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Distinct Inflammatory Changes of the Pancreas of Slowly Progressive Insulin-dependent (Type 1) Diabetes

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Objective: The aim of this study was to identify the distinct pathological changes on the endocrine and exocrine pancreas of slowly progressive insulin-dependent diabetes mellitus (SPIDDM) or latent autoimmune diabetes in adults.

Methods: The pancreases from 12 islet autoantibody-positive SPIDDM patients and 19 age-matched subjects with no diabetes were examined histologically for islet inflammation/insulinitis, expressions of cytokines, and enterovirus VP1 protein, exocrine pancreatic inflammation, pancreatic ductal changes, major histocompatibility complex class I hyperexpression, and amylin-positive amyloid in the islets.

Results: Insulinitis dominant for CD8⁺ T-cells and CD68⁺ macrophages was observed in all SPIDDM cases irrespective of duration of diabetes and weight of residual beta cells. Major histocompatibility complex class I hyperexpression on residual beta cells was observed in SPIDDM. All SPIDDM exocrine pancreases showed extensive inflammation, dilated pancreatic ducts, and periductal fibrosis. As many as 75% (9/12) of pancreases had pancreatic intraepithelial neoplasia, which is assumed to

be associated with ductal obstruction/narrowing and exocrine pancreatic inflammation, in SPIDDM. Amylin-positive amyloid deposition was not detected in SPIDDM.

Conclusions: Persistent insulinitis with preserved beta cells and major histocompatibility complex class I hyperexpression and exocrine pancreatic inflammation with pancreatic intraepithelial neoplasia are distinct histological features of SPIDDM pancreas.

Key Words: chronic pancreatitis, GADAbs, insulinitis, PanIN, SPIDDM (*Pancreas* 2018;47: 1101–1109)

Slowly progressive insulin-dependent (type 1) diabetes (SPIDDM),^{1–4} later also referred to as latent autoimmune diabetes in adults,^{5,6} is the most prevalent clinical subtype of type 1 diabetes mellitus.⁶ Late age at onset; progressive beta cell failure, which is associated with an initial non-insulin-requiring state and an ultimate insulin-dependent state over several years; and persistent islet cell autoantibodies including glutamic acid decarboxylase autoantibodies (GADAbs) and islet cell antibodies (ICAs) are characteristic clinical features of this subtype of type 1 diabetes.^{3,7,8} Slowly progressive insulin-dependent diabetes or latent autoimmune diabetes in adults is a conceptual phenotype between classic acute-onset type 1 diabetes (AT1DM) and type 2 diabetes (T2DM).⁶ Intervention trials to prevent progressive beta cell failure using insulin, metformin, DPP4 inhibitors, and/or GLP-1 analogs are currently challenging clinical issues in SPIDDM.^{9–12} However, reports on pathological changes in the pancreas of SPIDDM are lacking; the presence or absence of islet inflammation, a sign of type 1 diabetes, and islet amyloid deposition, a sign of T2DM, are obscure.¹³ Exocrine pancreatic inflammation and atrophy in type 1 diabetes are currently under discussion.^{14–16} To the best of our knowledge, this is the first systematic study of in situ changes of islets and exocrine pancreatic tissues in SPIDDM pancreases positive for GADAbs and ICAs. The pathological features including insulinitis, profiles of islet-infiltrating mononuclear cells (MNCs), major histocompatibility complex (MHC) class I hyperexpression, pancreatic ductal changes, and exocrine inflammation of SPIDDM were studied by comparison with age-matched control subjects with no diabetes.

MATERIALS AND METHODS

Subjects

Pancreatic tissue acquisition and data collection were performed between 1982 and 2014 and partly published previously.^{17–20} The pancreatic samples were obtained from Toranomon Hospital (Tokyo, Japan), University of Yamanashi (Yamanashi, Japan), Saitama Social Insurance Hospital (Saitama, Japan), and Tokyo Saiseikai Central Hospital (Tokyo, Japan). Twelve pancreases from SPIDDM patients and 19 age-matched control subjects with no diabetes were examined to minimize age-related bias (Table 1).

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Received for publication January 23, 2018; accepted July 15, 2018.

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This work was supported by a research grant from Japan Society for Promotion of Science KAKENHI (grant 24591319).

The authors declare no conflict of interest.

Author Contributions: K.A. and T. Fukui conducted immunohistochemical staining and discussed, reviewed, and edited the article. E.J. contributed to analysis of immunostained sample data and discussion. S.Y. contributed to planning and discussion and edited the article. T. Fukui, A.S., T. Fujii, and Y.O. sampled autopsied or biopsied pancreases and participated in discussion. Y.M., Y.N., and R.K. contributed to analysis of the data, discussion, and reviewed the article. T.K. planned the study, sampled autopsied pancreases, analyzed data, and wrote and edited the article. All authors approved the final version to be published. T.K. is the guarantor of this work and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pancreasjournal.com).

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DOI: 10.1097/MPA.0000000000001144

All subjects were of Japanese ethnic origin. This report presents the results from the pancreases obtained from autopsied samples, except for 1 biopsied case (case SP-10). Patients with SPIDDM had adult-onset diabetes with a non-insulin-requiring period of more than 3 months, as well as GADAbs and/or ICAs (Table 1). Their findings met the Japan Diabetes Society Diagnostic Criteria for SPIDDM.⁸ Patients with SPIDDM with heavy alcohol use and inflammatory bowel diseases were excluded. The causes of death of the 19 age-matched control subjects with no diabetes (ND-1 to ND-19, Table 1) were as follows: pneumonia (n = 1), chronic renal failure (n = 2), cerebral infarction (n = 1), acute myocardial infarction (n = 1), retroperitoneal tumor (n = 1), intestinal bleeding (n = 1), duodenal cancer (n = 6), gastrinoma (n = 1), colon cancer (n = 1), and biliary cancer (n = 4). No patients received treatment with glucocorticoids.

Tissue Preparation and Histochemical and Immunohistochemical Analyses

The tail and body part of each pancreas were obtained for pathological analyses of islet morphology, pancreatic ductal changes, and parenchymal changes. For quantification analyses including the beta cell area, insulinitis frequencies and MNC subtyping in the islets and exocrine pancreatic tissues were performed on the pancreatic tissues from the midpoint of the pancreas to the position one-quarter of the distance to the end of the pancreatic tail, because this part is regarded as fairly representative of the morphometric parameters of the entire pancreas.²¹ Pancreatic tissues were 4% formalin fixed and paraffin embedded. Methods for immunohistochemical analyses on MNCs for CD subtyping, class I MHC hyperexpression, expressions of innate immune receptors

TABLE 1. Clinical Characteristics of the Subjects With SPIDDM and Nondiabetic Control Subjects (Non-DC)

Case Subject	Age, y	Sex	Duration of Diabetes, y	GADAb, U/mL/ICA, JDF U	Pancreatic Weight, g	C-Peptide, ng/mL	Body Mass Index, kg/m ²	Treatment for Diabetes	Non-Insulin- Requiring Period, y	Cause of Death
SPIDDM										
SP-1	56	F	3	12.5/5	30	0.5	19.7	Insulin	1.5	Gastrointestinal bleeding
SP-2	58	F	21	3.4/ND	20.5	1.5	19.2	Insulin	9	Meningitis
SP-3	56	M	10	3.4/ND	23	1.8	20.2	Insulin	10	Gastric cancer
SP-4	42	M	17	4.2/ND	28	2.8	20.4	Insulin	12	Esophageal cancer
SP-5	43	M	13	4.6/ND	31	1.17	21.1	Insulin	10	Small intestinal cancer
SP-6	68	F	8	398.0/10	45	<0.02	18.2	Insulin	1.2	Chronic renal failure
SP-7	75	M	11	12.8/5	35.5	0.14	21.2	Insulin	0.8	Esophageal cancer
SP-8	87	M	14	5.8/5	18.4	<0.05	24.3	Insulin	1	Myocardial infarction,
SP-9	55	F	16	23.1/ND	33	<0.1	19.1	Insulin	10.1	Chronic renal failure
SP-10	65	F	0.3	151/+*	ND	9 µg/d [†]	26	Insulin	0.3	NA [‡]
SP-11	56	F	17	3.4/5	24.9	0.5	16.9	Insulin	5.8	Cerebral infarction, pneumonia
SP-12	77	F	24	3.5/5	40	0.6	17.2	Insulin	3.5	Acute respiratory distress, multiple cerebral infarction
Mean (SD)	62 (14)	M/F: 5/7	12.0 (6.9)	52.1 (116.6)	29.9 (8.2)	0.82 (0.89)	20.3 (2.7)		5.4 (4.5)	
Range	42–87	42–87	0.3–24	3.4–398.0/5–10	18.4–40.0	0–2.8	17.2–26.0		0.3–12.0	
Non-DC										
ND-1–ND-19 [§]										
Mean (SD)	67 (7)	M/F: 10/9	—	Negative/ND	79.2 (8.8)	ND	21.7 (2.9)		—	
Range	53–82				63.1–95.1		16.4–27.3		—	See the text

*Positive for IA-2Ab.

[†]Twenty-four-hour urine C-peptide (normal, 16–120 µg/d).

[‡]Biopsied sample.

[§]Nondiabetic control.

NA indicates not applicable; ND, not detected.

(RIG-I, MDA5, and Toll-like receptor 4 [TLR4]), cytokine (interferon γ [IFN- γ], and interleukin 18) expressions, enterovirus VP1 protein localization, and morphometric analysis of beta cells were done as reported.^{17–19} Two investigators judged independently in a double-blind manner.

Assessment of Islet Inflammation, Insulinitis, and Pseudoatrophic Islets

To minimize possible sampling bias for counting CD marker-positive MNCs in the islets, MNC counting was done on serial sections (4 μ m thick) stained by triple immune staining: beta cells and alpha cells were stained by immunofluorescence, and CD marker-positive MNCs were stained by immunoperoxidase staining. More than 10 islets with long diameters of 50 μ m or more and with more than 10 alpha cells were stocked in the captured image at magnification $\times 200$ (DP73; Olympus, Tokyo, Japan). Surface markers for CD45, CD3, CD8, CD4, CD20, CD11c, and CD68 were stained on the pancreatic sections.¹⁹ L-plastin (LPL), actin-binding molecule crucial for T-cell activation, polarization, and chemotactic actions to target sites,^{22,23} was also examined using specific antisera.²⁴ L-plastin was identified in inflamed islets of enterovirus-induced fulminant type 1 diabetes (FT1DM) by laser-capture microdissection followed by mass spectrometry.²⁴ In the captured images, the numbers of CD3⁺ cells stained by immunoperoxidase in the islet areas marked by glucagon-positive cells were counted. Insulinitis was defined as an islet with 6 or more CD3⁺ cells immediately adjacent to (peri-islet area) or within the islet (intra-islet area) in 3 or more islets per pancreas section.^{25,26} The average number of MNCs in the intra-islet area plus peri-islet area was used as an individual sample for further statistical analysis. Pseudoatrophic islets were defined as islets devoid of beta cells having a long diameter of more than 50 μ m.

Estimation of Islet Beta Cell Area and Beta Cell Weight

The beta cell and alpha cell areas were measured morphometrically by Cellsens software (version 1.16; Olympus). At least 5 pancreatic sections were measured, and the average values are expressed as percentages of the total section area (% beta cell area). The estimation of beta cell weight was calculated by (% beta cell area) \times pancreatic weight of each case.

Examination of Pancreatic Exocrine Tissue

The number of CD45⁺ MNCs and CD3⁺ MNCs in pancreatic exocrine cells was counted in 5 randomly selected photographs of pancreatic sections (1.4 \times 1.1 mm²) of each case at $\times 100$ magnification using Cellsens software. The average number of CD45⁺ and CD3⁺ MNCs in the exocrine tissues was used as an individual sample for further statistical analysis. Pancreatic ductal changes were examined as reported.¹⁵

Examination of Pancreatic Tissue on Amylin-Positive Amyloid Deposition

The presence of amylin-positive amyloid deposition in the islets of the subjects was examined using Congo red staining and specific sera against amylin.²⁷

Assay Methods for GADAbs, ICAs, and Human Leukocyte Antigen

Levels of GADAbs were measured by radioimmunoassay (RSR, Cardiff, United Kingdom), and GADAb titers of 1.5 U/mL or higher were judged positive.⁹ Islet cell antibodies were assayed by immunofluorescence methods.²⁸ Our laboratory participated in

the second through fifth International Workshop on Standardization of the ICA Assay, in which the quality of our Lab B was in the highest-quality class (detection limit, <5 JDF U; precision score, 0.65; and specificity, 100%).²⁹ The HLA gene, which encodes cell surface protein responsible for the regulation of the immune system in humans, was typed as previously.³

Ethics

Written informed consent was obtained from the next of kin on behalf of the autopsied patients and from the biopsied patient. All procedures used in this study were approved by the ethics committees of the University of Yamanashi and Toranomon Hospital.

Statistical Analysis

Fisher's exact test was used to evaluate the frequencies of lesions. Comparisons between groups were performed using the Kruskal-Wallis test and the Mann-Whitney *U* test. Values are expressed as means (standard deviation [SD]) or medians depicted by box-and-whisker plots. *P* < 0.05 was considered significant.

RESULTS

Islet Inflammation and Insulinitis

Mononuclear cells positive for CD45⁺ and CD3⁺ infiltrated the peri-islet and intraislet areas in all SPIDDM pancreases (Figs. 1A, B). The median frequency of defined insulinitis, observed in all 12 SPIDDM pancreases in a patchy distribution, was 14.0%, ranging from 3.5% to 33.3% (*n* = 524) (Fig. 1C). The cell numbers of CD45⁺ MNCs in the intraislet and peri-islet areas correlated well with those of CD3⁺ MNCs (*r* = 0.926, *P* < 0.001), suggesting that the insulinitis lesion is mainly composed of CD3⁺ T-cells. The mean number of CD3⁺ T-cells in the islets with insulinitis was 9 (SD, 5) (range, 6–29). The main subsets of MNCs infiltrating to the islets were CD8⁺ T-cells and CD68⁺ macrophages in SPIDDM (Fig. 1D). High proportions of CD8⁺ and CD68⁺ cells in peri-islet and intraislet areas were positive for LPL (Fig. 1E) in SPIDDM. The LPL-positive CD8⁺ T-cells showed the characteristic “uropod” appearance, indicating that the CD8⁺ T-cells were activated/polarized and chemotactic to target cells (Fig. 1E) in SPIDDM. These findings suggest that most activated CD8⁺ T-cells around the islets may target and migrate from peri-islet areas into the islets of SPIDDM, according to the previous findings in enterovirus-induced FT1DM and AT1DM.^{17,19,20,25,30,31} Insulinitis in SPIDDM persisted for long periods after the onset of diabetes (Fig. 1F) and differing previous reports of classic AT1DM.^{25,30,31} No correlation was found between the frequency of insulinitis and titer of GADAbs (Fig. 1G).

Beta Cell Weight, Fasting Serum C-Peptide Levels, and Pseudoatrophic Islets

The beta cell area and calculated beta cell weight were significantly decreased to $\sim 10\%$ of control subjects with no diabetes, but beta cells remained in 75% (9/12) of cases with SPIDDM, even in cases with a long duration of diabetes (Figs. 2A, B, C). Beta cell volume in SPIDDM cases correlated well with fasting serum C-peptide levels measured in outpatient clinics on routine visits within 3 years before their demise (Fig. 2D). Pseudoatrophic islets devoid of beta cells were observed in 67% (8/12) of SPIDDM cases at a frequency of 28.5% (SD, 43.4%) (range, 0%–100%; *n* = 524) of islets, and 5 of 8 cases had insulinitis in the pseudoatrophic islets (Supplemental Table 1, <http://links.lww.com/MPA/A674>). No pseudoatrophic islets were observed in age-matched control subjects with no diabetes.

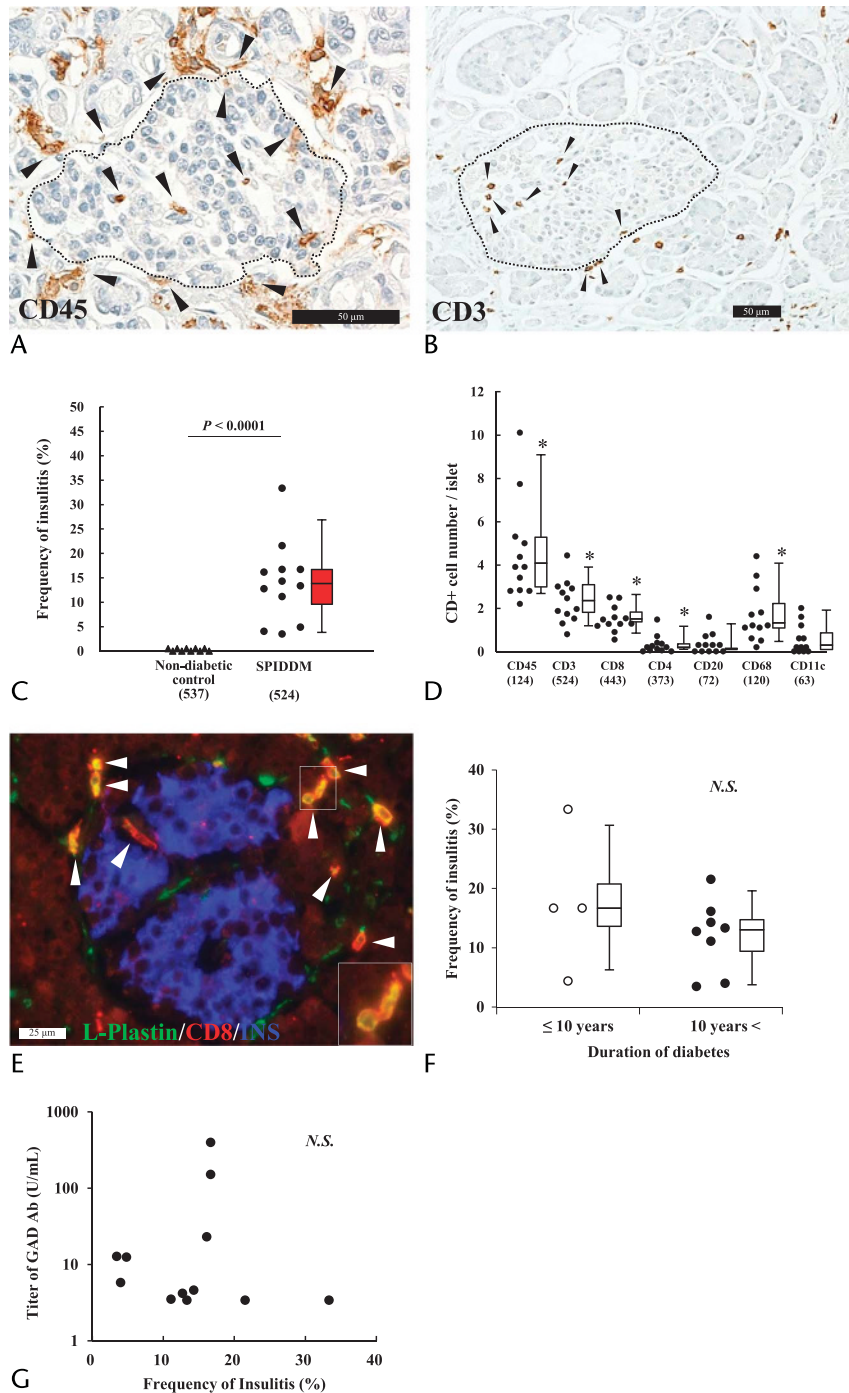


FIGURE 1. Islet inflammation in SPIDDM. A, CD45⁺ (leukocyte common antigen) MNCs (brown, arrowheads) infiltrate in and around the islet demarcated by the dashed line in SPIDDM (case SP-6) (scale bar, 50 μ m). B, CD3⁺ T-cells (brown, arrowheads) are observed in and around the islet demarcated by the dashed line (case SP-3) (scale bar, 50 μ m). C, Overall frequencies of insulinitis in SPIDDM patients and control subjects with no diabetes with medians depicted by box-and-whisker plots. The box represents the mid 50% of the data, and the high and low whiskers represent the 95th and 5th percentiles. Numbers in parentheses indicate the numbers of individual islets counted. D, The median number of leukocyte subtypes per islet in the SPIDDM pancreas with medians depicted by box-and-whisker plots. *Significantly higher than control subjects with no diabetes. Numbers in parentheses indicate total numbers of individual islets analyzed for each leukocyte marker. $P < 0.05$ versus control subjects with no diabetes. E, Merged image of triple immunostaining for LPL (green), CD8 (red), and insulin (blue) in the SPIDDM pancreas. Most CD8⁺ T-cells double positive for LPL and CD8 (arrowheads stained as yellow) are infiltrated to the islets and show the characteristic "uropod" appearance (inset) (case SP-1) (scale bar, 25 μ m). F, There are no differences of insulinitis frequencies between SPIDDM patients with short duration and long duration. N.S. indicates not significant. G, Relationship between frequencies of insulinitis and titers of GADAbs in SPIDDM.

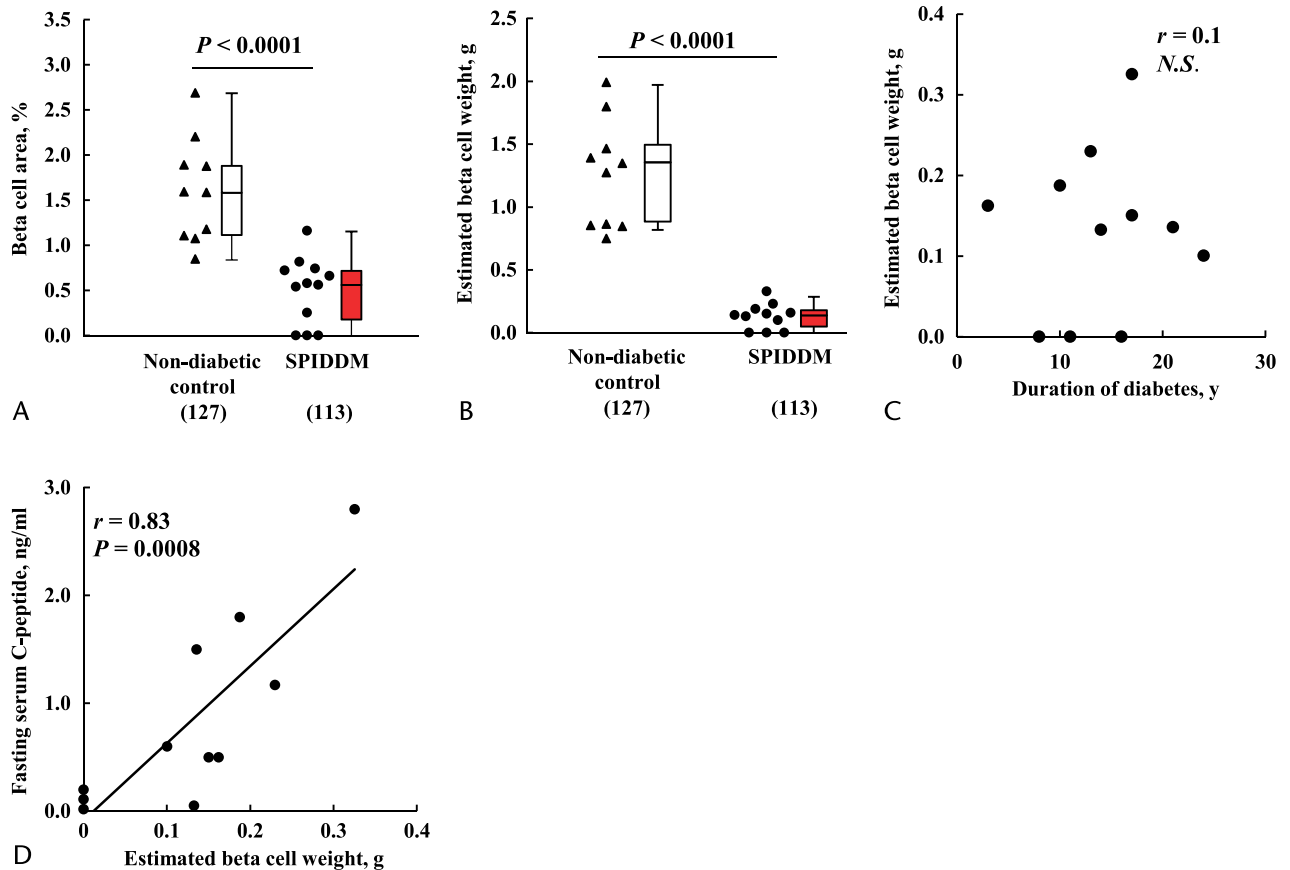


FIGURE 2. Beta cell volume in SPIDDM and control subjects with no diabetes. A, Beta cell areas (%) are decreased in SPIDDM compared with nondiabetic control pancreases. Numbers in parentheses are numbers of individual islets analyzed. B, Beta cell weights (g) in SPIDDM are decreased to approximately 10% to 20% of nondiabetic control pancreases. Numbers in parentheses are numbers of individual islets analyzed. C, Beta cell weight is preserved long after the onset of SPIDDM. N.S. indicates not significant. D, An excellent correlation between beta cell weight in the pancreas and fasting serum C-peptide levels is observed in SPIDDM patients.

Enterovirus-Related VP1 Proteins, Innate Immune Cascades, Cytokines, and Islet Amylin-Positive Amyloid

Enterovirus-related VP1 proteins were not detected in any parts of 12 SPIDDM pancreases immunohistochemically. The expressions of innate immune receptors including RIG-I, MDA5, and TLR4 and cytokines including interleukin 18, IFN- γ were not detected in the islet beta cells and exocrine pancreases in all SPIDDM pancreases immunohistochemically. These findings are in contrast to the reports of marked expressions of these molecules in AT1DM^{30–33} and FT1DM.^{17,19,32}

Amylin-positive amyloid deposition in islets, a marker of T2DM, was not observed immunohistochemically and Congo red staining in SPIDDM and control.

Pancreatic Ductal Changes and Exocrine Tissue Inflammation

Pancreatic Ductal Changes

As many as 75% (9/12) of SPIDDM pancreases had significantly higher frequencies of pancreatic intraepithelial neoplasia (PanIN) than age-matched control subjects with no diabetes: 5% (1/19) (Fig. 3A). The numbers of PanIN-positive pancreatic

ducts per centimeter squared of each section were significantly higher than those of control subjects with no diabetes (Fig. 3B). The PanIN grade ranged from PanIN-1A to PanIN-2 (Supplemental Table 1, <http://links.lww.com/MPA/A674>). The lesions were characterized by tall, columnar, and mucin-rich cells with various degrees of nuclear atypia in the branches and smaller ducts (Fig. 3C). The pancreatic ducts had dilated lumens filled with mucous plaques and sequestered contents (Fig. 3C), and the pancreatic lobules with PanIN showed chronic pancreatitis and lobular atrophy, whereas lobes without PanIN showed intact acinar tissues (Fig. 3D). Two SPIDDM pancreases without apparent PanIN in the pancreatic sections (cases SP-5 and SP-7) showed dilated pancreatic ducts filled with mucinous materials, pancreatic duct epithelial erosion, and periductal inflammation, suggesting the presence of ductal obstruction and subsequent chronic obstructive pancreatitis caused by occult PanIN at the downstream part of the pancreatic duct (Fig. 3E). The one remaining SPIDDM patient (case SP-10, duration of diabetes 0.3 years) without PanIN lesions had a recent history of recurrent bile stone attacks, and the pancreas biopsied at surgical cholecystectomy showed pancreatic ductal dilation and periductal fibrosis with focal parenchymal atrophy (Figs. 3F, G).

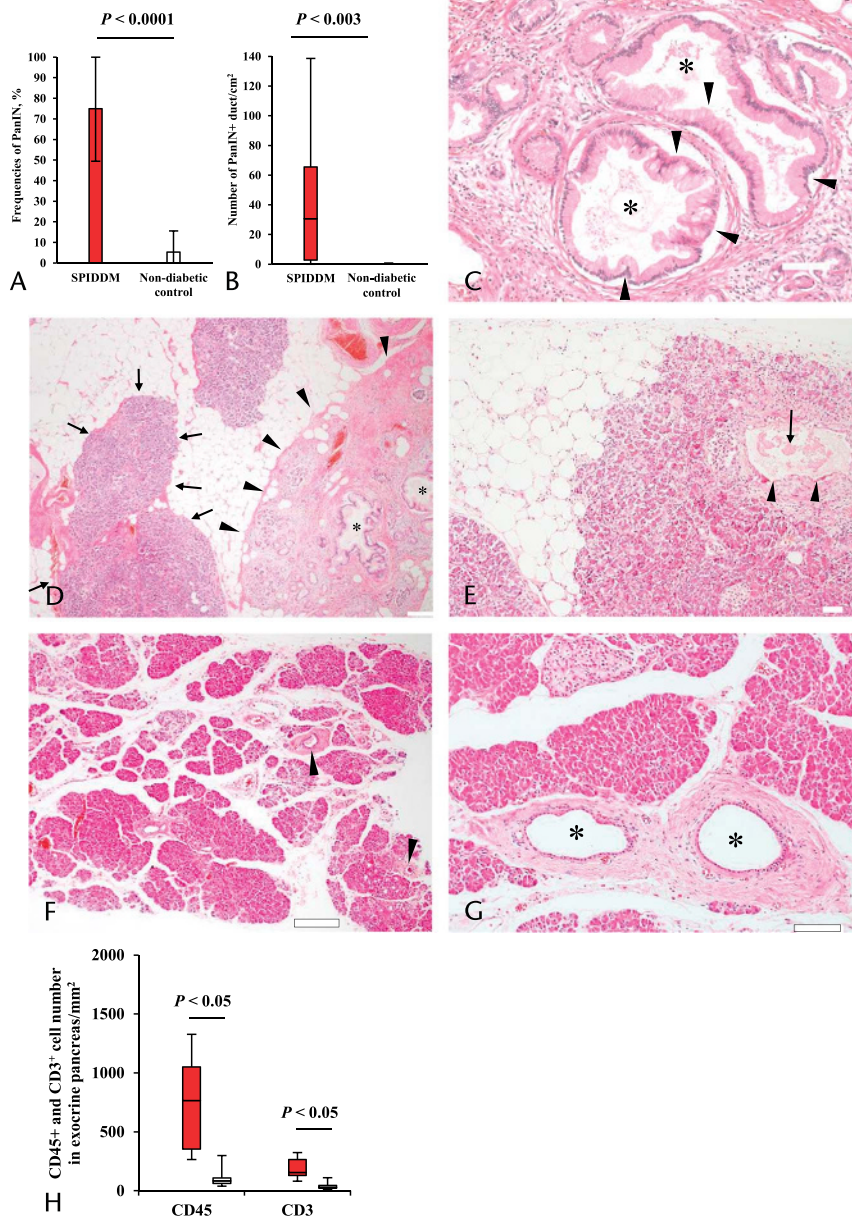


FIGURE 3. Changes in exocrine pancreases in SPIDDM. A, The frequencies of pancreatic intraductal neoplasia (PanIN) lesion in pancreatic duct with 95% confidence interval in SPIDDM and nondiabetic control pancreases. B, The number of PanIN-positive pancreatic ducts in SPIDDM and nondiabetic control pancreases with median depicted by box-and-whisker plots. C, Pancreatic intraductal neoplasia lesion with tall columnar cells and various degrees of atypia is observed (arrowheads, case SP-2). Note marked inflammatory cells, fibrosis, and atrophied exocrine parenchyma and intraductal mucous contents (asterisks) (hematoxylin-eosin [HE] staining, original magnification $\times 200$; scale bar, 50 μm). D, Pancreatic exocrine lobe with PanIN (asterisks) shows extensive lobular atrophy demarcated by arrowheads, whereas the adjacent lobe without PanIN appears nonatrophied (demarcated by arrows) (case SP-2) (HE staining, original magnification $\times 40$; scale bar, 250 μm : B–D). E, Changes in pancreatic exocrine tissue without apparent PanIN (case SP-5). Intraductal mucinous contents (arrow) and the erosive pancreatic ductal wall (arrowheads) indicate ductal fluid obstruction likely due to occult PanIN downstream of the pancreatic duct. F, Lobular atrophy and periductal fibrosis (arrowheads) in an SPIDDM patient (case SP-10) with a history of recurrent bile stone attacks. G, Another part of a section of case SP-10 shows pancreatic ductal dilation and periductal fibrosis (asterisks) indicating presence of intraductal hyperpressure presumably due to recurrent pancreatic ductal obstruction or narrowing by stacked bile stones in the duodenal papilla (HE staining, original magnification $\times 200$; scale bar, 50 μm). H, Increased numbers of CD45⁺ and CD3⁺ MNCs in exocrine pancreatic tissues (red box) indicate extensive exocrine pancreatic inflammation in SPIDDM. White box: control subjects with no diabetes.

Pancreatic Exocrine Inflammation

Exocrine pancreases with SPIDDM showed extensive inflammation, especially in the lobes with PanIN lesions (Figs. 3C, D). The numbers of CD45⁺ and CD3⁺ MNCs infiltrated into the exocrine pancreas were increased in SPIDDM (Fig. 3H).

MHC Class I Hyperexpression

Hyperexpression of MHC class I on beta cells was observed in all cases with SPIDDM (Figs. 4A, B). Percent MHC class I-positive islet beta cells were significantly low in 19 age-matched nondiabetic control pancreases (Figs. 4C–E).

DISCUSSION

The distinct pathological findings of the pancreases of SPIDDM were as follows.

First, CD3⁺ T-cell-dominant patchy islet inflammation/insulinitis was observed in all cases with SPIDDM. Insulinitis persisted for a long time after onset. CD8⁺ T-cells expressed LPL and were shaped in a characteristic “uropod” appearance, showing that the T-cells were activated and homing to islets, even in long-standing cases (Fig. 1F). In contrast, insulinitis commonly subsides after several months to years with a decreased volume of beta cells in typical AT1DM and FT1DM.^{18,25,30–34} Second, beta cells remained long after onset of diabetes in SPIDDM pancreases. In contrast, beta cell volume decreases rapidly with the duration of diabetes, and beta cells are mostly destroyed after long duration of diabetes in AT1DM.^{35,36} Third, significantly high frequencies of PanIN lesions and extensive chronic pancreatitis

were observed in SPIDDM. In our previous report,¹⁵ the frequencies of PanIN lesion in AT1DM and T2DM were 10% (1/10) and 13% (2/15), respectively, significantly lower than the values of SPIDDM. Pancreatic intraepithelial neoplasia lesions secrete mucinous products, and/or PanIN lesions themselves obstruct or narrow the pancreatic ducts, initiating intraductal hyperpressure and chronic pancreatitis causing pancreatic lobular atrophy.³⁷ No MHC class I hyperexpression and no pseudoatrophic islets were found in the pancreases of control subjects with no diabetes. It is therefore possible that PanIN lesion, insulinitis, MHC class I hyperexpression, and pseudoatrophic islets are distinct features of SPIDDM pancreas. Finally, evidence of enterovirus infection (VP1 protein expression), activation of innate immune cascades (MDA5, RIG-I, and TLR4), and expression of the cytokine axis (IFN- γ , IL-18), which was detected in FT1DM and AT1DM,^{17,19,32} were not observed in the islet cells of SPIDDM. Similar to FT1DM and AT1DM, pseudoatrophic islets devoid of beta cells with preserved non-beta cells were observed in SPIDDM, indicating that the destruction of islet cells is caused by beta cell-specific mechanisms. These findings in SPIDDM pancreases support the concept that the initial mechanisms of beta cell destruction are different from viral infection-triggered FT1DM and potentially from AT1DM, involving innate and autoimmune mechanisms.^{17,19,30–34}

Our hypothesis regarding beta cell destruction in SPIDDM is that pancreatic ductal hyperpressure and stasis/leakage of pancreatic digestive fluid induced by PanIN lesions, which were observed in a high proportion of SPIDDM cases, may cause chronic pancreatitis.³⁷ An extended and persisting/recurrent

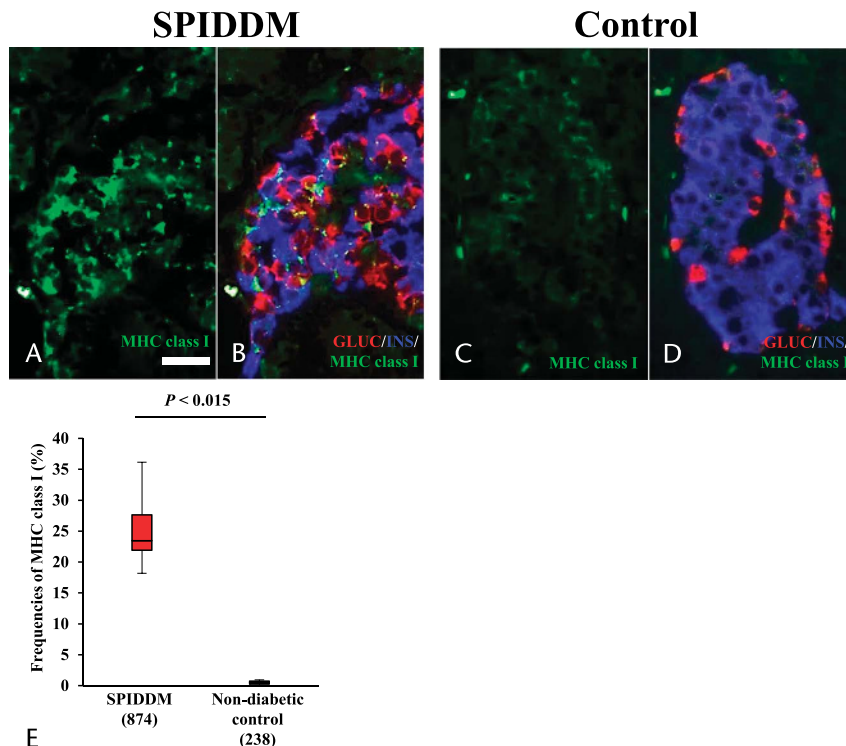


FIGURE 4. The MHC class I hyperexpression in SPIDDM. A, MHC class I hyperexpression (green) in SPIDDM pancreas (case SP-1) (scale bar: 50 μ m). B, Merged image of triple immunostaining for MHC class I (green), insulin (blue), and glucagon (red) in SPIDDM pancreas (case SP-1). Mainly beta cells show hyperexpression of MHC class I. C, MHC class I staining in nondiabetic control. D, Merged image of triple immunostaining for MHC class I (green), insulin (blue), and glucagon (red) in nondiabetic control. E, The percentage of MHC class I hyperexpression of islet beta cells in SPIDDM and control pancreases with medians depicted by box-and-whisker plots. Numbers in parenthesis indicate counted islet beta cell number.

inflammatory milieu in the islets and/or the exocrine pancreas potentially induces release of self-antigens from islets and the exocrine pancreas and promotes islet MHC class I hyperexpression of islet beta cells in SPIDDM. Ballooning and dilation of the pancreatic duct by pancreatic ductal hyperpressure in SPIDDM were demonstrated by endoscopic retrograde pancreatography.³⁸ One of the authors (T.K.) evaluated a non-insulin-requiring GADAb-negative 58-year-old woman with diabetes, who had pancreatic ductal cystic lesion with epithelial hyperplasia (PanIN) and type 1 diabetes-susceptible HLA haplotypes (HLA-DR 9/9). In this case, GADAbs became negative to positive during the follow-up period.³⁹ After the seroconversion, C-peptide responses decreased, and PanIN lesion advanced progressively to the features of intraductal papillary mucinous neoplasm. Considering together with these findings, combined processes including MHC class I hyperexpression associated with exocrine inflammation around the islet caused by PanIN lesion and genetic predisposition (ie, HLA gene, Supplemental Table 1, <http://links.lww.com/MPA/A674>) may be required to continue to activate, attract, and induce homing of autoreactive CD8⁺ T-cells to islet beta cells through a bystander mechanism,^{40,41} leading to full-blown insulinitis and complete beta cell destruction in SPIDDM. All SPIDDM patients typed for HLA (Supplemental Table 1, <http://links.lww.com/MPA/A674>) had susceptible one for type 1 diabetes in Japanese population.³

An absence of amylin-positive amyloid depositions in SPIDDM pancreases, which are closely related to T2DM, supports the concept that the mechanisms of beta cell damage of SPIDDM are different from those in T2DM.²⁷ The present study had a limitation due to its small sample size, especially samples from short duration of diabetes. Its strength was that most of the cases were recruited from our cohort in which patients were followed up throughout their clinical courses, and clinical data including fasting C-peptide levels, the exact time until the insulin-requiring state developed, and body mass index were obtained.^{1-3,7,8,17-20}

ACKNOWLEDGMENTS

The authors thank the late Dr Taro Maruyama, Saitama Social Insurance Hospital, for his generous assistance in obtaining the study samples and Fumie Takano, Okinaka Memorial Institute for Medical Research, for her excellent secretarial work.

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