Diabetology International (2020) 11:323–328
https://doi.org/10.1007/s13340-020-00462-6

MINI-REVIEW

Unique pathological changes in the pancreas of fulminant type 1 diabetes

Tetsuro Kobayashi[1,2] · **Shoichiro Tanaka**[3] · **Kaoru Aida**[4]

Received: 6 August 2020 / Accepted: 18 August 2020 / Published online: 29 August 2020

Abstract

Distinct features of the pancreas of fulminant type 1 diabetes (FT1DM) include (1) enterovirus infection of the islets and exocrine acinar tissue. (2) Activated innate immune responses: MDA5 and RIG-I expression and TLR4 and TLR9 expression in the islets of FT1DM. (3) Combined activation of the STAT/JNK and NFkB pathways, resulting in Type I interferon (IFN) and proinflammatory cytokine (i.e., IFNγ) expression in islet beta cells and MHC class I hyper-expression. (4) Activation of dendritic cells followed by effector cell infiltration of $CD8^+$ T cells and $CD68^+$ macrophages, resulting in apoptosis and neurosis of islet cells and exocrine acinar cells. (5) Many chemo-attractants (i.e., CXCL10) and chemotactic activators (i.e., L-plastin) were induced by a viral infection. (6) Mutual stimulating effect of cytokines expressed in beta cells in autocrine and paracrine mechanisms may enhance beta-cell destruction through the STA1-caspase pathway. (7) Proteomics analysis using laser capture microdissection followed by mass spectrometry found 38 molecules in inflamed islets of FT1DM, which were not highlighted before. Our pathologically verified model of beta-cell destruction in FT1DM will contribute to anti-virus therapy of type 1 diabetes in the near future.

Keywords Fulminant type 1 diabetes · Enterovirus · Innate immunity · MDA5 · RIG-I · Toll-like receptor · Insulitis · Pancreatitis

Introduction

The term "fulminant type 1 diabetes (FT1DM)" is derived from the characteristic symptoms documented as "an abrupt onset of diabetic symptoms, thirst and weakness, and the point at which symptoms first developed can be easily determined" [1–3]. At admission to hospital, such patients usually show normal HbA1 levels and the extent of their hyperglycemia and ketosis is more severe than in acute-onset type 1 diabetes [1–3]. Recent pathological analysis demonstrated that the destructive process progresses rapidly through virus-induced innate and adaptive immune processes and specific host immunogenetic backgrounds [1–9] in addition to many molecules not highlighted in type 1 diabetes previously [10, 11].

Pathogenic virus

The virus that causes FT1DM is an acutely cytolytic virus that exhibits tropism mainly to islets and occasionally to exocrine acinar cells [12, 13]. It generally assumed that the causative viruses are belongs to the family Picornaviridae: enterovirus (EV), human herpes virus 6, influenza B virus and cytomegalovirus [14]. Immunohistochemical methods (IHC) and in situ hybridization (ISH) are used to detect EV, by staining freshly cut/prepared sections with the commonly used monoclonal antibody 5D8/1 with the optimized condition to obtain specific IHC staining VP1 in the islet cells (Fig. 1a) [15].

In FT1DM, EVs are found to have tropism both to the islets and exocrine acinar cells of the pancreas (Fig. 1a, b)

✉ Tetsuro Kobayashi
tetsurou@yamanashi.ac.jp

1 Division of Immunology and Molecular Medicine, Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan

2 Department of Endocrinology and Metabolism, Toranomon Hospital, Tokyo, Japan

3 Ai Home Clinic Toshima, 4th Floor, INS Building, 2-32-2 Minamiotsuka, Toshima-ku, Tokyo 170-0005, Japan

4 Department of Diabetes Medicine, Kanoiwa Hospital, Kamijinnai River 1309, Yamanashi, Yamanashi, Japan

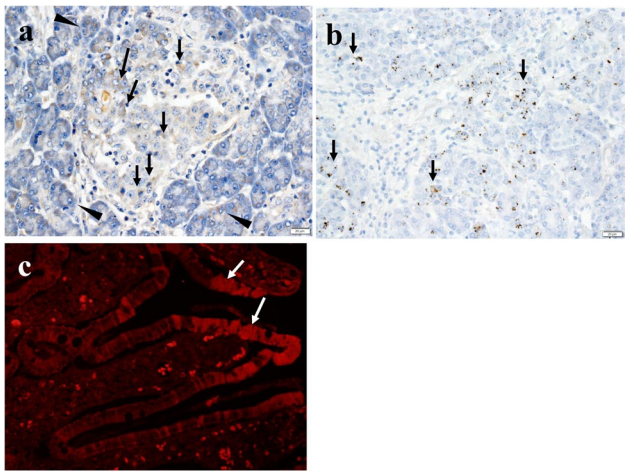

Fig. 1 Enterovirus infection in fulminant type 1 diabetes (FT1DM). **a** Enterovirus (EV) envelope protein 1 (VP1, brown, arrows) staining is positive mainly in beta cells in FT1DM. Acinar cells (arrowheads) are also positive for VP1. **b** In situ hybridization demonstrate EV-RNA (brown dots) in exocrine pancreatic acinar cells in FT1DM. Numerous RNA dots in acinar cells suggest strong tropism of EV to exocrine acinar cells in FT1DM. **c** Enterovirus VP1 (red) was stained in the duodenal mucosa in a case with FT1DM

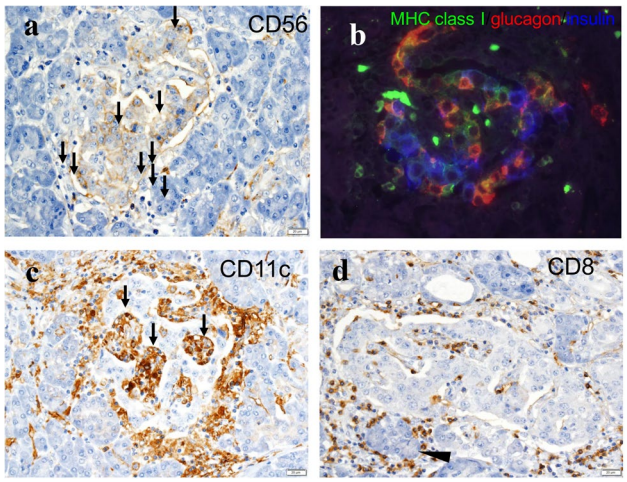

Fig. 2 Presence of natural killer (NK) cells, dendritic cells (DCs), $CD8^+$ T cells and MHC class-I expressing islet cells. **a** $CD56^+$ natured killer (NK) cells (arrows) to the islet in FT1DM. **b** MHC class I hyper-expressed islet cells in FT1DM. Merge image with MHC class I glucagon (red) and insulin (blue). Note that hyper-expression of MHC class I is observed in alpha cells as well as beta cells. **c** $CD11C^+$ dendritic cells (DCs) are infiltrated around and into the islets. **d** $CD8^+$ T cell (brown) infiltration to the islets and acinar cells (arrowhead)

[12, 15]. Interestingly, EV-positive acinar cells are pyknotic and sometimes de-granulated, and are eosinophilic by Hematoxylin–Eosin (HE) staining [12, 15]. EVs also reside on the duodenal mucosa (Fig. 1c) and are assumed to retrogradely spread from duodenal mucosa to the pancreas through pancreatic ducts or through the systemic circulation in FT1DM and acute-onset type 1 diabetes (AT1DM) [15].

Innate immune responses to viral invasion

The most profound feature of innate immunity against EV invasion in FT1DM is expression of the cytoplasmic pattern recognizing receptors, which include MDA5 and RIG-I receptors. Further, their membrane sensors TLR2, TLR4 and TLR9 are reported in FT1DM [13, 16, 17].

In FT1DM, MDA5, a cytoplasmic double stranded RNA sensor, is expressed in all islet cell subtypes, while RIG-I is expressed specifically in beta cells [16]. Downstream signals are the STAT1 and MyD88 pathways, which induce the expression of type I interferons (IFNα, β) and some proinflammatory cytokines and natural killer (NK) cells (Fig. 2a). The expression of type I IFNs induces class I MHC hyper-expression on the surface of beta cells (Fig. 2b). MHC-class I hyper-expression is a hallmark of migratory immune cells including $CD8^+$ T cells and $CD68^+$ macrophages. Dendritic cells (DCs), which connect innate and adaptive immunity, are activated and engulf beta-cell debris in the islets to present antigen to $CD8^+$ T cells in the regional lymph nodes (Fig. 2c) [16, 17].

Adaptive immunity and destructive mechanisms of islet beta cells

The most predominant pathological feature of enterovirus-induced inflammation in the islets and exocrine pancreas is insulitis and exocrine pancreatic inflammation [12, 15]. The frequency of insulitis among islets is 60%, and most islets are infiltrated by $CD8^+$ T cells, $CD68^+$ macrophages and DCs [15] (Fig. 2d). Characteristically, DCs reside mainly around the islets (Fig. 2c). Beta cells expressing caspase 3 showed T-cell mediated cell damage [16]. Destruction of Fas-positive beta cells by Fas ligand-positive cell-mediated mechanisms is also involved [10, 16].

Another interesting finding regarding islet inflammation is the migratory characteristic of effector cells [19]. They extravasate from the vascular wall near the target islets (Fig. 3a–d), and migrate to the interstitial space (rather than the vasculature of the islets) to reach the target islets, suggesting that specific attracting chemokines may influence their trafficking movements to the target. In addition, the peri-islet region is the final destination of migrating immune

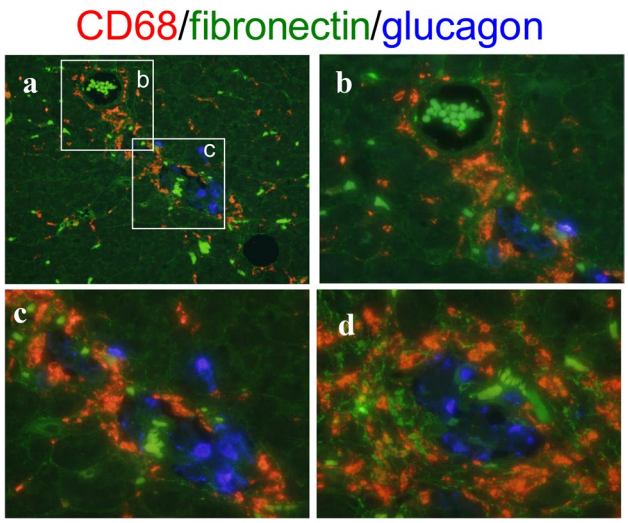

Fig. 3 Migration of CD68$^+$ macrophages to target islets through interstitial space of pancreatic exocrine glands. Fibronectin (green) represents the basement membrane surrounding each gland. **a** Migration of CD68$^+$ macrophages (red) to the target islet beta cells (blue), penetrating the vascular wall (green). **b** Magnified view of **b** in **a**. **c** Magnified view of **c** in **a**. **d** The CD68$^+$ macrophages reached and surrounded the target islets in FT1DM

cells, indicating that peri-insulitis is common in human type 1 diabetes (Fig. 3d). One of the attracting chemokines is C-X-C motif chemokine 10 (CXCL10), which is expressed on endangered beta cells, and specific ligand-bearing cells (CXCR3$^+$ cells) or macrophages are attracted to and accumulate in the islets [12]. Interestingly, CXCL10 was also expressed on EV-positive acinar cells, suggesting that EV-induced adaptive mechanisms involve the exocrine pancreas attracting virus-specific T cells in FT1DM [15]. Detailed mechanisms of accelerated beta cells in FT1DM was reviewed previously [10].

Recent mass spectrometry-based proteomic analysis of inflamed islets in FT1DM detected several proteins not highlighted by classical pathological observation [11] (Table 1).

Table 1 includes 38 molecules associated with cell repair, viral replication, anti-viral function, immune cell migration and viral infection. Plastin-2 (LCP1) was highly expressed on CD8$^+$ T cells and CD68$^+$ macrophages that aggressively infiltrated to or around the islets in FT1DM, indicating a sensitive marker of active migration of T cells or macrophages to the islets [11]. Thymidine phosphorylase (TYMP) is expressed in infiltrating MNCs to the islets of FT1DM, which presumably effector cells and the inhibitors of TYMP are potential targets of intervention of beta-cell destruction [11].

Structural alterations of islets

Structural alterations of islets frequently occur at the basement membranes (BMs), extra-cellular matrix (ECM) packing the islets and cell cluster named Acinar-cell cluster Touching Langerhans islets with Thin Interstitial Surrounding (ATLANTIS): the BMs and ECM were sometimes disrupted during islet inflammation (Fig. 4a) [18]. A detailed description of ATLANTIS is beyond the scope of this review. The mechanisms of structural change of islets and ATLANTIS are mainly related to infiltrating mononuclear cell (MNC), especially CD8$^+$ T cells, DCs and CD68$^+$ macrophages [18]. These cells infiltrate into islets by secreting proteases, which enable immune cells to penetrate the barriers (e.g., BMs and ECMs) surrounding the islets, and disrupt the microenvironment maintained by islets and ALANTIS. Another interesting feature of ATLANTIS in inflamed FT1DM is the increased expression of regenerating protein 1 (Reg 1α) in ATLANTIS. Reg 1α is located in ATLANTIS under non-inflammatory conditions. Under inflammatory conditions the cell cluster hyper-expresses Reg 1α, suggesting crucial roles of ATLANTIS to regenerate the islet cells in an inflammatory milieu of FT1DM [18, 19].

Regeneration of beta cells

Beta-cell kinetics at the onset of FT1DM includes an increased number of Ki67$^+$ beta cells as well as non-beta cells (Fig. 4b). We have also reported increased BrdU$^+$ beta cell regeneration in encephalomyocarditis (EMC) virus-induced diabetes in wild-type mice but not in Reg 1α$^{-/-}$ mice [19].

Exocrine pancreatic inflammation

Distinct pathological feature of the pancreas with FT1DM is exocrine pancreatic inflammation never reported previously [15]. Enterovirus is the primary cause of exocrine inflammation (Fig. 1b), which is sometimes associated with clinical symptoms of FT1DM including abdominal pain and hyperamylasemia. Most striking features in pancreatic inflammation go through a similar immunological pathway with insulitis. Enterovirus triggers innate immunity followed by adaptive immunity to cell apoptotic death also in exocrine pancreas in FT1DM. CXCL10 is also expressed in the acinar cells accelerating the processes [15]. As a consequence, autoantibodies against amylase α 2A and heat shock protein 10 are reported in FT1DM [20, 21].

Table 1 Proteins found only in the islets of FT1DM by mass spectrometry

	Accession number	Entry name	Protein names	Gene names	Molecular weight (kDa)
1	Q71U36	TBA1A_HUMAN	Tubulin alpha-1A chain	TUBA1A	50
2	P52272	HNRPM_HUMAN	Heterogeneous nuclear ribonucleoprotein M	HNRNPM	78
3	P13796	PLSL_HUMAN	Plastin-2	LCP1	70
4	P11678	PERE_HUMAN	Eosinophil peroxidase	EPX	81
5	P23381	SYWC_HUMAN	Tryptophan–tRNA ligase, cytoplasmic	WARS	53
6	P42224	STAT1_HUMAN	Signal transducer and activator of transcription 1-alpha/beta	STAT1	87
7	P30504	1C04_HUMAN	HLA class I histocompatibility antigen, Cw-4 alpha chain	HLA-C	41
8	P19971	TYPH_HUMAN	Thymidine phosphorylase	TYMP	50
9	P61313	RL15_HUMAN	60S ribosomal protein L15	RPL15	24
10	O60814	H2B1K_HUMAN	Histone H2B type 1-K	HIST1H2BK	14
11	P28838	AMPL_HUMAN	Cytosol aminopeptidase	LAP3	56
12	P46940	IQGA1_HUMAN	Ras GTPase-activating-like protein IQGAP1	IQGAP1	189
13	P61158	ARP3_HUMAN	Actin-related protein 3	ACTR3	47
14	Q06323	PSME1_HUMAN	Proteasome activator complex subunit 1	PSME1	29
15	O14950	ML12B_HUMAN	Myosin regulatory light chain 12B	MYL12B	20
16	P26038	MOES_HUMAN	Moesin	MSN	68
17	O60506	HNRPQ_HUMAN	Heterogeneous nuclear ribonucleoprotein Q	SYNCRIP	70
18	P48643	TCPE_HUMAN	T-complex protein 1 subunit epsilon	CCT5	60
19	P31946	1433B_HUMAN	14-3-3 protein beta/alpha	YWHAB	28
20	Q9UL46	PSME2_HUMAN	Proteasome activator complex subunit 2	PSME2	27
21	P10412	H14_HUMAN	Histone H1.4	HIST1H1E	22
22	P17844	DDX5_HUMAN	Probable ATP-dependent RNA helicase DDX5	DDX5	69
23	P35237	SPB6_HUMAN	Serpin B6	SERPINB6	43
24	Q9BQE5	APOL2_HUMAN	Apolipoprotein L2	APOL2	37
25	P08729	K2C7_HUMAN	Keratin, type II cytoskeletal 7	KRT7	51
26	P20700	LMNB1_HUMAN	Lamin-B1	LMNB1	66
27	P62847	RS24_HUMAN	40S ribosomal protein S24	RPS24	15
28	Q9Y3Z3	SAMH1_HUMAN	SAM domain and HD domain-containing protein 1	SAMHD1	72
29	O60361	NDK8_HUMAN	Putative nucleoside diphosphate kinase	NME2P1	16
30	O95154	ARK73_HUMAN	Aflatoxin B1 aldehyde reductase member 3	AKR7A3	37
31	P13473	LAMP2_HUMAN	Lysosome-associated membrane glycoprotein 2	LAMP2	45
32	P21333	FLNA_HUMAN	Filamin-A	FLNA	281
33	P31943	HNRH1_HUMAN	Heterogeneous nuclear ribonucleoprotein H	HNRNPH1	49
34	P52209	6PGD_HUMAN	6-phosphogluconate dehydrogenase, decarboxylating	PGD	53
35	P59998	ARPC4_HUMAN	Actin-related protein 2/3 complex subunit 4	ARPC4	20
36	P16401	H15_HUMAN	Histone H1.5	HIST1H1B	23
37	P34931	HS71L_HUMAN	Heat shock 70 kDa protein 1-like	HSPA1L	70
38	O60763	USO1_HUMAN	General vesicular transport factor p115	USO1	108

Conclusion

FT1DM is mainly caused by acute cytolytic EV infection to the islets and acinar cells. EVs initiate innate immune activation followed by adaptive immune activation. During innate and adaptive immune conditions, beta cells and acinar cells are affected by chemokine and cytokine exposure. It remains unclear whether EVs persistently infect the pancreas with established pathological features or not. Further study on long-standing features in the pancreas of FT1DM is required.

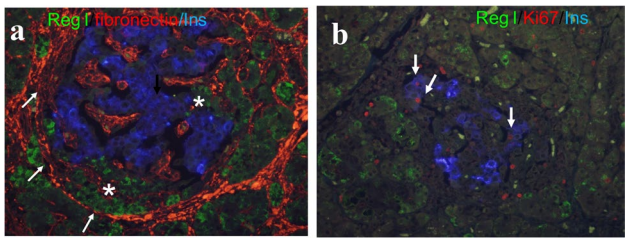

Fig. 4 Damaged islet basement membrane (red) by immune cell infiltrated into the islets and hyper-expressing Reg 1alpha in the ATLANTIS cell (green), which touch with beta cells (blue). **a** Basement membranes (red, fibronectin) encapsulating islets and ATLANTIS cells were damaged by infiltrating effector cells. ATLANTIS cells (asterisks), which is touching with beta cells (blue), hyper-express regenerating protein alpha (Reg 1α) (green, asterisks). **b** Ki-67^{+} cells (red, arrows) were increased in beta cells representing active regeneration in the islets of FT1DM (18)

Acknowledgements The authors would like to thank the late Taro Maruyama, Saitama Social Insurance Hospital, for his generous assistance in obtaining the study samples, Erika Jimbo, Okinaka Memorial Institute for Medical Research, for her excellent technical assistance with immunostaining of samples, and Fumie Takano, Okinaka Memorial Institute for Medical Research, for her excellent secretarial assistance.

Author contributions TK, KA and ST conducted the immunohistochemical staining and discussed, reviewed, and edited the manuscript. TK contributed to the planning and discussions and edited the manuscript.

Funding This work was supported by a research grant from Japan Society for the Promotion of Science KAKENHI (Grant 2459319).

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest in this work.

Ethical approval All procedures used in this study were approved by the ethics committees of the University of Yamanashi and Toranomon Hospital.

Informed consent Written, informed consent was obtained from the next of kin on behalf of the autopsied patients.

Statement on human research All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation of Toranomon Hospital and University of Yamanashi and with the Helsinki Declaration of 1964 and later versions.

References

1. Kobayashi T. Immunology and immunogenetics of type I diabetes in Japan. IDF Bull. 1990;35:34–7.
2. Kobayashi T. Subtype of insulin-dependent diabetes mellitus (IDDM) in Japan: slowly progressive IDDM-the clinical characteristics and pathogenesis of the syndrome. Diabetes Res Clin Pract. 1994;24(Suppl):S95–S9999.
3. Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y. A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. Osaka IDDM Study Group. N Engl J Med. 2000;342:301–7.
4. Tanaka S, Kobayashi T, Momotsu T. A novel subtype of Type 1 diabetes mellitus. N Engl J Med. 2000;342:1835–7.
5. Tanaka S, Kobayashi T, Nakanishi K, Koyama R, Okubo M, Murase T, Odawara M, Inoko H. Association of HLA-DQ genotype in autoantibody-negative and rapid onset type 1 diabetes. Diabetes Care. 2002;25:2302–7.
6. Kawabata Y, Nishida N, Awata T, Kawasaki E, Imagawa A, Shimada A, Osawa H, Tanaka S, Takahashi K, Nagata M, Yasuda H, Uchigata Y, Kajio H, Makino H, Yasuda K, Kobayashi T, Hanafusa T, Tokunaga K, Ikegami H. Genome-Wide association study confirming a strong effect of HLA and identifying variants in CSAD/lnc-ITGB7-1 on chromosome 12q13.13 associated with susceptibility to fulminant type 1 diabetes. Diabetes. 2019;68:665–75.
7. Imagawa A, Hanafusa T, Uchigata Y, Kanatsuka A, Kawasaki E, Kobayashi T, Shimada A, Shimizu I, Maruyama T, Makino H. Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes mellitus. Diabetologia. 2005;48:294–300.
8. Tsutsumi C, Imagawa A, Ikegami H, Makino H, Kobayashi T, Hanafusa T, Japan Diabetes Society Committee on Type 1 Diabetes Research. Class II HLA genotype in fulminant type 1 diabetes: a nationwide survey with reference to glutamic acid decarboxylase antibodies. J Diabetes Investig. 2012;3:62–9.
9. Kawabata Y, Ikegami H, Awata T, Imagawa A, Maruyama T, Kawasaki E, Tanaka S, Shimada A, Osawa H, Kobayashi T, Hanafusa T, Tokunaga K, Makino H. on behalf of the committee on type 1 diabetes, Japan diabetes society: Differential association of HLA with three subtypes of type 1 diabetes: fulminant, slowly progressive and acute-onset. Diabetologia. 2009;52:2513–21.
10. Tanaka S, Aida K, Nishida Y, Kobayashi T. Pathophysiological mechanisms involving aggressive islet cell destruction in fulminant type 1 diabetes. Endocr J. 2013;60:837–45.
11. Nishida Y, Aida K, Kihara M, Kobayashi T. Antibody-validated proteins in inflamed islets of fulminant type 1 diabetes profiled by laser-capture microdissection followed by mass spectrometry. PLoS ONE. 2014;9:e107664.
12. Tanaka S, Nishida Y, Aida K, Maruyama T, Shimada A, Suzuki M, Shimura H, Takizawa S, Takahashi M, Akiyama D, Arai-Yamashita S, Furuya F, Kawaguchi A, Kaneshige M, Katoh R, Endo T, Kobayashi T. Enterovirus infection, CXC chemokine ligand 10 (CXCL10), and CXCR3 circuit: a mechanism of accelerated β-cell failure in fulminant type 1 diabetes. Diabetes. 2009;58:2285–91.
13. Shibasaki S, Imagawa A, Tauriainen S, et al. Expression of toll-like receptors in the pancreas of recent-onset fulminant type 1 diabetes. Endocr J. 2010;57:211–9.
14. Yoneda S, Imagawa A, Fukui K, Uno S, Kozawa J, Sakai M, Yumioka T, Iwahashi H, Shimomura I. A histological study of fulminant type 1 diabetes mellitus related to human cytomegalovirus reactivation. J Clin Endocrinol Metab. 2017;102:2394–400.
15. Takita M, Jimbo E, Fukui T, Aida K, Shimada A, Oikawa Y, Yagihashi S, Miura J, Babazono T, Kobayashi T. Unique inflammatory changes in exocrine and endocrine pancreas in enterovirus-induced fulminant type 1 diabetes. J Clin Endocrinol Metab. 2019;104:4282–94.
16. Aida K, Nishida Y, Tanaka S, Maruyama T, Shimada A, Awata T, Suzuki M, Shimura H, Takizawa S, Ichijo M, Akiyama D, Furuya F, Kawaguchi A, Kaneshige M, Itakura J, Fujii H, Endo T,

Kobayashi T. RIG-I- and MDA5-initiated innate immunity linked with adaptive immunity accelerates β-cell death in fulminant type 1 diabetes. Diabetes Diabetes. 2011;60:884–9.
17. Kobayashi T, Nishida Y, Tanaka S, Aida K. Pathological changes in the pancreas of fulminant type 1 diabetes and slowly progressive insulin-dependent diabetes mellitus (SPIDDM): innate immunity in fulminant type 1 diabetes and SPIDDM. Diabetes Metab Res Rev. 2011;27:965–70.
18. Aida K, Saitoh S, Nishida Y, Yokota S, Ohno S, Mao X, Akiyama D, Tanaka S, Awata T, Shimada A, Oikawa Y, Shimura H, Furuya F, Takizawa S, Ichijo M, Ichijo S, Itakura J, Fujii H, Hashiguchi A, Takasawa S, Endo T, Kobayashi T. Distinct cell clusters touching islet cells induce islet cell replication in association with overexpression of regenerating gene (REG) protein in fulminant type 1 diabetes. PLoS ONE. 2014;9:e95110.
19. Aida K, Kobayashi T, Takeshita A, Jimbo E, Nishida Y, Yagihashi S, Hosoi M, Fukui T, Sugawara A, Takasawa S. Crucial role Reg 1 from acinar-like cell cluster touching with islets (ATLANTIS) on mitogenesis of beta cells in EMC virus-induced diabetic mice. Biochem Biophys Res Commun. 2018;503:963–9.
20. Endo T, Takizawa S, Tanaka S, Takahashi M, Fujii H, Kamisawa T, Kobayashi T. Amylase α-2A autoantibodies: novel marker of autoimmune pancreatitis and fulminant type 1 diabetes. Diabetes. 2009;58:732–7.
21. Takizawa S, Endo T, Wanjia X, Tanaka S, Takahashi M, Kobayashi T. HSP 10 is a new autoantigen in both autoimmune pancreatitis and fulminant type 1 diabetes. Biochem Biophys Res Commun. 2009;386:192–6.