (25 μmol/l) significantly reduced the ratio of stained cells at 4 h, but 25 μmol/l Ac-YVAD-cmk did not inhibit the apoptotic event (Fig. 6).

These results do not necessarily indicate that caspase-3 itself was involved in the induction of apoptosis, because other members of the caspase-3 subfamily, such as caspase-6, can be inhibited by Z-DEVD-fmk. To assess whether caspase-3 itself plays an essential role in Fas-mediated apoptosis of the β-cell line, we performed transfection of the antisense caspase-3 construct containing the translation initiation site into the mouse β-cell clone expressing human Fas and established five clones that expressed both human Fas mRNA and antisense mouse caspase-3 mRNA (Fig. 7*A*). The double transfectants, designated hFasCas3A/βTC1 clones, showed lower responses in caspase-3 activity to anti-Fas in comparison with hFas/βTC1. We also carried out transfection of the sense-oriented construct and isolated five clones expressing the frag-

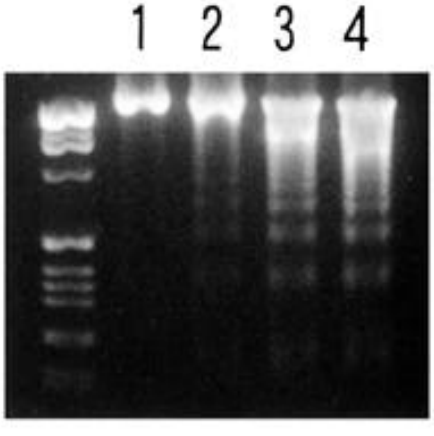

FIG. 3. Internucleosomal DNA fragmentation of hFas/βTC1 cells exposed to 0.5 μg/ml anti-Fas for 0 h (*lane 1*), 4 h (*lane 2*), 8 h (*lane 3*), or 12 h (*lane 4*).

TABLE 1
Attenuation of Fas-mediated β-cell apoptosis by caspase inhibitors

Treatment of βTC1 cells	Cytotoxicity (%)
Blank	3.4 ± 1.5
Anti-Fas (CH11) (0.5 μg/ml)	42.5 ± 7.1
Anti-Fas plus Ac-YVAD-cmk	
5 μmol/l	41.8 ± 4.0
25 μmol/l	43.2 ± 6.8
Anti-Fas plus Z-DEVD-fmk	
5 μmol/l	40.6 ± 4.9
25 μmol/l	21.4 ± 4.8*

Data are means ± SD. n = 4. *P = 0.003 vs. anti-Fas alone.

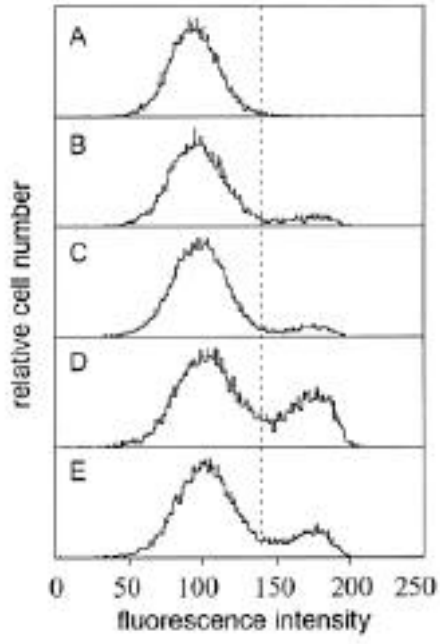

FIG. 5. Annexin V staining of hFas/βTC1 cells exposed to anti-Fas in the absence or presence of caspase-3 inhibitor. *A*: Cells cultured without anti-Fas or caspase-3 inhibitor. *B*: Cells after 2-h exposure to CH11 (0.5 μg/ml). *C*: Cells after 3-h preincubation with Z-DEVD-fmk (25 μmol/l) and 2-h incubation with CH11 and Z-DEVD-fmk. *D*: Cells after 4-h exposure to CH11. *E*: Cells after 3-h preincubation with Z-DEVD-fmk and 4-h incubation with CH11 and Z-DEVD-fmk. Cells were stained with FITC-conjugated annexin V and analyzed by flow cytometry.

ment of caspase-3 mRNA. Twelve hours after the addition of anti-Fas, the caspase-3–like activity of the antisense knockout clones was approximately 20% of that of original hFas/βTC1 or hFas/βTC1 clones transfected with the sense-oriented construct (Fig. 7*B*). Although the hFasCas3A/βTC1 clones expressed comparable levels of surface Fas (data not shown), the clones showed higher viability than the original hFas/βTC1 cells or the hFas/βTC1 clones transfected with the sense construct after 12-h exposure to anti-Fas (Table 2).

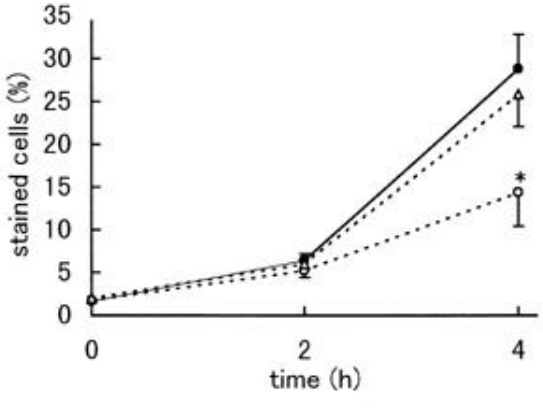

FIG. 6. Effects of caspase inhibitors on Fas-mediated apoptosis of hFas/βTC1 cells. Cells were preincubated with Ac-YVAD-cmk (△) or Z-DEVD-fmk (○) for 3 h and supplemented with anti-Fas (0.5 μg/ml CH11). ●, cells exposed to anti-Fas without caspase inhibitors. After 2- or 4-h exposure to anti-Fas, cells were stained with FITC-conjugated annexin V and analyzed by flow cytometry with a cut point of fluorescence intensity at 140. Data are means ± SD (n = 5). *P < 0.001 vs. cells without inhibitors.

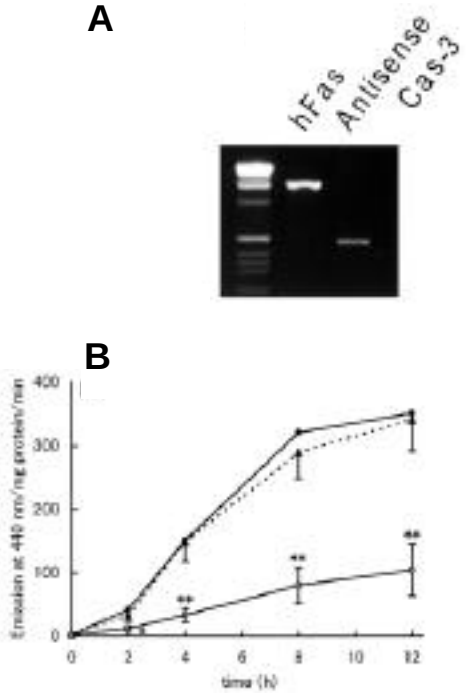

FIG. 7. *A*: RT-PCR of human Fas and antisense caspase-3 of mRNA from a hFas/βTC1 clone transfected with antisense caspase-3. The antisense caspase-3 sequence was amplified using a T7 primer and the forward PCR primer for 35 cycles with the annealing temperature of 52°C. *B*: Effects of antisense knockout of caspase-3 on anti-Fas–induced caspase-3 activation. Caspase-3–like activities were measured by Ac-DEVD-mca in lysate from original hFas/βTC1 cells (●), hFas/βTC1 transfected with antisense caspase-3 constructs (○, means ± SD of five clones), and hFas/βTC1 cells transfected with the sense-oriented construct (▲, means ± SD of five clones) 0–12 h after the addition of 0.5 μg/ml anti-Fas (CH11). Each assay was performed in quintuplicate. *P < 0.01, **P < 0.001 vs. clones transfected with the sense construct.

DISCUSSION

To assess the signaling mechanism of Fas-mediated apoptosis in pancreatic β-cells, we established a mouse β-cell clone expressing human Fas. The hFas/βTC1 cells underwent apoptosis when exposed to agonistic anti-Fas, indicating that the mouse β-cell line can functionally transduce Fas-mediated death signals and induce apoptosis without additional signals mediated by cytokine receptors. Hallmarks of apoptosis—chromatin condensation, nucleolar disintegration, internucleosomal fragmentation of DNA, and annexin V staining—were observed in the anti-Fas–treated cells.

Recent studies have revealed that coordinated actions of multiple caspases with different roles are required for apoptosis in mammalian cells (10–13,28). Caspases can be subdivided into three groups: the caspase-1 subfamily, the caspase-2 subfamily, and the caspase-3 subfamily (29). Caspase-1 and caspase-3 have been implicated as major proteases mediating the death signal; however, usage of caspases is tissue- and stimulus-dependent (23,24). In this study, we demonstrated that exposure of hFas/βTC1 cells to anti-Fas resulted in marked generation of caspase-3–like activity in cell lysates. PI staining and annexin V studies showed that the apoptosis was inhibited by the caspase-3 inhibitor Z-DEVD-fmk,

TABLE 2
Low susceptibility of hFas/βTC1 cells transfected with antisense caspase-3 (hFasCas3A/βTC1) to anti-Fas (CH11)

Cell	Anti-Fas (μg/ml)	Cytotoxicity (%)
hFas/βTC1	0	4.9
	0.5	53.8
hFasCas3A/βTC1 clones (n = 5)	0	4.4 ± 1.8
	0.5	19.5 ± 7.1*
Sense construct–transfected hFas/βTC1 clones (n = 5)	0	4.0 ± 1.4
	0.5	46.2 ± 5.1

Data for clones are means ± SD of five clones each. *P < 0.001 vs. sense construct–transfected hFas/βTC1 cells treated with 0.5 μg/ml anti-Fas. Each assay was carried out in quadruplicate.

suggesting that one or more caspase-3–like proteases is required for Fas-mediated apoptosis of hFas/βTC1 cells. In contrast, no increase in caspase-1–like activity was observed in hFas/βTC1 cells incubated with anti-Fas. The activation of caspase-1 might be transient or too low to be detected in β-cells, but the lack of protective effect of caspase-1 inhibitor on the apoptosis of hFas/βTC1 cells indicates that caspase-1 activation, if any, is not essential for the induction of Fas-mediated apoptosis in the cells. Although there is a possibility that transfection itself may upregulate caspase expression, the transfection of empty vector alone did not result in activation of caspase-1 or caspase-3 in βTC1 cells.

Because Z-DEVD-fmk is not a specific inhibitor of caspase-3, the protective effect of Z-DEVD-fmk could be attributable to the inhibition of other members of caspase-3 subfamily. To assess whether caspase-3 itself is involved in the apoptosis of the β-cell clone, we tested the effect of antisense knockout of caspase-3. After 12-h exposure to anti-Fas, the hFas/βTC1 clones transfected with antisense caspase-3 (hFasCas3A/βTC1) showed higher viability than original hFas/βTC1 cells or hFas/βTC1 clones transfected with the sense construct. Hence caspase-3 itself may play a major role in Fas-mediated apoptosis of β-cells. Other caspases might partially compensate for the deficiency of caspase-3, however, because the Fas-mediated β-cell death was not completely blocked by treatment with the tetrapeptide caspase-3 inhibitor or transfection with the antisense caspase-3 construct. It was shown that deficiency in caspase-3 prevented apoptosis in some cells, while it had no effect in others, indicating that caspase-3 is redundant in some cell types (19). In fact, the annexin V study showed no significant inhibition by Z-DEVD-fmk of the weak staining at 2 h, indicating that other caspases may be involved in the very early stages of apoptosis induction.

A number of studies (20,28) have indicated that there is a protease cascade in which caspase-1 is upstream of caspase-3 in Fas-mediated apoptosis. It is not known how caspase-3 could be activated by Fas-mediated signals in hFas/βTC1 cells without the activation of caspase-1. However, the activation of caspase-3 has been reported without detectable activation of caspase-1 in Fas-mediated apoptosis of several normal or transformed cells, such as rheumatoid arthritis synovial cells (21) and Hep G2 cells derived from hepatocellular carcinoma (22). The Fas-induced death signal apparently bypasses caspase-1 in those cells. Caspase-3 might be directly activated by the death-inducing signaling complex or through the activation of other caspases that mediate the signal.

In conclusion, the β-cell line proved to have the intact machinery of Fas-mediated apoptosis, supporting the hypothesis that Fas-mediated apoptosis is a major mechanism of β-cell death in autoimmune insulitis. Caspase-3 may be an essential caspase in the induction of Fas-mediated apoptosis in hFas/βTC1 cells, while the caspase-1 subfamily may not be involved in the apoptosis of the cells. Further studies on the Fas-mediated signaling mechanisms in β-cells may provide clues to devise new treatments to suppress autoimmune β-cell damage.

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