Fas and Fas ligand expression in inflamed islets in pancreas sections of patients with recent-onset Type I diabetes mellitus

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Abstract

Aims/hypothesis. Type I (insulin-dependent) diabetes results mainly from T-cell-mediated autoimmune destruction of pancreatic beta cells. Cytotoxic T lymphocytes destroy target cells via a perforin-based or Fas-based mechanism. Our previous study indicated that the Fas-Fas ligand (FasL) pathway is required for the development of autoimmune diabetes in the NOD mouse. We now investigated whether or not the Fas-FasL system is involved in the beta-cell destruction in human Type I diabetes.
Methods. We immunohistochemically analysed pancreas biopsy specimens of 13 recent-onset patients.
Results. Pancreatic islets were identified but showed various degrees of reduction in beta-cell volume in all patients. Out of 13 patients 6 had insulitis. In these 6 patients Fas was expressed in both the islets and in-filtrating cells but not in either cell type in the 7 other patients without insulitis. Double immunostaining showed that Fas was positive in 92.2 to 97.7 % of beta cells but only in 17.6 to 46.7 % of alpha cells in Fas-positive, insulin-remaining islets. We found FasL was expressed exclusively in islet-infiltrating cells in patients with insulitis. Double immunostaining revealed that the most prevalent phenotype of FasL-positive cells was CD8, which was followed by macrophages and CD4.
Conclusion/interpretation. The interaction between Fas on beta cells and FasL on infiltrating cells might trigger selective apoptotic beta-cell death in inflamed islets, leading to immune-mediated Type I diabetes. [Diabetologia (1999) 42: 1332–1340]

Keywords Type I diabetes, Fas, Fas ligand, insulitis, human pancreas, apoptosis.

Type I (insulin-dependent) diabetes mellitus is thought to be a cell-mediated autoimmune disease characterized by the selective destruction of pancreatic beta cells [1, 2]. Several lines of evidence have established a critical role for autoreactive lymphocytes in the pathogenesis of this disease. Peripheral blood mononuclear cells from people with or at increased risk for Type I diabetes showed increased reactivity to beta cell autoantigens, such as insulin secretory granule proteins and glutamic acid decarboxylase (GAD) [3–5]. Type I diabetes was transferred by bone marrow transplantation between HLA-matched siblings [6]. Mononuclear cell infiltration into the pancreatic islets (insulitis) and the reduction of insulin-producing beta cells have been recognized as pathological features of the pancreas of recent-onset Type I diabetes [7–10]. The infiltrate was composed of CD8+ T, CD4+ T, B lymphocytes and macrophages: in most cases, CD8+ T-cells were predominant [8–10]. There was also the hyperexpression of MHC class I molecules in inflamed islet cells [8–11]. CD8+ T-cells interact with target antigens in the context of self-MHC class I molecules [12] and CD8+ T-cells are major effectors of cell-mediated cytotoxicity, and the killing of beta cells by CD8+ cyto-

toxic T lymphocytes (CTLs) is thought to play a major part in the insulitis lesion.

T-cell-mediated cytotoxicity comprises two major pathways: Fas-dependent and perforin-dependent mechanisms [13–15]. Fas (APO-1/CD95) is a Type I membrane protein that belongs to the TNF/nerve growth factor receptor family and mediates apoptosis [16]. Fas mRNA is abundantly expressed in a variety of organs including the thymus, liver, heart and kidney [17]. As a natural ligand for Fas, Fas ligand (FasL/CD95L) was identified as a type II membrane protein that belongs to the TNF family [18]. FasL is predominantly expressed in activated T-cells and mediates Fas-bearing target cell death [19–21].

The considerable contribution of the Fas-FasL system in autoimmune diabetes has been shown recently by studies of Fas-deficient, non-obese diabetic (NOD)-lymphoproliferation (*lpr*) /*lpr* mice, which are devoid of both spontaneous and adoptively transferred diabetes [22, 23]. Most recent studies suggested, however, that Fas-FasL system plays only a minor part in beta-cell destruction in NOD mice [24, 25]. To investigate whether or not the Fas-FasL system is involved in the pathogenesis of human Type I diabetes, we analysed pancreas biopsy specimens of patients with recent-onset Type I diabetes for the expression of Fas and FasL.

Materials and methods

Subjects. We examined 13 patients with recent-onset Type I diabetes (11 men and 2 women). All patients fulfilled the criteria of the National Diabetes Date Group for insulin-dependent diabetes mellitus [26] and presented with the typical abrupt disease onset with ketosis. They were assumed to have Type I diabetes under the new classification reported by the expert committee on the diagnosis and classification of diabetes mellitus [27]. The patients included seven (cases 1–7) from our former study [9]. The mean age of the 13 patients was 25.5 (range 16–46) years and the mean duration of Type I diabetes was 2.7 (range 1–5) months. Islet cell antibodies were detected in seven patients by an indirect immunofluorescence method and GAD antibodies were determined in six patients with a radio-immunoassay kit (Hoechst-Japan, Tokyo, Japan).

Pancreas sections. The study protocol was primarily approved by the ethics committee of Osaka University Medical School and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before the study. Pancreas biopsy was as described previously [28] without any complications. Briefly, a laparoscope was introduced into the abdominal cavity from the skin incision site near the navel. Under direct inspection, one to three pieces of tissue were obtained from the body of the pancreas with biopsy forceps. Blocks of the biopsy specimen tissue were frozen immediately in dry ice-isopentane, and 5-μm-thick sections were cut on a cryostat. About 300 consecutive sections were cut from each biopsy specimen block and stored at –80 °C until use. Parts of the pancreas body from four subjects who underwent partial pancreatectomy for gastric cancer were used as the normal control.
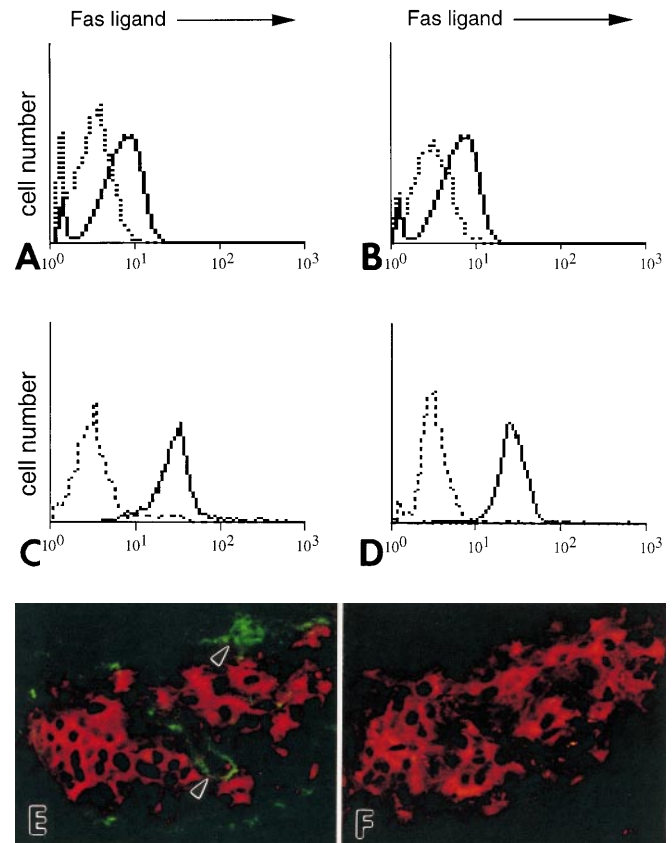

Fig. 1 A–F. Expression of FasL in human PBLs stimulated with 5 μg/ml ConA, 10 ng/ml IL-2, 10 ng/ml PMA and 500 ng/ml of ionomycin (**A**, **B**). See Methods for details. The PBLs were incubated with anti-human FasL antibody, H707 (solid line), and with normal rabbit serum (dashed line) (**A**). The PBLs were incubated with anti-human FasL antibody, H707 (solid line) and with H707 pre-absorbed with FasL peptide (dashed line) (**B**). Expression of FasL in Jurkat cells stimulated with 1 μg/ml of anisomycin (**C**) and 3300 rad of gamma irradiation (**D**) in the presence of 30 mmol/l of Z-VAD. The stimulated Jurkat cells were incubated with anti-human FasL antibody, H707 (solid line) and with normal rabbit serum (dashed line). Two consecutive pancreas sections of a recent-onset Type I diabetic patient (**E**, **F**) were stained with non-absorbed, anti-FasL antibody, H707 (E) or H707 pre-absorbed with FasL peptide (**F**) (green) and anti-insulin (red), respectively (× 400). FasL-positive cells were detected in and around the islet, as indicated by arrowheads (**E**). In the next control section, positive FasL staining was completely absorbed using H707 pre-incubated with FasL peptide (**F**)

Antibodies. The following antibodies were used in this study : monoclonal anti-Fas antibody (4B4-B3) was prepared by one of the authors (S. Nagata) and the specificity was confirmed by western blotting. Polyclonal rabbit anti-human Fas ligand antibody (H707, Nichirei, Tokyo, Japan) was reported to be suitable for immunohistochemistry [29]. The specificity of the H707 antibody was assessed by an absorption test. Human PBLs were cultured at 37 °C for 3 days with 5 μg/ml ConA and 10 ng/ml IL-2, then stimulated with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) and 500 ng/ml of ionomycin for 12 h. Under these stimulations, PBLs have been shown to express FasL [30]. And Jurkat cells were stimulated for 3300 rad of gamma irradiation and 1 μg/ml of anisomycin (Sigma chem-
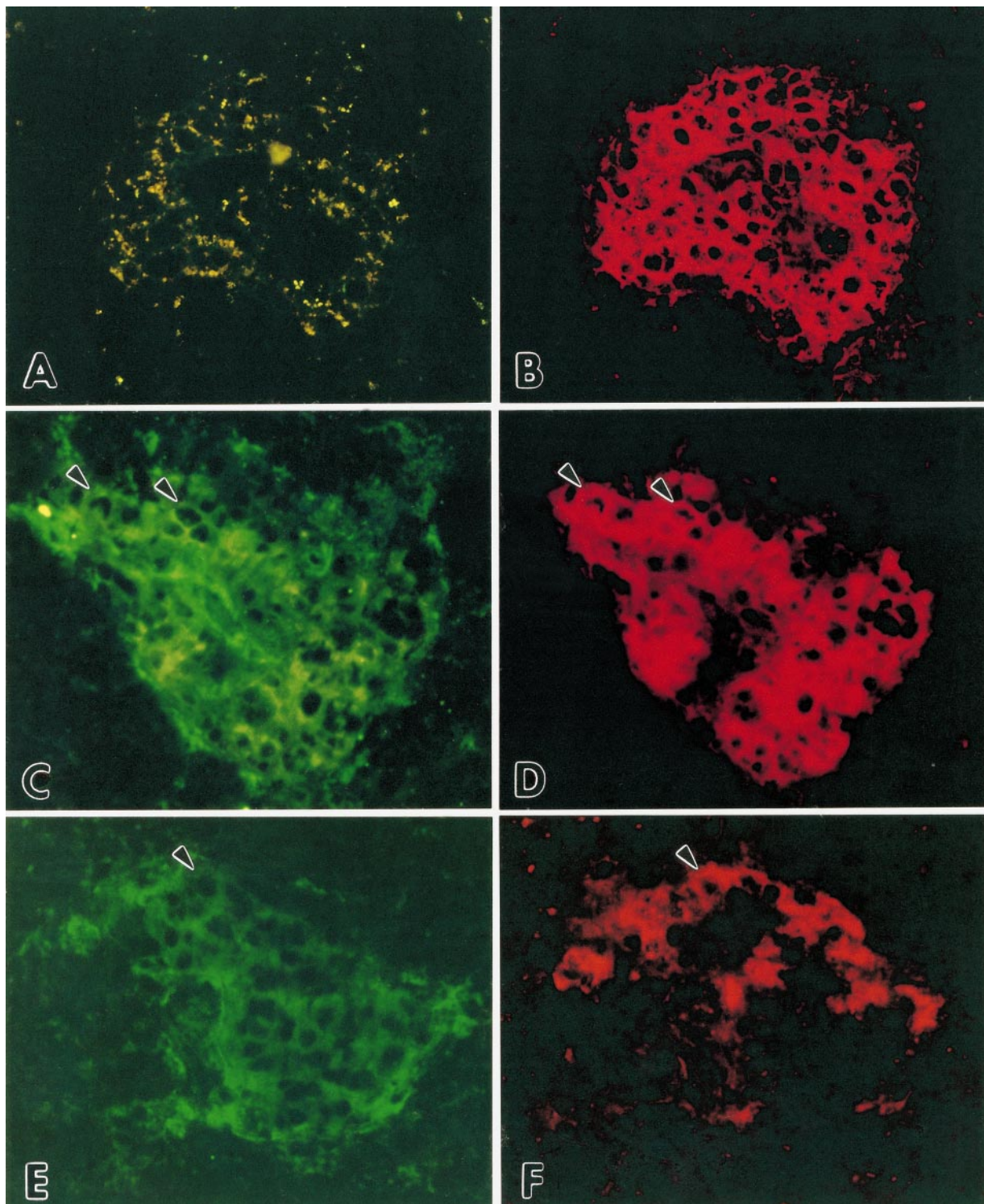
Fig. 2 A–F. Pancreas sections of a control subject (**A**, **B**) and a recent-onset Type I diabetic patient (**C**, **D**, **E**, **F**) were stained with anti-Fas antibody (green; **A**, **C**, **E**) and anti-insulin (red; **B**, **D**) and anti-glucagon antibody (red; **F**) ($\times$400). Fas is positive in the islet of a Type I diabetic patient (**C**, **E**) but not in the islet of the control subject (**A**). Double immunostaining of Fas-stained sections with anti-insulin (**B**, **D**) and anti-glucagon (**F**) antibodies (red) shows that Fas-positive cells are mostly insulin-positive cells and partly glucagon-positive cells. Representative double-positive cells are indicated by arrowheads

ical company, Saint Louis, Mo., USA) in the presence of 30 mmol/l of Z-VAD (carbobenzoxy-Val-Ala-Asp-CH2–2,6-dichlorobenzolate) (Phoenix Pharmaceuticals, Mountain View, Calif., USA) which interferes with the activation of CPP32 and IL-1β converting enzyme (ICE)-like caspase [31]. Under these stimulations, Jurkat cells have been shown to express FasL [31]. The stimulated PBLs and Jurkat cells were fixed with 1% paraformaldehyde containing PBS for 20 min and made permeable with 0.1% Saponin/0.1% FCS/PBS for 5 min. The fixed cells were then incubated for 30 min at 4°C

with anti-human FasL antibody or the antibody pre-incubated overnight with excessive human FasL peptide (molar ratio: peptide/antibody = 11.1). The cells were washed with PBS, incubated for 30 min at 4 °C with fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit IgG (H + L) (Zymed Laboratories, San Francisco, Calif., USA), then analysed on a FACScan (Fig. 1A–D). In addition, the specificity of the antibody in immunostaining was also assessed by an absorption test. After air-drying for 30 min, the sections were prefixed in cold acetone for 10 min. Endogenous biotin was blocked using a biotin blocking system (Dako-Japan, Kyoto, Japan). The first incubation was conducted overnight at 4 °C either with non-absorbed anti-FasL or antibody pre-incubated overnight with excessive human FasL peptide. After washing in PBS, the sections were incubated at room temperature for 60 min with biotinylated goat anti-rabbit immunoglobulins (Igs) (Vector Laboratories, Burlingame, Calif., USA) and for an additional 30 min with FITC-avidin D (Vector Laboratories). The above mentioned procedure was followed by two consecutive incubations with the following anti-insulin antibodies (Dako-Japan) combined with the rhodamine-conjugated goat anti-guinea pig Igs (Chemicon International, Temecula, Calif., USA). The section was washed in PBS, mounted with PermaFluor (Shandon/Lipshaw, Pittsburgh, Pa., USA) and observed using a fluorescence microscope (AX-80 T and AX80TR, Olympus, Tokyo, Japan).

PBLs were positively stained with the anti-FasL antibody, and the positive staining was completely abolished when the anti-FasL antibody H707 was replaced with antibody that had been pre-absorbed with the FasL peptide against which H707 had been generated (Fig. 1A, B). Stimulated Jurkat cells that have been shown to express FasL, were positively stained with the anti-FasL antibody (Fig. 1C, D). FasL positive cells (green) were seen in and around the islet (Fig. 1E). This positive staining was completely absorbed using H707 pre-absorbed with FasL peptide (Fig. 2). This absorption experiment in immunostaining further confirmed the feasibility of our anti-FasL antibody, H707.

Monoclonal mouse anti-human CD3 (T3–4B5), CD8 (DK25), CD56 (T199) and CD68 (EBM11) were purchased from Dako-Japan. Anti-CD4 (NU-TH/I) was from Nichirei and guinea pig anti-insulin from Dako-Japan. Rabbit anti-glucagon was kindly provided by Dr. S. Iwasa (Takeda Chemical, Osaka, Japan). Secondary antibodies were biotinylated horse anti-mouse immunoglobulins (Igs), biotinylated goat anti-rabbit Igs (both from Vector Laboratories), rhodamine-conjugated goat anti-guinea pig Igs (Chemicon International), Texas red-conjugated donkey anti-rabbit Igs (Amersham International, Amersham, UK) and Texas red-conjugated sheep anti-mouse Igs (Amersham International).

Immunohistochemistry. An indirect immunofluorescence method was used to detect the expression of Fas and FasL. After air-drying for 30 min, the sections were prefixed in cold acetone for 10 min. Endogenous biotin was blocked using a biotin blocking system (Dako-Japan). The first incubation was conducted with anti-Fas or anti-FasL overnight at 4 °C. After washing in PBS, the sections were incubated at room temperature for 60 min with biotinylated secondary antibodies and for an additional 30-min with FITC-avidin D (Vector Laboratories). Each section was washed in PBS, mounted with PermaFluor (Shandon/Lipshaw) and observed using a fluorescence microscope (AX-80 T and AX80TR). A double immunofluorescence method was used to determine which cells expressed Fas and FasL in and around the islet.

The above-mentioned procedure was followed by two consecutive incubations with the following antibodies: one of anti-insulin, anti-glucagon, anti-CD3, anti-CD4, anti-CD8, anti-CD56 and anti-CD68 combined with the corresponding secondary antibody. In the use of anti-CD68, Fc-receptors on macrophages were blocked with human γ-globulin (Teijin, Osaka, Japan) to exclude non-specific binding of the antibody. As a routine examination, sections were also stained with antibodies against mononuclear cells to detect insulitis, as described previously [9].

In situ TUNEL method (apoptosis detection studies). Apoptotic cells were examined by terminal dideoxynucleotidetransferase (TdT)-mediated X-dUTP nick end labelling (TUNEL) method using an in situ apoptosis detection kit (Takara Shuzo, Otsu, Japan) according to the manufacturer's instructions. Sections were air-dried for 30 min, prefixed in 4 % paraformaldehyde and made permeable with a supplied buffer. We tailed 3′-OH fragmented DNA ends with fluorescein-labelled dUTP and detected them with horseradish peroxidase-conjugated antifluorescein antibody. Liquid DAB (3,3,-diaminobenzidine tetrahydrochloride substrate kit, Zymed Laboratories) was used as substrate. To identify the islet area, double staining with anti-insulin antibody was done.

Results

Pathological changes of the islet. Pancreatic islets were identified but showed pronounced reduction in beta cell volume in all patients which is compatible with the diagnosis of Type I diabetes. In contrast, glucagon-positive cells were not significantly reduced in the patients ($p = 0.66$ data not shown). Infiltration of lymphocytes to the islet, namely insulitis, was detected in 6 out of 13 patients, but in none of the control subjects.

Expression of Fas in the pancreas. Fas was not immunohistochemically detected in the pancreas of four control subjects (Fig. 2A). In contrast, immunoreactivity of Fas was identified in the pancreas of six patients and the Fas-positive cells were localized exclusively in the islet area (Fig. 2C, E). These 6 patients with Fas expression corresponded completely to the 6 patients with insulitis in a total of 13 patients examined (Table I). There were seven other patients without insulitis who were all negative for Fas staining in the islet. Staining of consecutive sections with anti-Fas and anti-mononuclear cell antibodies revealed that all the islets with the mononuclear cell infiltrate were Fas-positive.

Phenotypic analysis of Fas-positive cells. Double immunostaining for Fas and pancreatic hormones showed that islet endocrine cells were included among the Fas-positive cells. Fas-immunoreactive cells were mostly insulin-positive cells (Fig. 2C, D) and partly glucagon-positive cells (Fig. 2E, F). The prevalence of Fas expression in insulin-positive and glucagon-positive cells was analysed in five out of six Fas-positive patients. Immunoreactivity of Fas was seen in 92.2 to 97.7 % (average 94.5 %) of insulin-pos-

Table 1. Immunohistochemical findings of the pancreas biopsy specimens

Case	Insulitis	Remaining beta cells	Hyperexpression of MHC class I in islets	Fas expression in islets	Number of islets with Fas expression/total number of islets examined	FasL expression in islets	Number of islets with FasL expression/total number of islets examined
1	–	+	–	–	0/10	–	0/10
2	–	+	–	–	0/6	–	0/10
3	–	–	–	–	0/12	–	0/15
4	–	+	–	–	0/9	–	0/10
5	+	+	+	+	13/14	+	14/35
6	–	+	–	–	0/11	–	0/6
7	+	+	+	+	3/5	+	1/1
8	+	+	+	+	12/12	ND	ND
9	+	+	+	+	13/16	+	19/33
10	–	+	–	–	0/13	–	0/4
11	+	+	+	+	44/50	+	39/43
12	–	+	–	–	0/12	–	0/20
13	+	+	+	+	21/21	+	38/38

ND, not determined

Table 2. Prevalence of Fas-positive cells in insulin-positive and glucagon-positive cells

Case	Number of Fas-positive cells/total number of insulin-positive cells	%	Number of Fas-positive cells/total number of glucagon-positive cells	%
5	83/90	92.2	9/51	17.6
7	0/2	0	4/38	10.5
8	109/114	95.6	7/15	46.7
11	104/110	94.5	15/112	22.3
13	84/86	97.7	25/58	43.1
Total	380/402	94.5	60/274	21.9

itive cells (Table 2). Patient 7 was not included for the calculation of average because of the extremely small number of insulin-positive cells. Fas was detected in 10.5 to 46.7% of the glucagon-positive cells (average 21.9%) (Table 2).

Expression of FasL in the pancreas. FasL was detected in the islet area in 5 of 12 patients examined. The islets of all these five patients showed both insulitis and Fas expression. One patient with such phenomena who was included initially in the present study (case 8) could not be analysed for FasL because of the shortage of his pancreas sections. FasL-positive cells were not detected in the pancreas of seven other patients without insulitis and four control subjects.

Phenotypic analysis of FasL-positive cells and TUNEL analysis. The phenotype of FasL-positive cells was determined in four of the five patients. In double immunostaining, FasL-positive cells were CD4+ T or CD8+ T-cells or CD68+ macrophages (Fig. 3). A few CD56+ natural killer cells were identified in the insulitis lesion, but were FasL-negative. The prevalence of FasL expression in CD4+ T, CD8+ T and macrophages was 37.5 to 43.5% , 52.4 to 62.5% and 63.6 to

100% , respectively (Table 3). The percentages of CD4+ T, CD8+ T and macrophages among FasL-positive cells were 18, 49 and 33% , respectively (Table 3). This indicates that the most prevalent phenotype was CD8, followed by macrophages and CD4. In any case, FasL expression was not observed in islet endocrine cells. DNA strand break was not detected in the pancreas of any examined subject when assessed by the TUNEL method.

Fas and FasL expression and clinical characteristics. Fas and FasL expression were observed only in the islets of the patients with insulitis and neither Fas nor FasL was detectable in insulitis-negative patients without exception. Clinical characteristics such as patient age, sex, disease duration, urinary C peptide excretion, islet cell antibody status, GAD Ab status and HLA phenotype did not correlate significantly with Fas and FasL expression at the time of the disease onset (vs islet cell antibody, $p = 0.79$; vs GAD ab, $p = 0.73$; vs HLA-DR4, $p = 0.88$; vs HLA-DR9, $p = 0.88$).

Discussion

Fas is immunohistochemically detected in many organs including the liver, kidney, intestine, lung and ovary but not in the pancreatic islet [32]. We have shown that Fas is expressed in the pancreatic islet in patients with recent-onset Type I diabetes but not in normal subjects. A clear correlation between Fas-expression and insulitis indicates that inflammatory cytokines secreted by islet-infiltrating mononuclear cells are possible inducers of the Fas expression in endocrine cells. T-cells and macrophages are the major subpopulations of the infiltrate and it is widely accepted that these cells produce various cytokines. The transcripts of interferon (IFN)-α, IFN-γ, TNF-
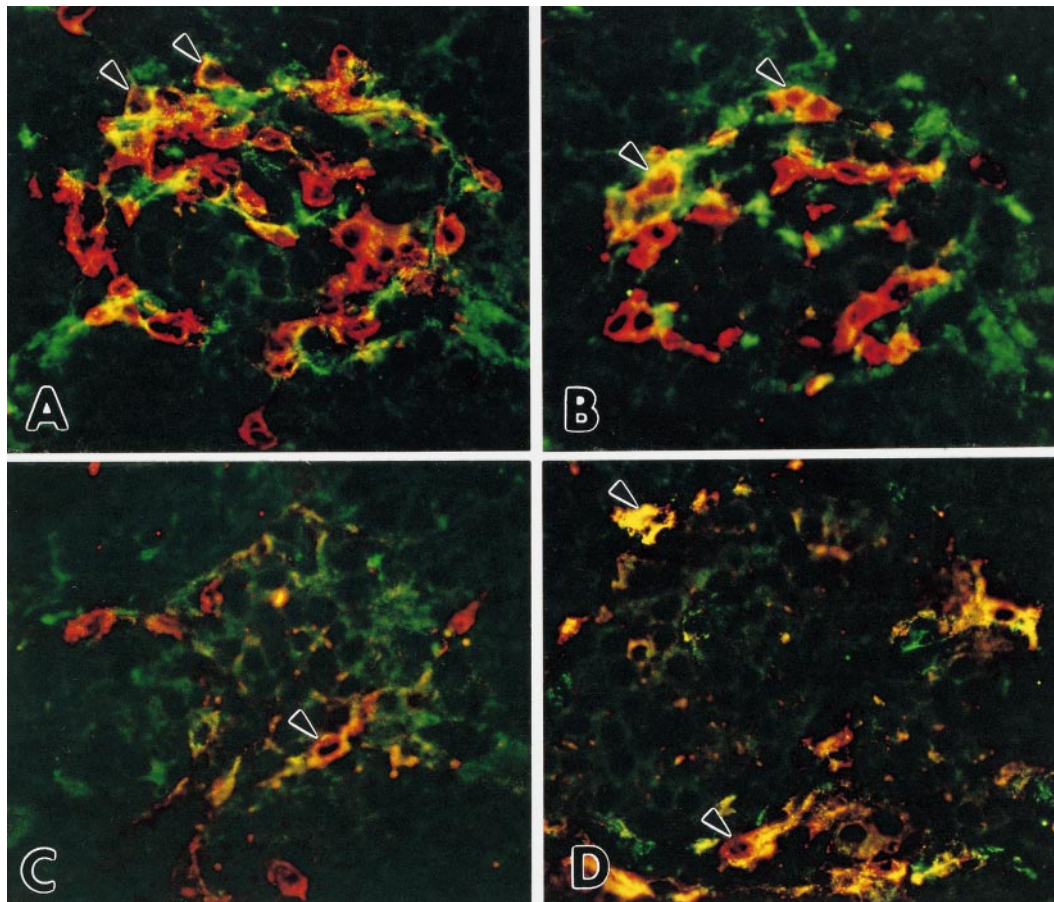
Fig. 3 A–D. Consecutive pancreas sections from a recent-onset Type I diabetic patient stained with a combination of anti-FasL antibody (green) and one of anti-mononuclear cell antibodies (red; **A**, CD3; **B**, CD8; **C**, CD4; **D**, CD68) (× 400). Representative double positive cells (yellow-orange) are indicated by arrowheads. These photographs indicate that FasL-positive cells are a fraction of CD4$^+$ T or CD8$^+$ T-cells or CD68$^+$ macrophages

α, IL-1, IL-4, and IL-6 have been identified in the pancreas of Type I diabetic patients so far [10, 33, 34]. Among these, IFN-γ, TNF-α or the combination of the two cytokines induces Fas mRNA and antigen expression in tumour cell lines [32, 35], whereas IL-1, IFN-γ and TNF-α induces Fas expression in isolated islet and beta cells [36, 37, 38]. It is, therefore,

postulated that infiltrating mononuclear cells secrete cytokines leading to Fas expression on islet cells at the insulitis lesion.

Double immunostaining surprisingly showed a discrepancy in Fas expression between islet cells. Most insulin-positive beta cells expressed Fas but only some glucagon-positive alpha cells showed Fas immunoreactivity. This could be due to the different reactivity between alpha and beta cells in response to the same cytokine signals. Although the reason is not clear, such a possibility has been raised by the results of several experiments. The beta cells produced inducible nitric oxide synthase or TNF-α or both more efficiently than alpha cells by the stimulation of cytokines [39, 40]. Mouse insulinoma-derived cell line, βTC1, was more susceptible to cytokine-induced apoptosis than glucagonoma-derived cell line, αTC1,

Table 3. Prevalence of FasL-positive cells in islets-infiltrating mononuclar cells

Case	FasL$^+$ CD4$^+$/ CD4$^+$ cells	%	FasL$^+$ CD8$^+$/ CD8$^+$ cells	%	FasL$^+$ Mø/ Mø cells	%	CD4:CD8:Mø(%) among FasL-positive cells
5	3/8	37.5	5/8	62.5	7/11	63.6	20:33:47
8	5/12	41.7	11/21	52.4	5/5	100	24:52:24
11	ND	ND	34/57	59.6	10/12	83.3	ND
13	10/23	43.5	32/58	55.2	21/22	95.5	16:51:33
Total[a]	18/43	41.8	48/87	55.2	33/38	86.8	18:49:33

Mø, Macrophages; ND, not determined; [a] Case 16 is not included in the Total due to the lack of CD4 data

probably because of lower endogenous Bcl-2 expression in βTC1 [41]. These facts indicate that the same inflammatory environment might elicit a more cytotoxic effect on beta cells than on alpha cells.

The results on expression of FasL in the infiltrating cells in our study were in good accordance with our recent finding that FasL is expressed on infiltrating cells in inflammatory lesions of ulcerative colitis, another cell-mediated autoimmune disease [42]. Another notable finding in our study was that FasL expression was observed on CD4+ T-cells and macrophages, as well as on CD8+ T-cells, which is the most prevalent phenotype among the FasL-positive infiltrating cells. The Fas-FasL system works not only in the pathway of CTL-mediated cytotoxicity but also in a variety of regulatory mechanisms. For example, CD4+ T-cells regulate immune systems by presenting FasL and leading Fas-bearing activated T-cells into activation-induced cell death [21]. It was also shown that macrophages could offer FasL and participate in lymphocyte depletion in human immunodeficiency virus-infected subjects [43]. As these CD4+ T-cells and macrophages did not act in an antigen-specific or MHC class I-restricted manner, a simple interaction with Fas-bearing cells might induce a bystander killing. Although CD8+ T-cell-predominant infiltration in the human and mouse islets suggest that CTL-mediated beta-cell destruction is important for the development of Type I diabetes [8–10, 44], the roles of CD4+ T-cells and macrophages have also been illustrated [45, 46]. If beta cells are prone to express Fas by cytokine stimulation, it is conceivable that Fas-FasL interaction occurs by chance between beta cells and islet-infiltrating cells, resulting in Fas-mediated apoptotic death of beta cells. Of note, a recent report on two Type I diabetic patients showing the Fas expression in beta cells and FasL expression in infiltrating cells [47] is in accordance with our results. On the other hand, we did not see any FasL expression in islet endocrine cells including beta cells, although human islet cells were reported to express FasL in response to interleukin-1β using anti-FasL antibody from Transduction Laboratories [48].

A certain polyclonal rabbit anti-FasL Ab (C-20, Santa Cruz Biotechnology) has been reported to produce false positive reaction in flow-cytometry [49]. We had tested several anti-FasL antibodies for immunohistochemistry and flow-cytometry before starting our study. The feasibility of the anti-FasL antibody was investigated by an absorption test. In our hands, some of the commercially available antibodies showed false positive staining in the exocrine pancreas tissue. After several attempts, we decided to use H707 (Nichirei) in this study because it did not elicit false positive staining in our immunohistochemical procedure. In addition, the positive FasL staining by H707 antibody was shown to be abolished by the pre-absorption of H707 antibody with the FasL peptide, confirming the specificity of H707 antibody. The result of the absorption study of other anti-FasL antibodies (C-20, or anti-FasL from Transduction Laboratories) have, however, not been reported. Thus, the discrepancy of FasL staining between studies was probably due to the anti-FasL antibody used.

Apoptosis is a form of cell death induced by Fas-FasL interaction. It can be recognized as DNA strand breaks and the TUNEL method is commonly used for its detection in situ. Based on the finding of Fas-FasL expression in the Type I diabetic pancreas, we expected and looked for in situ DNA strand breaks in the insulitis lesion. The TUNEL method did not, however, show obvious apoptotic signals in any pancreas sections. This could be explained by the very short term of completion of apoptosis [50] and the considerably reduced volume of the remaining target beta cells [9]. Using NOD.scid mice that had islet cell antigen-specific T-cell receptor transgene and an accelerated course of insulitis and diabetes, it has been calculated that the average apoptotic beta cell was cleared in only 1.7 min [50]. If this rate was applied to wild-type NOD, fewer than four apoptotic beta cells in the whole pancreas of one mouse were expected at any one time. Because it can be assumed that the time course of human Type I diabetes is slower than that of the NOD mouse, the difficulty in detecting in situ DNA fragmentation of beta cells is not surprising in a small piece of human biopsy specimen from a pancreas.

In conclusion, our study showed that beta cells expressed Fas, and islet-infiltrating mononuclear cells expressed FasL in inflamed islets of patients with recent-onset Type I diabetes. Among FasL-positive cells, the most prevalent phenotype was CD8, followed by macrophages and CD4. These results suggest that Fas-positive beta cells could be destroyed by an interaction with FasL-positive infiltrating cells in the inflamed islets, hence Fas-FasL-mediated apoptosis might play an important part in the destruction of pancreatic beta cells in insulitis-positive, immune-mediated Type I diabetes.

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