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Pancreatic beta and alpha cells are both decreased in patients with fulminant type 1 diabetes: a morphometrical assessment

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Abstract *Aims/hypothesis:* We have previously reported that fulminant type 1 diabetes is characterised by an absence of diabetes-related antibodies and a remarkably abrupt onset. However, little is known about the mechanism of beta cell destruction in this diabetes subtype, and to obtain insights into the aetiology of the disease, we investigated residual endocrine cells and the expression of Fas and Fas ligand in fulminant type 1 diabetes. *Methods:* Residual beta and alpha cells were morphologically assessed in pancreatic tissue obtained by biopsy from five patients with recent-onset fulminant type 1 diabetes and five patients with recent-onset typical autoimmune type 1 diabetes. In addition, the expression of Fas and Fas ligand was evaluated by immunohistochemistry. *Results:* In fulminant type 1 diabetes, beta and alpha cell areas were decreased significantly, compared with autoimmune type 1 diabetes and control subjects. In contrast, the alpha cell area was not decreased significantly in autoimmune type 1 diabetes, compared with that in control subjects. No Fas expression in islets and Fas ligand expression in CD3⁺ cells in the exocrine pancreas were found in the fulminant type 1 diabetes

patients who underwent this evaluation. *Conclusions/interpretation:* Our study showed that beta and alpha cells are damaged in fulminant type 1 diabetes. In addition to the lack of Fas and Fas ligand expression, the results suggest that the mechanism of beta cell destruction in fulminant type 1 diabetes is different from that in autoimmune type 1 diabetes.

Keywords Alpha cell · Biopsy · Fas · Fas ligand · Fulminant type 1 diabetes · Human · Immunohistochemistry · Morphometry · Type 1 diabetes

Introduction

Type 1 diabetes is caused by insulin deficiency resulting from the destruction of pancreatic beta cells. Type 1 diabetic patients without evidence of autoimmune disorder are classified as having type 1B or idiopathic diabetes by ADA/WHO criteria [1]. We have previously reported a novel subtype of type 1 diabetes characterised by an absence of diabetes-related antibodies, a remarkably abrupt onset, and a high serum pancreatic enzyme concentration, and we refer to this as fulminant type 1 diabetes [2, 3]. This condition belongs to the type 1B diabetes classification, according to ADA/WHO criteria, and accounts for approximately 20% of acute-onset type 1 diabetes in Japan [2, 3]. The clinical features of fulminant type 1 diabetes are significantly different from those of autoimmune type 1 (type 1A) diabetes, but little is known about the cause of fulminant type 1 diabetes.

To obtain insight into the mechanism of beta cell destruction in fulminant type 1 diabetes, we investigated in this study the residual beta and alpha cells and the expression of Fas and Fas ligand.

Subjects, materials and methods

Subjects Inclusion criteria for fulminant type 1 diabetes were: (1) presence of ketosis or ketoacidosis within a week

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after onset of hyperglycaemic symptoms; (2) urinary C-peptide excretion <10 µg/day, or fasting serum C-peptide level <0.3 ng/ml (0.1 nmol/l), or peak serum C-peptide level <0.5 ng/ml (0.17 nmol/l) after glucagon (1 mg) injection or a meal soon after disease onset; and (3) HbA_{1c} <8.5% on the first visit [2, 3]. Autoimmune type 1 diabetic patients were ketosis-prone and positive for GAD antibody or islet cell antibodies. The duration of hyperglycaemic symptoms before the start of insulin therapy was less than 3 months. Patients with slowly progressive type 1 diabetes or latent autoimmune diabetes of adults were excluded from the study.

We examined five patients with fulminant type 1 diabetes (two men and three women) and five patients with autoimmune type 1 diabetes (two men and three women). The mean age±SD of the fulminant type 1 diabetic patients was 32.4±11.1 years and that of the autoimmune type 1 diabetic patients was 21.5±4.8 years. The mean period of insulin treatment±SD in the fulminant type 1 diabetic patients was 2.8±1.9 months and that in the autoimmune type 1 diabetic patients was 4.8±2.5 months. There was no significant difference between the patients with fulminant type 1 diabetes and those with autoimmune type 1 diabetes in terms of age of onset and disease duration. Islet cell antibodies and/or GAD antibody were positive in all patients with autoimmune type 1 diabetes. Serum amylase or elastase-1 levels were elevated in all patients with fulminant type 1 diabetes at the onset of overt diabetes. The pancreatic tissues of six patients with gastric cancer (three men and three women, 53.2±10.1 years old) was examined as

control. Some of the results for T-cell infiltration and the expression of Fas and Fas ligand have been reported previously [4].

The study protocol was approved by the Ethics Committee of Osaka University Medical Hospital and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before the study.

Immunohistochemistry and morphometry Pancreatic sections from patients and controls were prepared and stained with haematoxylin and eosin, as reported previously [4, 5]. Each pancreatic biopsy specimen was 20–40 mg. To visualise pancreatic beta cells and alpha cells, sections were stained by the indirect immunoperoxidase technique, using one of the following primary antibodies: guinea-pig anti-insulin (Dako Japan, Kyoto, Japan) or rabbit anti-glucagon (generously provided by Dr Iwasa, Takeda Chemical, Osaka, Japan). The following biotinylated secondary antibodies were used in the indirect immunoperoxidase technique: rabbit anti-guinea-pig Igs (Vector, Burlingame, CA, USA) and goat anti-rabbit Igs (Vector). The reactions were developed with avidin–biotin complex (ABC) (Vector) and 3,3'-diaminobenzidine tetrahydrochloride (Zymed, South San Francisco, CA, USA) as substrate. All sections were counterstained with haematoxylin.

The total area of all sections was examined with a tablet-measuring device for micrometre measurement (Krypton-40; Flovel, Tokyo, Japan). The areas of the insulin- and glucagon-positive cells were measured with a Mac Scope,

Table 1 Immunohistological and morphometric analysis of pancreatic biopsy specimens

Classification/patient no.	Insulinitis	Beta cell area		Alpha cell area	
		Section (mm ²)	Beta cells (%)	Section (mm ²)	Alpha cells (%)
Fulminant					
1	No	5.960	0.005	3.239	0.068
2	No	6.078	0.001	6.315	0.047
3	No	8.532	0.013	10.843	0.049
4	No	18.499	0.003	18.124	0.095
5	No	5.599	0.016	5.806	0.089
Mean±SE	–	–	0.007±0.003*	–	0.069±0.010*
Autoimmune					
6	Yes	4.817	0.307	5.306	0.175
7	Yes	6.808	0.431	6.241	0.100
8	Yes	4.366	0.221	9.281	0.129
9	Yes	5.306	0.008	4.439	0.115
10	Yes	6.945	0.328	6.659	0.258
Mean±SE	–	–	0.259±0.071*	–	0.156±0.029
Control					
11	No	15.184	3.669	14.493	0.143
12	No	26.004	1.198	23.154	0.110
13	No	13.705	1.759	13.479	0.303
14	No	55.033	1.657	57.413	0.246
15	No	29.828	0.979	26.486	0.129
16	No	18.241	1.442	16.995	0.319
Mean±SE	–	–	1.784±0.395	–	0.209±0.037

**p*<0.05 vs. controls

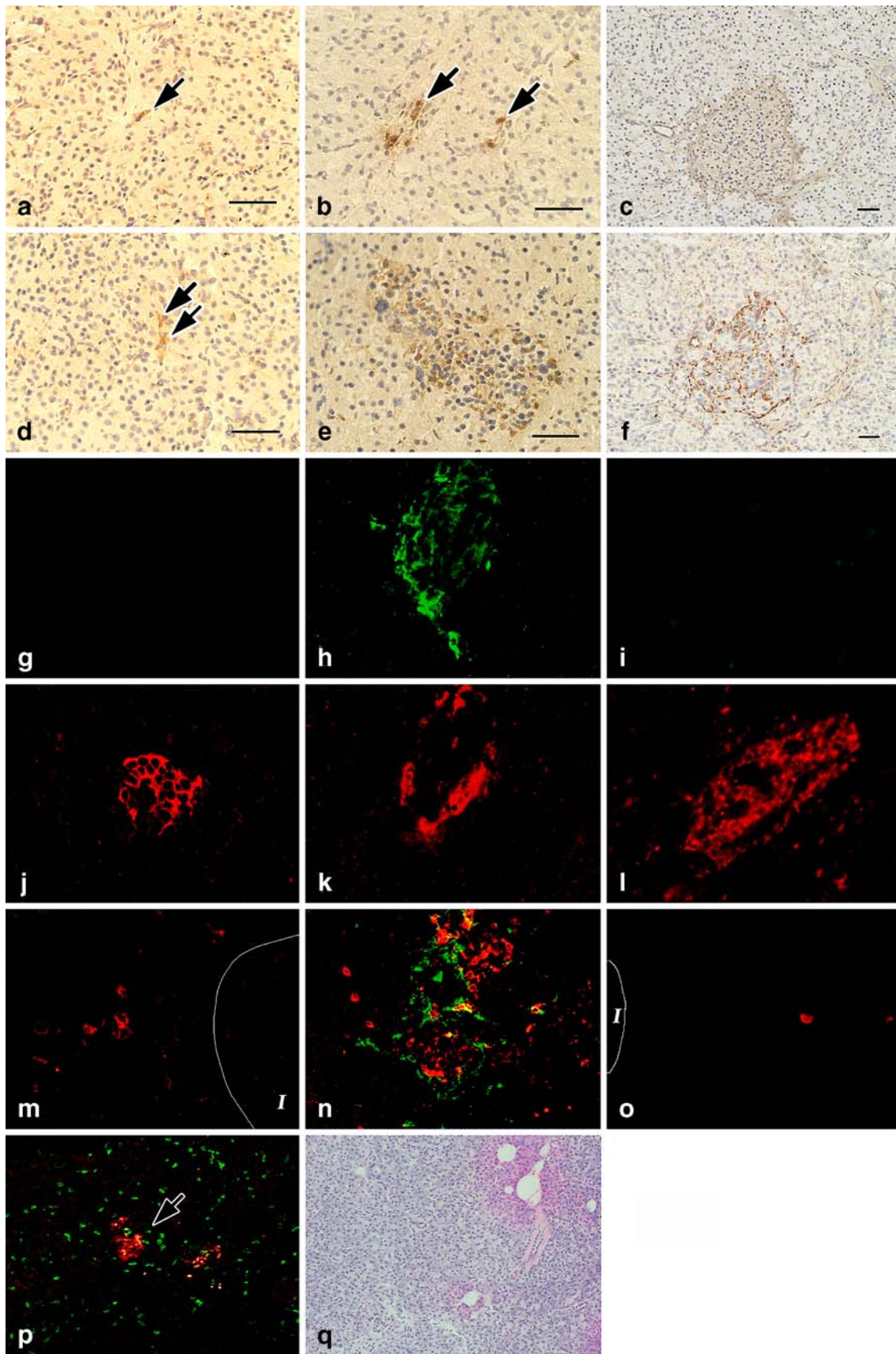


Fig. 1 Pancreatic sections of a fulminant type 1 diabetic patient. (a, d, g, j, m, p, q), an autoimmune type 1 diabetic patient (b, e, h, k, n) and a control subject (c, f, i, l, o). These sections were stained with anti-insulin antibody (brown; a–c), anti-glucagon antibody (brown; d–f), both anti-Fas (green; g–i) and anti-glucagon (red; j–l) antibodies, both anti-Fas ligand (green) and anti-CD3 (red) antibodies (m–o), both anti-glucagon (red) and anti-CD3 (green) antibodies (p), and haematoxylin and eosin (q). The arrows (a, b) indicate insulin-positive cells, glucagon-positive cells (d) and an islet (p). Bar=50 μ m. I, islet area (m, o)

Version 2.5 (Mitani Corporation, Fukui, Japan). For each subject, two sections separated by more than 500 μm were assessed to avoid measurement dispersion. The insulin- or glucagon-positive cell area was expressed as a percentage of the total area of the section.

To examine Fas and Fas ligand expression and the infiltration of CD3⁺ T cells, we performed immunohistochemical analysis, as reported previously [4, 5].

Statistical analysis The significance of differences between two groups was evaluated by Student's *t*-test. A level of $p < 0.05$ was considered to be statistically significant.

Results

Beta and alpha cell areas As shown in Table 1, the insulin- and glucagon-positive cell areas were 0.007 ± 0.003 and $0.069 \pm 0.010\%$ in fulminant type 1 diabetes, 0.259 ± 0.071 and $0.156 \pm 0.029\%$ in autoimmune type 1 diabetes, and 1.784 ± 0.395 and $0.209 \pm 0.037\%$ in control subjects respectively (means \pm SE). The beta cell area in fulminant type 1 diabetes and autoimmune type 1 diabetes was decreased significantly, in comparison with that in control subjects ($p = 0.0028$ and $p = 0.0072$ respectively). In fulminant type 1 diabetes, the beta cell area was also decreased significantly, in comparison with that in autoimmune type 1 diabetes ($p = 0.0077$).

The area of alpha cells in autoimmune type 1 diabetic patients did not differ from that in control subjects. However, in fulminant type 1 diabetic patients, the alpha cell area was decreased significantly, in comparison with that in autoimmune type 1 diabetic patients ($p = 0.0218$) and control subjects ($p = 0.0099$).

Fas and Fas ligand expression Fas expression was examined in five islets from three patients with fulminant type 1 diabetes, 71 islets from two patients with autoimmune type 1 diabetes, and 78 islets from four normal control subjects. There were no Fas-positive islets in fulminant diabetic patients or normal control subjects, but 65 Fas-positive islets were found in autoimmune diabetic patients. We also examined Fas ligand expression in four patients with fulminant type 1 diabetes, two patients with autoimmune type 1 diabetes, and four normal controls. CD3⁺ cells were observed in the exocrine area but not in the islets of any fulminant type 1 diabetes patients, as shown in Fig. 1. In a total of 1,470 CD3⁺ cells from four fulminant type 1 diabetes patients, Fas ligand was positive in only ten cells. In autoimmune type 1 diabetic patients, Fas ligand was positive in islet-infiltrating T cells in both patients examined (66 positive cells out of 115 cells). Fas-ligand-positive T cells were not observed in normal controls.

Exocrine pancreas in fulminant type 1 diabetes Haematoxylin and eosin staining showed that all islets were atrophic and distorted in all five patients with fulminant type 1 dia-

betes, but no oedematous change, necrosis, haemorrhage, suppuration, cyst formation or apparent atrophy of the exocrine pancreas was apparent in any patients (Fig. 1).

Discussion

Fulminant type 1 diabetes is a subtype distinct from typical type 1A diabetes, especially from a clinical point of view. In this subtype, a non-autoimmune mechanism of beta cell destruction is likely [2, 3]. Our present results show that both beta and alpha cells were decreased in recent-onset fulminant type 1 diabetes. In contrast, in autoimmune type 1 diabetes, only beta cells were decreased in the pancreatic biopsy specimens, and alpha cells were unaffected. In other words, pancreatic beta cells are specifically destroyed in autoimmune type 1 diabetes. Hence, insulin-deficient islets composed of normal numbers of alpha cells were found in the autopsied pancreas in 11 children with recent-onset type 1 diabetes [6], and no difference in alpha cell number was found between 11 juvenile diabetic patients and control subjects [7]. Furthermore, the mass of alpha, delta and pancreatic polypeptide cells and the ratio of delta to alpha cells in four type 1 diabetes patients did not differ from those in control subjects [8]. All these findings suggest that beta cells undergo specific damage in autoimmune type 1 diabetes, which may be promoted by the selective recognition of beta cell antigens by T cells. In contrast, both beta and alpha cells are damaged in fulminant type 1 diabetes, indicating that a cytotoxic mechanism that operates against both beta and alpha cells is involved in the pathogenesis of fulminant type 1 diabetes.

Fas was not observed in islet cells and Fas ligand was not observed in T cells in recent-onset fulminant type 1 diabetes patients in this study. In autoimmune type 1 diabetes, beta cell antigens are recognised through over-expressed MHC class I antigens by T-cell receptors of cytotoxic T cells, and then beta cells are destroyed by cytotoxic T cells through the Fas–Fas ligand system [4, 9]. In addition to the lack of expression of Fas in islet cells, hyperexpression of MHC class I molecules in islet cells was not observed in any fulminant type 1 diabetes patients, as shown in our previous report [2]. Fas ligand was also not expressed in infiltrating T cells in the exocrine pancreas in fulminant type 1 diabetes, although it was observed in islet-infiltrating cells in typical autoimmune type 1 diabetes. These facts also suggest different mechanisms of beta cell damage in autoimmune and fulminant type 1 diabetes.

Lernmark et al. [10] have reported that the presence or absence of insulinitis depends on age of onset and duration from onset to biopsy or autopsy in type 1 diabetic patients. In this study, however, there was no significant difference in age and disease duration between fulminant type 1 diabetes patients and autoimmune type 1 diabetes patients.

In conclusion, our present study shows that both beta and alpha cells are injured in fulminant type 1 diabetes. In addition to the lack of Fas and Fas ligand expression, the

results suggest that the mechanism of beta cell destruction in fulminant type 1 diabetes differs from that in autoimmune type 1 diabetes.

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