Diabetologia (1996) 39: 1306–1312

Diabetologia


# Mouse islet cell lysis mediated by interleukin-1-induced Fas

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**Summary** This study was conducted to investigate the possible involvement of Fas in β-cell death in insulitis of Type 1 (insulin-dependent) diabetes mellitus. Although primary cultured Balb/c mouse islet cells did not express Fas mRNA, 4–12 hours of treatment with $10^2$-$10^5$ U/l of mouse interleukin-1 α (IL-1α) induced the expression of Fas mRNA. Surface Fas expression was detected by immunofluorescence flow cytometry using a non-cytolytic anti-Fas monoclonal antibody after 6 or 12 h of incubation with $10^3$ U/l of IL-1α. Primary islet cells were resistant to an agonistic anti-Fas monoclonal antibody. However, 12 h pretreatment with IL-1α sensitized islet cells to its cytolytic effect. Significant cell death was observed 24 h after the addition of anti-Fas, and progressively increased until 72 h, when specific $^{51}$Cr release was 72 ± 6 %. Agarose gel electrophoresis of DNA extracted from cells exposed to IL-1α and agonistic anti-Fas showed internucleosomal DNA fragmentation, a hallmark of apoptotic cell death. Since the Fas antibody showed no cross-reactive activity of tumour necrosis factor (TNF), the cytotoxic effect was not mediated by TNF receptors. A protein synthesis inhibitor cycloheximide augmented Fas-mediated islet cell death. The Fas-mediated killing of islet cells was not L-arginine-dependent, or blocked by $N^G$-monomethyl-L-arginine. β-TC1 cells also expressed Fas mRNA when exposed to IL-1α or IL-1α plus interferon-γ. These observations suggest that Fas-mediated apoptosis may be a mechanism of islet cell death in autoimmune insulitis. [Diabetologia (1996) 39: 1306–1312]

**Keywords** Islets of Langerhans, Fas, CD95, apoptosis, interleukin-1, interferon-γ, DNA cleavage.

A bulk of evidence indicates that β-cell destruction in type 1 (insulin-dependent) diabetes mellitus results from cell-mediated autoimmunity. Pancreatic infiltrates of human type 1 diabetes [1, 2] and rodent models [3, 4] include $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, natural killer cells, and macrophages. It is known that a part of T-cell-mediated cytotoxicity is $Ca^{2+}$-independent [5, 6], whereas a well-known perforin/granzyme-based mechanism is $Ca^{2+}$-dependent. Recently, it was shown that the former is mediated by Fas (APO-1, CD95). Fas is a cell-surface receptor belonging to nerve growth factor/tumour necrosis factor (TNF) receptor superfamily [7, 8]. The Fas-mediated death mechanism plays an important regulatory role in autoimmune disorders of *lpr* mice [9, 10] and *gld* mice [11, 12]. Fas ligand (FasL) identified in activated T-cell lines has significant homology with TNF-α and TNF-β [13, 14]. Engagement of FasL and Fas on target cells results in the activation of an intrinsic suicide programme in target cells leading to apoptotic cell death. Fas-FasL interaction is involved in the cytotoxic action of $CD4^+$ cytotoxic T lymphocytes (CTLs) and, to a lesser extent, in that of $CD8^+$ CTLs [15–17]. Fas is also

Received: 5 April 1996 and in revised form: 7 August 1996

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*Abbreviations:* IL-1, Interleukin-1; TNF; tumour necrosis factor; IFN, interferon; FasL, Fas ligand; CTL, cytotoxic T-lymphocyte; RT-PCR, reverse transcription polymerase chain reaction; FITC, fluorescein isothiocyanate; NO, nitric oxide; NMMA, $N^G$-monomethyl-L-arginine; MHC, major histocompatibility complex.

involved in the cytotoxic activity of natural killer cells [18, 19]. Several organs including thymus and liver constitutively express Fas [20]. If islet cells express Fas in autoimmune insulitis, the cells could be killed not only by the perforin/granzyme-based mechanism but also by the Fas-mediated mechanism. In this study we examined the possible effect of interleukin-1 (IL-1) on Fas expression and Fas-mediated death of mouse islet cells.

## Materials and methods

*Islet cells, cytokines and antibodies.* Pancreatic islets were isolated by collagenase digestion of pancreases from 10-week-old male Balb/c mice, and purified by Percoll gradient and hand picking to eliminate exocrine cells. Islets were incubated with 1 mmol/l glycoletherdiaminetetra acetic acid for 10 min, and dispersed with 2 g/l collagenase type IV (Worthington, Freehold, N.J., USA) and $10^5$ U/l DNase (Sigma, St. Louis, M.O., USA). After adherent nonendocrine cells were removed, endocrine cells were cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum. β-TC1, a β-cell line derived from a transgenic mouse with insulin-promoted SV40 large T antigen [21], was also maintained in the culture medium. Recombinant mouse IL-1α ($8 \times 10^6$ U/mg) and recombinant mouse interferon-γ (IFN-γ) ($1 \times 10^7$ U/mg) were purchased from Genzyme (Cambridge, Mass., USA). Cytolytic hamster anti-mouse Fas monoclonal antibody (clone RK8) and non-cytolytic rat anti-mouse Fas monoclonal antibodies (clone RMF6) were provided by MBL (Nagoya, Japan).

*Reverse transcription polymerase chain reaction (RT-PCR) of Fas mRNA.* Islet cells were cultured at the density of $5 \times 10^4$ cells/well for 2 days, followed by 1–12 h exposure to IL-1α at the concentrations of $10^2$–$10^5$ U/l. β-TC1 cells inoculated at a density of $10^5$ cells/well in 24-well trays were cultured for 24 h and exposed to IL-1α and/or IFN-γ for 6 h. RNA was extracted by the single-step method using RNAzol B (Biotecx, Houston, Texas, USA), and 200 ng of total RNA was reverse transcribed with 100 U of RNase $H^-$ Moloney murine leukaemia virus reverse transcriptase (Gibco BRL, Grand Island, N.Y., USA) for 60 min at 37 °C and for 30 min at 42 °C. After completion of the reaction, the reverse transcriptase was inactivated at 94 °C for 3 min. Amplification of mouse Fas cDNA was performed by PCR with $5 \times 10^4$ U/l of *Taq* DNA polymerase (Roche, Branchburg, N.J., USA) using a sense primer 5′-ACAGTTAAGAGTTCATAC and an antisense primer 5′-GGTTGCTGTGCATGGCTC, resulting in a PCR product of 424 bp between bases 45 and 468 of the Fas coding region. Although the whole structure of mouse Fas gene has not been reported yet, at least one intron exists between the primer sites [10]. A cDNA sequence of β-actin was amplified as control with primers 5′-ATCCGTAAAGACCTCTATGC (945–964) and 5′-AACGCAGCTCAGTAACAGTC (1212–1231). A thermal cycle was 1 min 20 s at 94 °C, 1 min at 55 °C and 2 min at 72 °C. The Fas and β-actin sequences were amplified by 35 and 30 cycles, respectively. Aliquots of 10 μl from each reaction were analysed by electrophoresis together with 1 kb DNA ladder (Gibco BRL) in 2.5% agarose gel and stained with ethidium bromide. The PCR products of Fas were ligated into the pCR vector of the TA cloning kit (Invitrogen, Leek, The Netherlands). Competent cells were transformed according to the manufacture's instructions. DNA from positive clones were extracted using the QIAprep plasmid kit (QIAGEN, Hilden, Germany), and sequenced bidirectionally using the Cy5 AutoRead sequencing kit (Pharmacia Biotech, Uppsala, Sweden).

*Flow cytometric analysis of Fas expression.* After 2-day primary culture, dispersed islet cells at a density of $2 \times 10^5$ cells/well were exposed to $10^3$ U/l of mouse IL-1α for 6 or 12 h. Islet cells resuspended with trypsin/EDTA were incubated in 100 μl of RPMI-1640 containing 1% bovine serum albumin and 10 mg/l non-cytolytic anti-Fas monoclonal antibody (RMF6) or rat IgG (Jackson Immunoresearch, West Grove, Pa., USA) for 2 h at 4 °C, and washed twice with cold RPMI-1640. Cells were then incubated with fluorescein isothiocyanate (FITC) conjugated rabbit anti-rat Ig (Dako, Glostrup, Denmark) for 30 min at 4 °C. After washing fluorescence intensity was analysed by flow cytometry (Ortho, Tokyo, Japan).

*$^{51}Cr$ release assay.* Islet cell microcultures ($2 \times 10^4$ cells/well) were incubated with 4 μCi $^{51}Cr$ sodium chromate (New England Nuclear, Boston, Mass., USA) in 100 μl complete medium for 2 h at 37 °C, and washed four times with warm RPMI-1640 medium. After 4-h reincubation in $^{51}Cr$-free complete medium at 37 °C, cells were washed twice with the medium, and refed with 100 μl/well of complete medium containing $10^3$ U/l of IL-1α. After 12 h, anti-Fas monoclonal antibody (RK8) was added to cultures at a concentration of 5 mg/l. Aliquots from each reaction mixture were counted in a gamma counter. Total $^{51}Cr$ release was determined by the addition of 2% Triton-X100, and specific $^{51}Cr$ release was calculated by the formula of 100 × (test cpm – spontaneous cpm)/(total cpm – spontaneous cpm).

*Determination of nucleosomal DNA cleavage.* After 2-day primary culture, $3 \times 10^5$ dispersed mouse islet cells were incubated with $10^3$ U/l of IL-1α for 12 h and then exposed to 5 mg/l of agonistic anti-Fas monoclonal antibody (RK8) for 48 h. Cells were washed twice with RPMI-1640, precipitated by centrifugation, and incubated in 100 μl of lysis solution containing 50 μg proteinase K (Sigma), 50 μg RNase A (Sigma), and 1 μg sodium dodecyl sulphate at 37 °C for 30 min. The lysates were added with 150 μl of NaI solution containing 6 mol/l NaI, 13 mmol/l EDTA, 0.5% sodium-N-lauroyl sarcosinate, 1% glycogen, and 26 mmol/l Tris-HCl pH 8.0, and incubated at 60 °C for 15 min. DNA was precipitated with an equal volume of isopropanol, and washed with 50% and 100% isopropanol. DNA samples were analysed together with 1 kb DNA ladder (Gibco BRL) by electrophoresis on 2% agarose containing 0.4 mg/l of ethydium bromide.

*TNF activity assay.* TNF bioactivity was measured by the amido black method [22] using L929 cells as targets. Recombinant mouse TNF-α (Genzyme) or anti-Fas antibody was diluted in RPMI-1640 containing 1 mg/l of actinomycin D, and added to L929 microcultures in 96-well culture plates. After 18-h incubation, the cells were fixed with 10% formalin in 0.1 mol/l sodium acetate/9% acetic acid, stained with 0.05% amido black in 0.1 mol/l sodium acetate/9% acetic acid, washed with water, and added with 0.025 N NaCl. The optical density of each sample was measured at 575 nm.

### *Statistical analysis*

All data are presented as means ± SD for the indicated number of observations. Data were analysed by Student's *t*-test for unpaired data. Differences were considered significant at $p$ less than 0.05.

## Results

No expression of Fas mRNA was detected by RT-PCR in primary cultured mouse islet cells. To examine the possible effect of IL-1α on Fas mRNA expression, islet cells were incubated with IL-1α at concentrations of $10^2$, $10^3$, $10^4$, or $10^5$ U/l for up to 12 h (Fig. 1). Although the cells were still negative for Fas mRNA after 1-h incubation, 4-h exposure to IL-1α at any dose used here resulted in the induction of Fas mRNA. Fas mRNA was also detectable at 12 h. Whereas a sequence of 287 bp of β-actin cDNA was amplified from all the samples. This experiment was performed three times with essentially the same result. To confirm that the RT-PCR of Fas amplified relevant sequence, the 424-bp PCR products were cloned and sequenced bidirectionally. The nucleotide sequence was completely identical with bases 45–468 of the previously published sequence of mouse Fas cDNA [20].

The capacity of IL-1α to induce cell surface Fas expression was then tested. Primary cultured dispersed islet cells incubated with or without IL-1α for 6 or 12 h were stained with non-cytolytic anti-Fas antibody (RMF6) and analysed by immunofluorescence and flow cytometry. Figure 2 shows that the exposure of islet cells to $10^3$ U/l of IL-1α resulted in the surface expression of Fas molecules. At 12 h, the peaks of relative fluorescence intensity of cells incubated without and with IL-1α were 58 and 75, respectively. Whereas, the IL-1-induced shift of peaks was not obtained when rat IgG was used as a control.

To assess whether islet cell surface Fas can transduce death signal, IL-1α-treated cells were exposed to agonistic anti-Fas antibody (RK8). Figure 3 shows the time course of islet cell lysis evaluated by the $^{51}$Cr-release assay. Spontaneous $^{51}$Cr release at 24, 48, and 72 h was 14, 25, and 33 %, respectively. The lysis of IL-1α-pretreated cells was observed 24 h after the addition of anti-Fas antibody, and the specific $^{51}$Cr release was progressively increased to 72 ± 6 % at 72 h. Incubation for 48 h with 0.5 or 5 mg/l of anti-Fas antibody alone did not cause significant islet cell lysis (Fig. 4). However, islet cell damage was evoked when the cells had been treated with $10^2$–$10^3$ U/l of IL-1α before exposure to anti-Fas antibody. Although IL-1 is cytotoxic to islet cells at higher concentrations, islet cell lysis was not caused by $10^2$–$10^3$ U/l of IL-1α alone.

To exclude the possibility that islet cell lysis was mediated by TNF receptors with which anti-Fas antibody might cross-react, we examined the effect of anti-Fas RK8 on TNF-sensitive L929 cells. Exposure of the cells to TNF-α for 18 h in the presence of 1 mg/l of actinomycin D resulted in cell lysis in a dose-dependent manner. However, RK8 antibody at the levels of 1–10 mg/l in combination with 1 mg/l actinomycin D did not induce L929 cell lysis (Table 1).

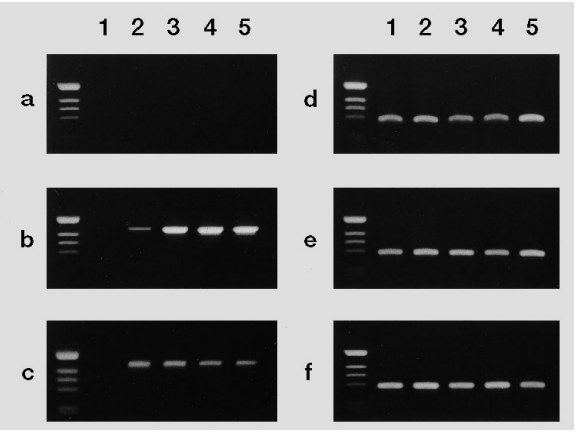


**Fig. 1. a–f.** IL-1α-induced Fas mRNA expression by mouse islet cells. Islet cells were exposed to IL-1α for 1 h (**a, d**), 4 h (**b, e**) or 12 h (**c, f**). IL-1α concentrations were 0 (lane 1), $10^2$ U/l (2), $10^3$ U/l (3), $10^4$ U/l (4) or $10^5$ U/l (5). Fas mRNA (**a, b, c**) and β-actin mRNA (**d, e, f**) were reverse transcribed and amplified by PCR by 35 cycles and 30 cycles, respectively. DNA molecular weight markers were 506, 396, 344, and 298 bp from top

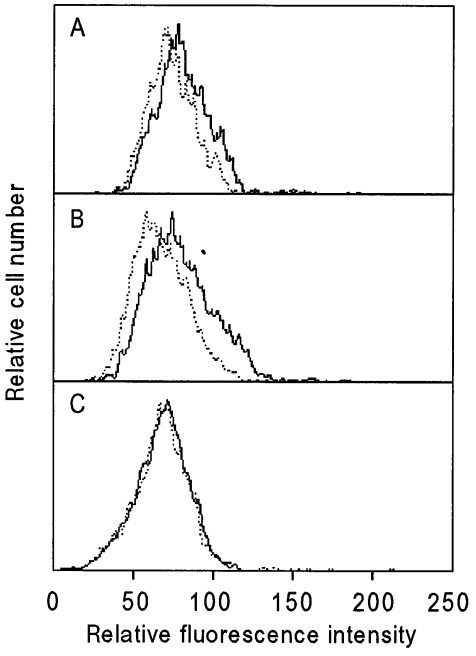


**Fig. 2. A–C.** Induction by IL-1α of Fas expression. Mouse islet cells were cultured in the presence (solid line) or absence (broken line) of $10^3$ U/l of IL-1α for 6 h (**A**) or 12 h (**B, C**). Then the cells were incubated with non-cytolytic rat anti-Fas monoclonal antibody (**A, B**) or rat IgG (**C**), and stained with FITC-conjugated rabbit anti-rat IgG. Fluorescence intensity was analysed by flow cytometry. Experiments **A**, **B**, and **C** were performed independently four times, and representative data are shown

Since protein synthesis inhibitors potentiate Fas-mediated apoptosis of various cells, we examined the effect of cycloheximide on anti-Fas antibody-induced islet cell lysis. Islet cells were incubated with 5 mg/l of anti-Fas antibody (RK8) after 12-h pretreatment with

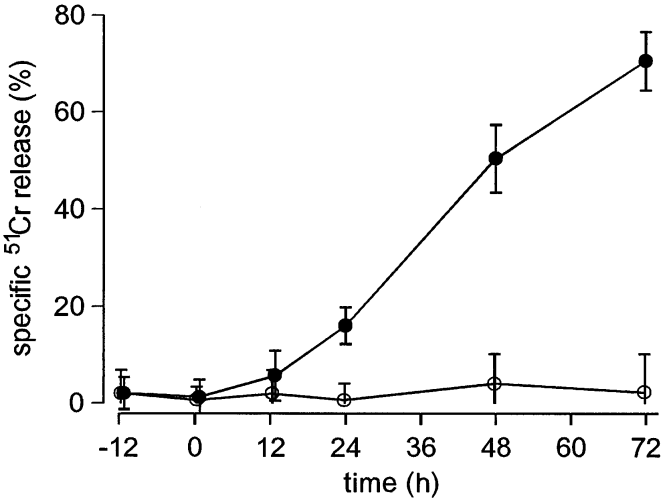


**Fig. 3.** Islet cell destruction induced by IL-1α and anti-Fas antibody. Islet cells of $1 \times 10^4$ cells/well were exposed to IL-1α of $10^3$ U/l. After 12 h agonistic anti-Fas monoclonal antibody (RK8) was added to the cultures at the concentration of 5 mg/l. Cell lysis was evaluated by the $^{51}$Cr-release assay. Specific $^{51}$Cr release = 100 × (test cpm – spontaneous cpm)/(total cpm – spontaneous cpm). ○, blank; ●, IL-1α plus anti-Fas. Data are means ± SD ($n$ = 4)

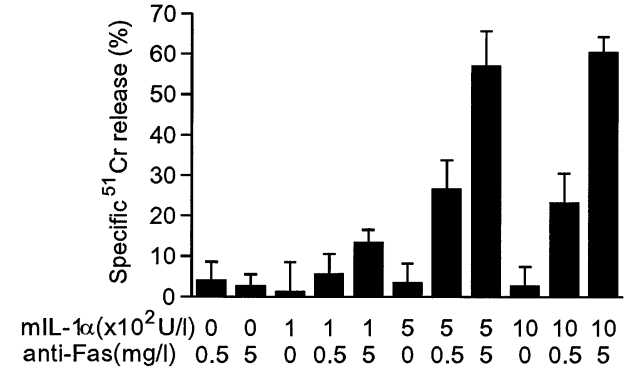


**Fig. 4.** Islet cell lysis induced by IL-1α and anti-Fas antibody measured by the $^{51}$Cr release assay. $^{51}$Cr-labelled mouse islet cells were incubated for 48 h with agonistic anti-Fas monoclonal antibody added 12 h after the addition of IL-1α. Data are means ± SD ($n$ = 4)

$10^3$ U/l of IL-1α. Anti-Fas-induced islet cell damage was still mild at 24 h without cycloheximide. The supplementation of cycloheximide together with anti-Fas antibody significantly enhanced the lysis of IL-1α-pretreated islet cells ($p < 0.01$). Incubation for 24 h with cycloheximide alone did not result in islet cell lysis (Table 2).

Although nitric oxide (NO) production from L-arginine by islet cells is one of the mechanisms by which IL-1 exerts its cytotoxic effects on islet cells [23–25], 0.2 and 1 mmol/l of $N^G$-monomethyl-L-arginine (NMMA), an inhibitor of NO synthetase, did not significantly attenuate islet cell lysis induced by anti-Fas antibody (Table 3). Furthermore, IL-1α-treated

**Table 1.** Cytotoxicity of anti-Fas antibody (RK8) on TNF-sensitive L929 cells

| Sample | | Absorbance at 575 nm |
|---|---|---|
| Blank | | 0.855 ± 0.024 |
| Mouse TNF-α | 10 U/l | 0.838 ± 0.026 |
| | $10^2$ | 0.744 ± 0.033[a] |
| | $10^3$ | 0.579 ± 0.051[a] |
| | $10^4$ | 0.287 ± 0.029[a] |
| RK8 | 1 mg/l | 0.848 ± 0.013 |
| | 5 | 0.862 ± 0.043 |
| | 10 | 0.851 ± 0.024 |

L929 cells were incubated with control TNF-α or RK8 in the presence of 1 mg/l actinomycin D for 18 h. Viability was determined by the amido black assay. Data are means ± SD ($n$ = 6)
[a] $p < 0.001$ vs blank

**Table 2.** Effects of IL-1α pretreatment and protein synthesis inhibition on cytotoxicity of anti-Fas antibody to mouse islet cells

| Treatment | Specific $^{51}$Cr release (%) |
|---|---|
| IL-1α ($10^3$ U/l) | 2.1 ± 3.1 |
| Anti-Fas (5 mg/l) | 1.0 ± 3.9 |
| CHX (10 μmol/l) | 3.3 ± 2.5 |
| IL-1α ($10^3$ U/l) and anti-Fas (5 mg/l) | 17.7 ± 4.5 |
| IL-1α ($10^3$ U/l), anti-Fas (5 mg/l) and CHX (10 μmol/l) | 41.3 ± 5.9[a] |

Islet cell death was evaluated by the $^{51}$Cr release assay 24 h after the addition of anti-Fas (RK8). IL-1α and cycloheximide (CHX) were supplemented 12 h and 10 min respectively before the addition of anti-Fas. Data are means ± SD ($n$ = 6)
[a] $p < 0.001$ vs IL-1α and anti-Fas

islet cells were damaged by anti-Fas antibody in the absence of L-arginine as well as in the presence of L-arginine in culture medium.

To assess whether anti-Fas antibody induces DNA fragmentation in IL-1α-treated islet cells, islet cell nuclear DNA was analysed by electrophoresis on agarose gels after 48-h incubation with agonistic anti-Fas antibody (RK8) following 12-h culture with $10^3$ U/l of IL-1α. Ethydium bromide staining showed internucleosomal fragmentation of DNA, indicating that the cell lysis was apoptosis (Fig. 5). No internucleosomal DNA cleavage was detected in cells incubated with either $10^3$ U/l of IL-1α alone for 60 h or 5 mg/l of RK8 alone for 48 h.

Next we analysed Fas mRNA of β-TC1 cells by RT-PCR to confirm that β-cells are capable of Fas expression. Although β-TC1 cells did not constitutively express Fas mRNA, 6-h incubation of the cells with $10^3$ or $10^4$ U/l mouse IL-1α resulted in slight but apparent expression of Fas mRNA (Fig. 6). Incubation with $10^5$ U/l of IFN-γ alone did not induce the expression of Fas mRNA. The combination of IL-1α and IFN-γ, however, caused obvious Fas mRNA expression by β-TC1 cells. The augmentation of

**Table 3.** Effects of NMMA and L-arginine on Fas-mediated islet cell damage

| Treatment | Specific $^{51}$Cr release (%) |
|---|---|
| IL-1α ($10^3$ U/l) and anti-Fas (5 mg/l) | 45.0 ± 4.8 |
| plus NMMA (0.2 mmol/l) | 42.3 ± 4.3 |
| plus NMMA (1 mmol/l) | 43.7 ± 3.1 |
| minus L-arginine | 48.0 ± 6.9 |

$^{51}$Cr-labelled mouse islet cells were incubated with IL-1α in the absence or presence of NMMA or L-arginine for 12 h, followed by the addition of anti-Fas (RK8) to the medium. Islet cell death was evaluated 48 h after the addition of anti-Fas. Data are means ± SD ($n$ = 4)

IL-1α-induced Fas mRNA expression by IFN-γ was observed in all of three independent experiments. However, the induction of Fas molecules was not detected by fluorescence-activated flow cytometry on β-TC1 cells treated with IL-1α ($10^4$ U/l) plus IFN-γ ($10^5$ U/l) for 6 h (data not shown).

## Discussion

It has been shown that Fas is constitutively expressed in the thymus, liver, heart, lung, and ovaries, but not in brain, spleen, testis, uterus and kidney [20]. Primary and viral infected hepatocytes have sensitivity to Fas-mediated apoptosis in vitro [26, 27] and an intraperitoneal injection of monoclonal anti-Fas into mice causes hepatic failure and death [28]. In this study we showed that mouse islet cells were negative for Fas mRNA, and resistant to the agonistic anti-Fas monoclonal antibody. However, the exposure of islet cells to IL-1α resulted in the expression of Fas mRNA. Fluorescence flow cytometry using non-cytolytic anti-Fas monoclonal antibody showed surface Fas expression after 6- or 12-h incubation with IL-1α. IL-1 has been implicated as an immunological effector molecule that mediates the destruction of β-cells in type 1 diabetes [29–31]. Incubation of pancreatic islets with IL-1 alone results in a time-dependent inhibition of glucose-stimulated insulin release which has been demonstrated to be dependent on the metabolism of L-arginine to NO [23–25]. Although this is the first report showing cytokine-induced Fas expression by islet cells, it has been described that IFN-γ up-regulates Fas expression in various cell lines, and enhances the cytotoxic activity of anti-Fas antibody to the cells [7, 8, 20].

IL-1α rendered islet cells sensitive to the cell-killing activity of cytolytic anti-Fas antibody. The anti-Fas antibody-induced destruction of islet cells was associated with DNA fragmentation. Internucleosomal DNA cleavage, the characteristic phenomenon of

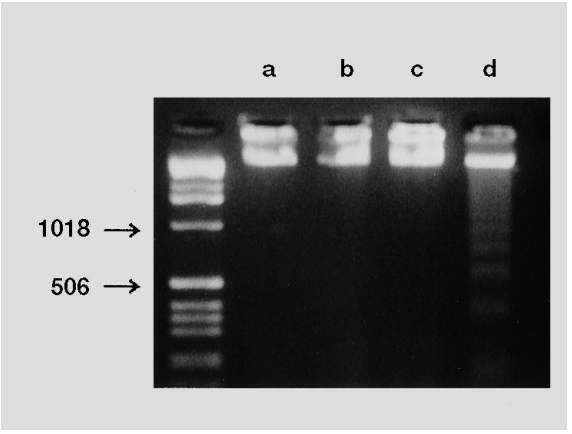


**Fig. 5.** Agarose gel electrophoresis of DNA from islet cells treated with IL-1α and anti-Fas antibody. Primary cultured Balb/c mouse islet cells were incubated with $10^3$ U/l of IL-1α for 12 h and the supplemented with 5 mg/l of agonistic anti-Fas monoclonal antibody (RK8). After 48-h exposure, cells were lysed with proteinase K, RNase A, and sodium dodecyl sulphate. Extracted DNA samples were subjected together with 1 kb DNA ladder to electrophoresis. a, DNA from cells cultured without IL-1α or anti-Fas; b, cells treated with IL-1α; c, cells treated with anti-Fas; d, both of IL-1α and anti-Fas

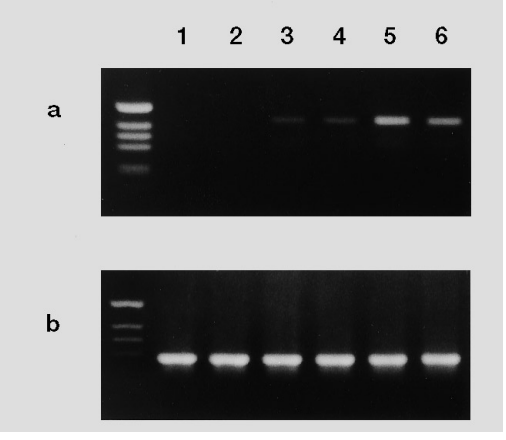


**Fig. 6 a, b.** Cytokine-induced Fas mRNA expression by β-TC1 cells. β-TC1 cells were cultured without cytokine (lane 1) or exposed to $10^5$ U/l of IFN-γ (2), $10^3$ U/l of IL-1α (3), $10^4$ U/l IL-1α (4), or the combination of $10^5$ U/l of IFN-γ and $10^3$ U/l of IL-1α (5) or the combination of $10^5$ U/l of IFN-γ and $10^4$ U/l of IL-1α (6) for 6 h. Fas mRNA (**a**) and β-actin mRNA (**b**) were reverse transcribed and amplified by PCR by 35 cycles and 30 cycles, respectively. Representative data of three experiments are shown

apoptotic cell death, could be observed 24 h after the addition of anti-Fas antibody. Recently, it was reported that IL-1 itself [32–34] or a combination of IL-1, IFN-γ and TNF-α [35] induces DNA damage in rat islet cells and insulinoma cell lines through NO generation. Exogenous NO also causes DNA cleavage leading to apoptosis in islet cells [34]. This study, however, showed that islet cell Fas could be induced by lower concentrations of IL-1α; Fas mRNA

was induced by $10^2$ U/l of IL-1α, and Fas-based cell death and DNA fragmentation were observed when islet cells had been incubated with $10^3$ U/l of IL-1α. IL-1α alone of this dose did not cause significant death or DNA fragmentation in islet cells. Furthermore, anti-Fas monoclonal antibody killed IL-1α-treated islet cells in the absence of L-arginine, and NMMA did not significantly protect islet cells against the antibody. Therefore, the anti-Fas antibody-induced lysis of IL-1α-treated islet cells is not mediated by NO generation. Previously we have shown that islet cell cytotoxicity of the combination of IFN-γ and TNF-α was almost completely suppressed in the absence of L-arginine or in the presence of 0.5 mmol/l of NMMA [36]. Although Fas and TNF receptor-1 have homologous intracellular death domains, death transducing signals of Fas and TNF receptor-1 are probably different. Since the anti-Fas antibody was not cytotoxic to TNF-sensitive L929 cells, the cytolytic action of the antibody on IL-1-treated islet cells cannot be attributable to possible cross reaction to TNF receptors. The relatively long period for maximum cytotoxicity may be attributable to the low density of Fas molecules on the islet cell surface. The mechanism of Fas-mediated islet cell killing requires further studies.

A protein synthesis inhibitor cycloheximide enhanced Fas-based cytolysis of various cells, such as thymocytes [37] and hepatocytes [26]. It was suggested that cycloheximide inhibits de novo synthesis of a protein which protects the cells against Fas-mediated killing. The augmentation by cycloheximide of anti-Fas antibody-induced islet cell death indicates the presence of protective mechanism requiring de novo protein synthesis. It may be the reason why it took a long time for anti-Fas antibody to kill islet cells. However, the presence of the inhibitor was not essential to Fas-mediated islet cell killing. Since anti-Fas antibody progressively killed IL-1-treated islet cells even in the absence of cycloheximide, the protective mechanism may be insufficient in islet cells. Unlike thymocytes and hepatocytes, the absence of constitutive Fas in islet cells may allow the incomplete protective mechanism.

Exposure to IL-1 alone or in combination with IFN-γ induced Fas mRNA expression by β-TC1 cells, although surface Fas molecules were not detectable on cytokine-treated β-TC1 cells by immunofluorescence flow cytometry. One possible explanation for the discrepancy is that Fas expression may be down-regulated by SV40 T antigens in the cells. Cytokine-induced Fas mRNA expression by β-TC1 cells suggests that beta-cells are capable of Fas expression.

If cytokines released by infiltrating cells induced Fas expression by β-cells in insulitis, islet cell surface Fas may be bound by FasL on infiltrating cells. $CD4^+$ T cells, the classical helper T subset, do not express perforin-based mechanism but express FasL and lyse in a Fas-based manner. Among the $CD4^+$ T subpopulations, TH1 cells kill Fas-bearing cells more readily than TH2 cells [38, 39]. Whereas, $CD8^+$ CTLs usually express both the Fas-based and the perforin-based mechanisms [40, 41]. Up-regulated class I major histocompatibility complex (MHC) antigens on islet cells [1, 2] may enhance the interaction between $CD8^+$ T cells and islet cells. Although it is controversial whether class II MHC antigens are expressed on islet cells in insulitis, $CD4^+$ cells could participate in Fas-based islet cell killing, because FasL may transduce death signal to Fas-bearing cells without recognition of MHC antigens [13]. Recently, it was shown that freshly isolated natural killer cells express FasL and exert Fas-based cytotoxic activity [18]. Furthermore, the soluble form of FasL released by activated lymphocytes can lyse Fas-bearing cells [42].

Our results provide the first evidence that islet cells express Fas when exposed to cytokines. Fas-mediated apoptosis may be one of the mechanisms by which β-cells are killed in autoimmune insulitis.

*Acknowledgements.* This work was supported in part by grants-in-aid for scientific research from the Japanese Ministry of Education, Science and Culture. We thank Dr. T. Hoshino, Department of Immunology, Kurume University, for helpful comments.

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