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A NOVEL SUBTYPE OF TYPE 1 DIABETES MELLITUS CHARACTERIZED BY A RAPID ONSET AND AN ABSENCE OF DIABETES-RELATED ANTIBODIES

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ABSTRACT

Background and Methods Type 1 diabetes mellitus is now classified as autoimmune (type 1A) or idiopathic (type 1B), but little is known about the latter. We classified 56 consecutive Japanese adults with type 1 diabetes according to the presence or absence of glutamic acid decarboxylase antibodies (their presence is a marker of autoimmunity) and compared their clinical, serologic, and pathological characteristics.

Results We divided the patients into three groups: 36 patients with positive tests for serum glutamic acid decarboxylase antibodies, 9 with negative tests for serum glutamic acid decarboxylase antibodies and glycosylated hemoglobin values higher than 11.5 percent, and 11 with negative tests for serum glutamic acid decarboxylase antibodies and glycosylated hemoglobin values lower than 8.5 percent. In comparison with the first two groups, the third group had a shorter mean duration of symptoms of hyperglycemia (4.0 days), a higher mean plasma glucose concentration (773 mg per deciliter [43 mmol per liter]) in spite of lower glycosylated hemoglobin values, diminished urinary excretion of C peptide, a more severe metabolic disorder (with ketoacidosis), higher serum pancreatic enzyme concentrations, and an absence of islet-cell, IA-2, and insulin antibodies. Immunohistologic studies of pancreatic-biopsy specimens from three patients with negative tests for glutamic acid decarboxylase antibodies and low glycosylated hemoglobin values revealed T-lymphocyte-predominant infiltrates in the exocrine pancreas but no insulinitis and no evidence of acute or chronic pancreatitis.

Conclusions Some patients with idiopathic type 1 diabetes have a nonautoimmune, fulminant disorder characterized by the absence of insulinitis and of diabetes-related antibodies, a remarkably abrupt onset, and high serum pancreatic enzyme concentrations. (N Engl J Med 2000;342:301-7.)

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TYPE 1 diabetes mellitus is caused by loss of insulin-secreting capacity due to selective autoimmune destruction of the pancreatic beta cells.^{1,2} Insulinitis (i.e., mononuclear-cell infiltration of the pancreatic islets) is the direct result of the autoimmune process. Antibodies to the cytoplasm of islet cells, glutamic acid decarboxylase, insulin, and tyrosine phosphatase-like protein (IA-2 or IA-2 β), which appear before the clinical onset of diabetes, are good markers of the autoimmune process.^{1,2}

Several lines of evidence have suggested that autoimmunity is not the only cause of beta-cell destruction. We and others have described young patients who presented with the abrupt onset of symptoms of hyperglycemia and who were prone to the development of ketoacidosis, as is characteristic of patients with type 1 diabetes, but who did not have insulinitis on either biopsy³⁻⁵ or autopsy.^{6,7} Furthermore, at least 10 percent of patients with newly diagnosed type 1 diabetes do not have any diabetes-related antibodies.^{8,9}

The American Diabetes Association and the World Health Organization have proposed that type 1 diabetes be subdivided into autoimmune (immune-mediated) diabetes (type 1A) and idiopathic diabetes with beta-cell destruction (type 1B).^{10,11} However, the specific characteristics of the idiopathic subtype are largely unknown. In a previous study,⁵ we found that the presence of glutamic acid decarboxylase antibodies, but not islet-cell antibodies, was closely correlated with direct evidence of insulinitis in patients with type 1 diabetes. In this report, we describe the results of detailed clinical and histologic studies of patients with idiopathic type 1 diabetes.

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*Other members of the Osaka IDDM Study Group are listed in the Appendix.

METHODS

Patients

We studied 56 consecutive patients with type 1 diabetes who came to our hospitals within one year after receiving the diagnosis, during the period from 1994 to 1998. All 56 patients met the criteria of the American Diabetes Association for type 1 diabetes — that is, pancreatic beta-cell destruction as the primary cause of the disorder and a tendency toward ketoacidosis^{10,11} — as determined by at least two physicians independently. Patients who had a period of remission that lasted for six months or more after the diagnosis had been made were excluded.¹² None of the enrolled patients consumed moderate or large amounts of alcohol. The study was approved by the ethics committee of Osaka University Medical Hospital, and written informed consent was obtained from each patient.

Clinical Characteristics and Serum Glutamic Acid Decarboxylase Antibodies

At the time of the onset of overt diabetes, all patients were hospitalized. Their clinical characteristics were recorded, and plasma glucose, serum electrolytes, arterial pH, glycosylated hemoglobin, and serum total or pancreatic amylase and elastase I were measured within two days after the initial diagnosis, and an ultrasonographic study of the pancreas was performed. Patients with arterial pH values lower than 7.35 and serum bicarbonate concentrations lower than 18 mmol per liter received the diagnosis of metabolic acidosis. Urinary C-peptide excretion was measured daily for at least three days, and the mean value was calculated. Subsequently, all patients underwent clinical examinations and measurements of glycosylated hemoglobin at monthly intervals.

The patients were divided into two groups according to the presence or absence of glutamic acid decarboxylase antibodies in serum samples obtained within three months after the initial diagnosis of diabetes, with the use of a radioimmunoassay kit (Rip-GAD, Hoechst Japan, Tokyo, or GAD-Ab Cosmic, Cosmic, Tokyo). A value greater than 5 units per milliliter (with the first kit) or 1.5 units per milliliter (with the second) was considered positive. The specificity and sensitivity of the first kit were 100 percent and 89.5 percent in the Second International GADAb Workshop, respectively,¹³ and the specificity and sensitivity of the second kit were both 100 percent in the Second and Third GAD Proficiency Test Results Evaluations (University of Florida, Gainesville).

Diabetes-Related and Thyroid Antibodies

Serum antibodies were measured within three months after the initial diagnosis of diabetes. Islet-cell antibodies were measured by an indirect immunofluorescence method (with an abnormal value defined as more than 5 Juvenile Diabetes Foundation units),⁴ IA-2 antibodies with an immunoprecipitation assay kit (with an abnormal value defined as more than 0.75 unit per milliliter) (Cosmic),¹⁴ and insulin antibodies with a liquid-phase radioimmunoassay (with an abnormal value defined as higher than the 99th percentile for 140 normal subjects).^{15,16} Thyroid antimicrosomal antibodies were measured by a hemagglutination assay (with an abnormal value defined as greater than 1:100).¹⁷

HLA Typing and Mitochondrial-DNA Analysis

Genomic and mitochondrial DNA were extracted from peripheral-blood leukocytes. The HLA class II antigen haplotype and the presence or absence of a guanine-for-adenine substitution at position 3243 in mitochondrial DNA were determined.^{5,18} No mitochondrial mutations were detected.

Pancreatic Studies

Pancreatic biopsies were performed in six patients within five months after the initial diagnosis of diabetes, as reported previously.^{3,5} The biopsy specimens were examined after staining with hematoxylin and eosin and by indirect immunohistochemical methods. To detect insulinitis, a double immunofluorescence method was

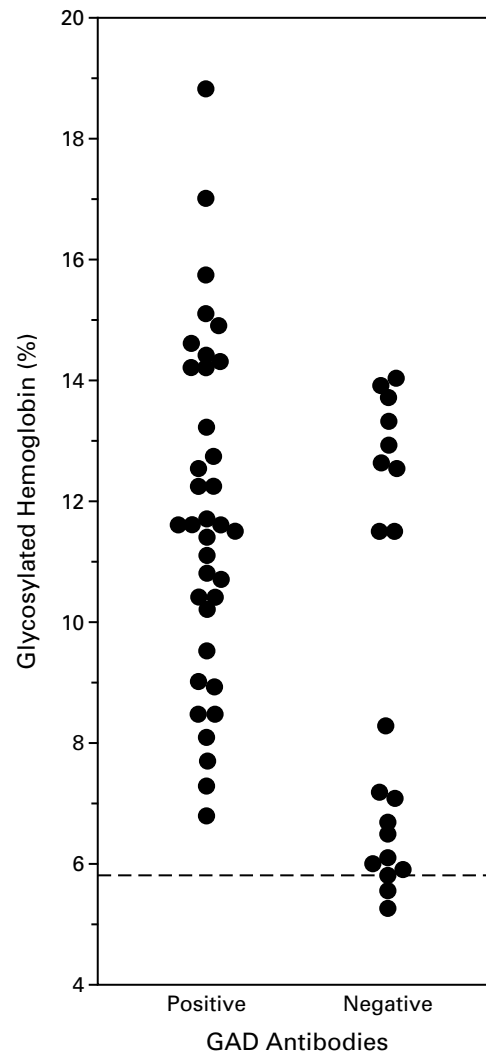


Figure 1. Glycosylated Hemoglobin Values at the Time of the Diagnosis of Diabetes in 56 Patients, According to Whether the Test for Glutamic Acid Decarboxylase (GAD) Antibodies Was Positive or Negative.

The values for glycosylated hemoglobin in the patients with positive antibody tests are scattered, whereas the values in the patients with negative antibody tests are clearly divided into two groups: those below 8.5 percent and those above 11.5 percent. The broken line indicates the upper limit of the normal range for glycosylated hemoglobin.

used with monoclonal antihuman CD3+ T-lymphocyte antibody, B-lymphocyte antibody, or macrophage antibody and anti-insulin or anti-glucagon antibodies. We also examined the expression of major histocompatibility complex (MHC) class I antigens in islets with the use of antihuman HLA-A, B, and C antibodies. The sources of these antibodies have been reported previously.⁴

To examine the relation between lymphocytes or macrophages and pancreatic exocrine tissue, we stained the cells with peroxidase substrate solution containing diaminobenzidine tetrachloride-nickel chloride (Zymed Laboratories, South San Francisco, Calif.). The sections were then incubated with antihuman alpha-amylase antibody (Sigma Chemical, St. Louis), and the pancreatic exocrine

TABLE 1. CHARACTERISTICS OF 56 PATIENTS WITH TYPE 1 DIABETES, ACCORDING TO WHETHER THE TEST FOR GLUTAMIC ACID DECARBOXYLASE ANTIBODIES (GAD) WAS POSITIVE OR NEGATIVE.*

CHARACTERISTIC	GAD-POSITIVE (N=36)	GAD-NEGATIVE, HIGH HbA _{1c} (N=9)	GAD-NEGATIVE, LOW HbA _{1c} (N=11)	P VALUE	
				GAD-NEGATIVE, LOW HbA _{1c} VS. GAD-POSITIVE	GAD-NEGATIVE, LOW HbA _{1c} VS. GAD-NEGATIVE, HIGH HbA _{1c}
HbA _{1c} (%)	11.7±2.8	12.9±1.0	6.4±0.9	<0.001	<0.001
Age (yr)					
Mean	32	27	38		0.05
Range	14–75	14–52	25–57		
Male sex (no.)	14	5	6		
Body-mass index†	19.1±2.6	20.0±2.9	21.0±3.8		
First-degree relative with diabetes (no.)‡	7	1	4		
Duration of hyperglycemic symptoms before diagnosis (days)	52.4±54.1	45.9±36.2	4.0±1.7	0.005	0.001
Abdominal pain (no.)	0	0	1		
Abnormal findings on pancreatic ultrasonography (no.)	0	0	0		
Plasma glucose (mg/dl)	398±198	439±179	773±250	<0.001	0.004
Urinary C peptide (µg/day)	21.0±11.2	19.7±10.3	3.2±1.9	<0.001	<0.001
Arterial pH	7.36±0.07	7.34±0.11	7.09±0.22	0.001	0.03
Serum bicarbonate (mmol/liter)	20.6±6.2	19.5±7.3	9.8±6.8	0.004	0.03
Serum amylase§	0.39±0.16	0.63±0.74	4.24±4.27	<0.001	0.02
Serum elastase I§	0.45±0.15	0.14±0.02	3.47±2.15	0.006	
Insulin dose during first yr (U/kg of body weight)	0.43±0.21	0.34±0.24	0.61±0.14	0.02	0.01

*Data were obtained at the time of diagnosis. Plus-minus values are means ±SD. To convert the values for glucose to millimoles per liter, multiply by 0.056. To convert the values for C peptide to millimoles per day, multiply by 0.33. HbA_{1c} denotes glycosylated hemoglobin.

†The body-mass index was calculated as the weight in kilograms divided by the square of the height in meters.

‡All affected relatives had type 2 diabetes except that one relative of each of two patients in the antibody-positive group had type 1 diabetes.

§Values for amylase and elastase I are expressed as multiples of the upper limit of the normal range.

cells were stained with 3-amino-9-ethylcarbazole (Dakopatts, Glostrup, Denmark).

Statistical Analysis

Statistical analysis was performed with Student’s t-test or Fisher’s exact probability test, as appropriate.

RESULTS

Serum glutamic acid decarboxylase antibodies were detected in 36 patients (64 percent) and were not detected in 20 patients (36 percent). The patients without glutamic acid decarboxylase antibodies were divided into two subgroups according to the initial glycosylated hemoglobin value: those with values of less than 8.5 percent, and those with values of more than 11.5 percent (Fig. 1).

The clinical characteristics of the patients with positive tests for glutamic acid decarboxylase antibodies and those of the two groups of patients with negative tests are shown in Table 1. The mean duration of hyperglycemic symptoms in the patients with negative tests for glutamic acid decarboxylase antibodies

and low glycosylated hemoglobin values was only 4.0 days. This group had a significantly higher mean glucose concentration, despite lower glycosylated hemoglobin values, and a significantly lower mean value for urinary C-peptide excretion than did the other two groups. All the patients with negative antibody tests and low glycosylated hemoglobin values had diabetic ketoacidosis, as compared with 20 percent of the patients with positive antibody tests and 40 percent of the patients with negative tests and high glycosylated hemoglobin values. Serum pancreatic enzyme concentrations were high in all the patients who had negative antibody tests and low glycosylated hemoglobin values but not in the other two groups (Table 1), and the values fell to the normal range in 3 to 38 days (median, 17).

All patients received multiple insulin injections, with a higher mean dose during the first year in the group of patients with negative antibody tests and low glycosylated hemoglobin values than in the other two groups (Table 1). Diabetes-related antibodies were not

TABLE 2. RESULTS OF OTHER ANTIBODY TESTS AT THE TIME OF DIAGNOSIS, ACCORDING TO WHETHER THE TEST FOR GLUTAMIC ACID DECARBOXYLASE ANTIBODIES (GAD) WAS POSITIVE OR NEGATIVE.*

TEST	GAD- POSITIVE (N=36)	GAD- NEGATIVE, HIGH HbA _{1c} (N=9)	GAD- NEGATIVE, LOW HbA _{1c} (N=11)
	no. with positive test/no. tested		
Islet-cell antibodies	15/22	3/7	0/11†
IA-2 antibodies	16/19	3/7	0/11†
Insulin antibodies	8/22	1/4	0/10‡
Thyroid microsomal antibodies	12/30	0/7	0/10§

*HbA_{1c} denotes glycosylated hemoglobin.

†P<0.001 for the comparison with the patients who had positive tests for glutamic acid decarboxylase antibodies, and P=0.04 for the comparison with the patients who had negative tests for glutamic acid decarboxylase antibodies and high HbA_{1c} values.

‡P=0.04 for the comparison with the patients who had positive tests for glutamic acid decarboxylase antibodies.

§P=0.02 for the comparison with the patients who had positive tests for glutamic acid decarboxylase antibodies.

detected in serum samples from any of the patients with negative tests for glutamic acid decarboxylase antibodies and low glycosylated hemoglobin values (Table 2).

The characteristics of the 11 patients with negative tests for glutamic acid decarboxylase antibodies and low glycosylated hemoglobin values are shown in Table 3. The HLA class II haplotype was determined in 10 of the patients. The haplotypes most often associated with type 1 diabetes in Japanese patients,¹⁹ HLA-DRB1*0405,DQA1*0303,DQB1*0401 and HLA-DRB1*0901,DQA1*0302,DQB1*0303, were present in five and three patients, respectively. Two haplotypes associated with resistance to type 1 diabetes, HLA-DRB1*1501,DQA1*0102,DQB1*0602 and HLA-DRB1*1502,DQA1*0103,DQB1*0601, were found in two patients and one patient, respectively.

Pancreatic biopsies were performed in one patient with a negative test for glutamic acid decarboxylase antibodies and a low glycosylated hemoglobin value (Patient 2 in Table 3) and in two other patients with similar findings (described previously⁵) who had been hospitalized before we started this study. In all three patients, no islet-cell antibodies were detected, hyperglycemic symptoms lasted for fewer than six days,

TABLE 3. CLINICAL CHARACTERISTICS OF THE 11 PATIENTS WITH NEGATIVE TESTS FOR GLUTAMIC ACID DECARBOXYLASE ANTIBODIES AND LOW GLYCOSYLATED HEMOGLOBIN (HbA_{1c}) VALUES AT THE ONSET OF DIABETES.*

PATIENT No.	AGE	SEX	BMI†	DURATION	SERUM GLUCOSE	HbA _{1c}	URINARY C PEPTIDE	pH	SERUM AMYLASE§	SERUM ELASTASE I§	HLA-DRB1, DQA1, DQB1 HAPLOTYPE
	yr			days‡	mg/dl	%	µg/day				
1	33	F	15.8	7	854	8.3	3.6	7.23	2.88	ND	0405,0303,0401/ 0803,0103,0601
2	40	M	17.6	6	643	6.7	1.1	7.29	0.32	1.28	0101,0101,0501/ 0405,0303,0401
3	57	M	29.8	2	811	6.5	6.5	7.22	6.96	1.64	ND
4	48	F	18.1	3	591	5.3	2.1	ND	1.16	3.38	0901,0302,0303/ 1502,0103,0601
5	25	F	21.0	3	1272	5.8	3.0	6.83	9.38	2.75	0901,0302,0303
6	29	M	19.7	5	555	6.0	0.6	ND	1.00	4.73	0405,0303,0401
7	35	F	19.1	4	993	5.6	5.7	6.81	12.21	7.05	1301,0103,0603/ 1501,0102,0602
8	36	F	22.1	3	726	7.2	1.3	6.86	1.13	ND	0802,0401,0302/ 1501,0102,0602
9	35	M	19.9	3	1041	6.1	<3.6	7.10	3.07	ND	0101,0101,0501/ 0405,0303,0401
10	34	M	22.7	6	447	5.9	3.0	7.33	ND	ND	0405,0303,0401/ 1405,0303,0303
30	41	M	24.4	2	569	7.1	4.7	ND	ND	ND	0901,0302,0303

*To convert the values for glucose to millimoles per liter, multiply by 0.056. To convert the values for C peptide to nanomoles per day, multiply by 0.33. ND denotes not determined.

†The body-mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

‡Duration refers to the period of hyperglycemic symptoms before the diagnosis of diabetes.

§Values are expressed as multiples of the upper limit of the normal range.

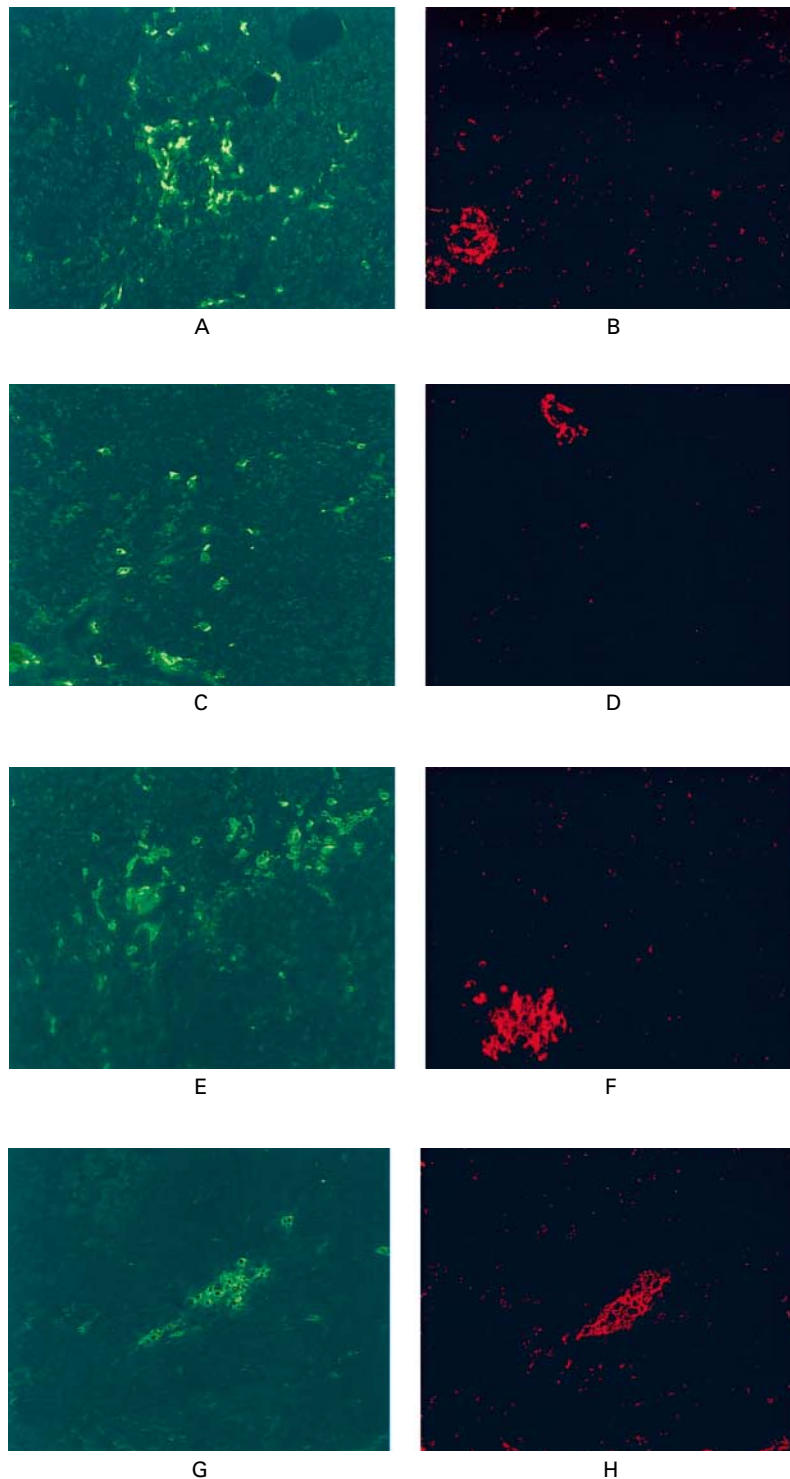


Figure 2. Photomicrographs of Double-Stained Pancreatic-Biopsy Specimens Showing Diffuse Infiltration of T Lymphocytes in the Exocrine Pancreas ($\times 140$).

Each pair of panels shows CD3+ T lymphocytes (green) and pancreatic alpha cells (red) in a biopsy specimen from one patient. Panels A and B, C and D, and E and F show specimens from three patients with negative tests for glutamic acid decarboxylase and islet-cell antibodies, and Panels G and H show specimens from one patient with positive tests for glutamic acid decarboxylase and islet-cell antibodies.

and at least one serum pancreatic enzyme value was elevated initially. As a control, pancreatic studies were also performed in three patients with positive tests for glutamic acid decarboxylase antibodies or islet-cell antibodies and glycosylated hemoglobin values of 8.5 to 15.1 percent.

Light-microscopical examination of sections of pancreas stained with hematoxylin and eosin revealed small, atrophic, distorted islet cells in all six patients. However, none of the patients had edema, necrosis, hemorrhage, suppuration, cyst formation, fibrosis, or apparent atrophy of the exocrine pancreas. Immunohistochemical examination revealed a markedly reduced beta-cell mass in all six patients, as would be expected in patients with type 1 diabetes. The sections from all three patients with negative tests for glutamic acid decarboxylase antibodies had T-lymphocyte–predominant infiltration of the exocrine pancreas but no insulinitis (Fig. 2 and 3). On the other hand, insulinitis but no cellular infiltration of the exocrine pancreas was seen in the sections from all three patients with positive antibody tests (Fig. 3). Hyperexpression of MHC class I molecules, another immunologic abnormality in islets, was seen only in the sections from the patients with insulinitis.

DISCUSSION

Among 56 consecutive Japanese patients who had type 1 diabetes, we identified 11 with a subtype of diabetes that differed from autoimmune diabetes in three respects. First, no autoimmune features were detected. The patients did not have diabetes-related serum antibodies, such as islet-cell, glutamic acid decarboxylase, IA-2, or insulin antibodies. Pancreatic biopsies revealed neither insulinitis nor hyperexpression of MHC class I molecules in islets.

Second, the onset of overt diabetes was rapid, and diabetic ketoacidosis occurred soon after the onset of hyperglycemic symptoms. The mean duration of hyperglycemic symptoms before the diagnosis was only four days. The short duration of hyperglycemia was reflected by the patients' nearly normal glycosylated hemoglobin values. Insulin-secretory capacity, estimated on the basis of urinary C-peptide excretion, was low, and the metabolic derangement at the onset was severe.

Third, the patients had markedly elevated serum pancreatic enzyme concentrations, a finding in accordance with the lymphocytic infiltration of the exocrine pancreas seen in the biopsy specimens. In contrast, the other patients had normal serum pancreatic enzyme concentrations and insulinitis but did not have lymphocytic infiltrates in the exocrine pancreas. The edema, necrosis, hemorrhage, suppuration, cyst formation, and fibrosis that characterize classic acute or chronic pancreatitis were not present in our patients.²⁰ In addition, none of the patients with negative tests for glutamic acid decarboxylase antibodies and low gly-

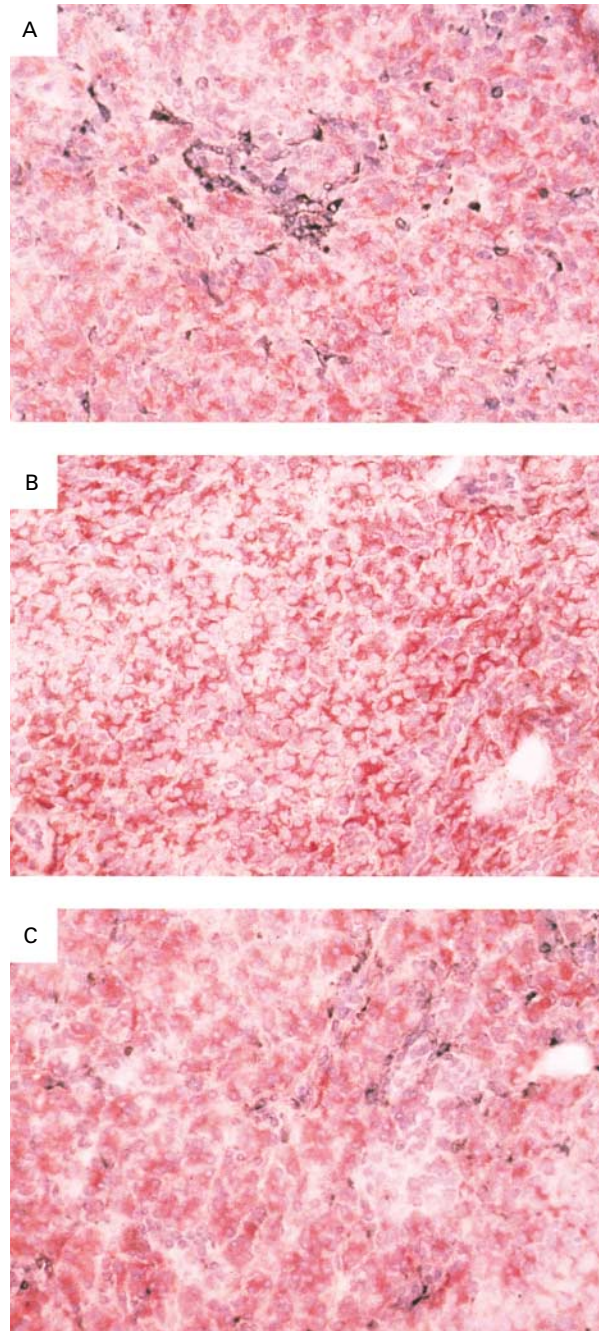


Figure 3. T-Lymphocyte–Predominant Infiltration of the Exocrine Pancreas in a Patient with a Positive Test for Glutamic Acid Decarboxylase Antibodies ($\times 200$).

T lymphocytes (Panel A), B lymphocytes (Panel B), and macrophages (Panel C) were stained with peroxidase substrate solution containing diaminobenzidine tetrahydrochloride–nickel chloride (black), and exocrine pancreas was stained with antihuman alpha-amylase antibody followed by 3-amino-9-ethylcarbazole (pink). Counterstaining was performed with hematoxylin.

cosylated hemoglobin values drank moderate or large amounts of alcohol, only 1 had abdominal pain, and all 11 had normal findings on ultrasonography of the pancreas. Therefore, these 11 patients had a type of diabetes other than that caused by classic pancreatitis.^{10,11}

On the basis of these findings, we believe that diabetes characterized by the absence of glutamic acid decarboxylase antibodies and low glycosylated hemoglobin values should be classified as nonautoimmune, fulminant type 1 diabetes, a subtype of idiopathic (type 1B) diabetes. Some similar cases have been reported previously.^{21,22}

The precise mechanism of beta-cell destruction in patients with this subtype of diabetes is not known. A viral cause is suggested by the abrupt onset of diabetes, the presence of lymphocytic infiltrates in the exocrine pancreas, and the affinity of several viruses for exocrine pancreatic tissue.^{23,24} In preliminary studies, however, none of our patients had high titers of antiviral antibodies (data not shown).

Further studies with younger patients and other ethnic groups may provide a better understanding of this subtype of diabetes. All our patients were adults, and the clinical features of type 1 diabetes differ to some extent in children and adults.²⁵ Diabetes-related antibodies are more often detected in white patients than in Japanese patients,²⁶ suggesting that nonautoimmune, fulminant type 1 diabetes may be rare in whites and that this subtype may therefore have been overlooked in studies of autoimmune diabetes in whites.

In conclusion, nonautoimmune, fulminant type 1 diabetes mellitus in Japanese adults is a novel subtype of type 1 diabetes characterized by the absence of both insulinitis and diabetes-related antibodies, an abrupt onset, and high serum pancreatic enzyme concentrations.

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APPENDIX

Other members of the Osaka IDDM Study Group included M. Namba, H. Nakajima, K. Yamamoto, H. Iwahashi, K. Yamagata, M. Moriwaki, T. Nanmo, S. Kawata, and S. Tamura (Osaka University); N. Itoh and T. Matsuyama (Toyonaka Municipal Hospital); I. Minco and C. Nakagawa (Otemae Hospital); Y. Yamada (Sumitomo Hospital); H. Itoh (Ikeda Municipal Hospital); M. Kawachi (Izumi-otsu Municipal Hospital); H. Toyoshima (Mino Municipal Hospital); N. Watanabe, M. Hashimoto, and T. Kinoshita (Nishinomiya Prefectural Hospital); and H. Asakawa (Itami Municipal Hospital).

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