

Pathogenesis of fulminant type 1 diabetes: Genes, viruses and the immune mechanism, and usefulness of patient-derived induced pluripotent stem cells for future research

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Keywords

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ABSTRACT

We reviewed fulminant type 1 diabetes, a recently established subtype of type 1 diabetes, from the aspects of genes, viruses, immune mechanism and usefulness of patient-derived induced pluripotent stem cells (iPSCs). In an analysis of the pancreas of patients with fulminant type 1 diabetes, viral antigens and viral receptors were expressed in β -cells, as well as macrophages and T lymphocytes surrounding the β -cells. These findings suggest that the β -cells of patients with fulminant type 1 diabetes are destroyed during an immune response against viral infection of the pancreas. Recently, fulminant type 1 diabetes was induced by treatment with anti-programmed cell death 1 antibodies, suggesting that immune regulatory mechanisms are also involved in the onset of this disease. We generated iPSCs from patients with fulminant type 1 diabetes for the first time. We also successfully differentiated patient-derived iPSCs into insulin-producing cells *in vitro*, and produced a disease model. The proportion of cytokine-induced apoptotic cells among insulin-positive cells was higher in the iPSCs from patients with fulminant type 1 diabetes than in iPSCs from healthy control participants. We carried out ribonucleic acid sequencing in insulin-producing cells differentiated from patient-derived iPSCs, and are now attempting to identify new biomarkers for the disease.

INTRODUCTION

Type 1 diabetes is characterized by the destruction of pancreatic β -cells, inducing hyperglycemia accompanied by ketosis or ketoacidosis, with patients eventually becoming finally dependent on insulin therapy. In most patients with type 1 diabetes, β -cell destruction is caused by an immune response against β -cells, and islet-related autoantibodies are detected in patients' sera. This destruction typically progresses over several months or several years¹. Fulminant type 1 diabetes is a subtype of type 1 diabetes mellitus characterized by the drastic onset of ketoacidosis within a few days after the development of hyperglycemic symptoms^{2–6}. Almost all pancreatic β -cells are rapidly destroyed, leading to a state of insulin dependency. In Japan, fulminant type 1 diabetes accounts for approximately 20% of acute onset type 1 diabetes cases, and the peak age of onset is

approximately 30–40 years, with most patients developing the disease at ≥ 20 years-of-age. Rapid and drastic onset of fulminant type 1 diabetes is reflected in the discrepancy between high glucose levels as compared with low glycated hemoglobin levels. Islet-related autoantibodies are usually not detectable, and many patients have digestive or flu-like symptoms just before onset, suggesting that several viruses, including enteroviruses, might be associated with disease onset. Serum amylase and lipase levels are elevated, reflecting the damage of not only pancreatic β -cells, but also pancreatic exocrine cells. Patients with fulminant type 1 diabetes are mainly found in East Asia, but were recently reported in Western countries^{4–9}. As there is no description of fulminant type 1 diabetes, even in a standard guidebook on diabetes care¹⁰, there might be less opportunity to diagnose fulminant type 1 diabetes in Western countries. Table 1 shows the characteristics of fulminant type 1 diabetes, but the precise mechanisms of β -cell destruction have not been fully clarified.

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PATHOGENESIS

Our current hypothesis regarding the pathogenesis of fulminant type 1 diabetes is summarized as follows: patients with genetic susceptibility to fulminant type 1 diabetes become virally infected. Once the infection reaches the pancreas and antiviral immunological processes occur, this leads to the destruction of almost all β -cells in the organ^{4,5,11}. Specific class II human leukocyte antigen genes have been reported to be involved in the onset of fulminant type 1 diabetes^{12,13}. *DRB1*04:05-DQB1*04:01* or *DRB1*09:01-DQB1*03:03* were the susceptible haplotypes, while *DRB1*01:01-DQB1*05:01*, *DRB1*15:02-DQB1*06:01* and *DRB1*08:03-DQB1*06:01* were the haplotypes resistant to the disease. *DRB1*04:05-DQB1*04:01* is the most common (32.6%) haplotype among patients in Japan. In contrast, the prevalence was 14.2% in healthy individuals, and the odds ratio was 2.9^{3,13}. Recently, a genome-wide association study of patients with Japanese fulminant type 1 diabetes was reported¹⁴. Single-nucleotide polymorphisms in the human leukocyte antigen region, especially the class II DR region, were strongly associated with development of fulminant type 1 diabetes. In addition, *CSAD/Inc-ITGB7-1* was strongly associated with the disease in Japanese patients. This is a unique region not associated with autoimmune type 1 diabetes, and further study to show its association with the onset of fulminant type 1 diabetes is warranted (Table 1).

Viral infection in the pancreas is also strongly correlated with the onset of fulminant type 1 diabetes. Approximately 70% of patients with fulminant type 1 diabetes presented with flu-like or gastroenteritis-like symptoms⁴⁻⁶. Fulminant type 1 diabetes develops after infection with either enterovirus, rotavirus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6 or others⁴⁻⁶. This means that while infection with one specific virus does not cause fulminant type 1 diabetes, several viruses possibly contribute to the onset of fulminant type 1 diabetes. Evidence supporting this hypothesis was shown when an enterovirus antigen, VP1, was found in autopsy samples from the pancreas of patients with fulminant type 1 diabetes^{15,16}. Virus

presence in the pancreas triggers an immune response involving macrophages and T lymphocytes, and these immune cells infiltrate and destroy β -cells.

There were several studies carried out to show the pathogenesis of fulminant type 1 diabetes by using pancreatic samples obtained from autopsy soon after disease onset, and evidence suggests the key players were islet cells and immune cells (Figure 1). In patient β -cells, melanoma differentiation associated protein 5 and retinoic acid inducible gene I, intracellular receptors of ribonucleic acid (RNA) associated with anti-viral responses, were detected¹⁷. Caspases 3, 8 and 9, which are associated with apoptotic signaling, and C-X-C motif chemokine 10 were also detected in the β -cells¹⁵⁻¹⁷. Pancreatic α -cells were also destroyed in patients with fulminant type 1 diabetes¹⁰, and the presence of melanoma differentiation-associated protein 5 and retinoic acid inducible gene I was also confirmed in these cells^{17,18}. We also reported that Z-DNA-binding protein 1, an intracellular receptor for deoxyribonucleic acid associated with viral response, was detected in the α -cells of patients¹⁸. Fas, major histocompatibility complex class I, interferon (IFN)- α and IFN- β were detected in the intra-islet cells of patients¹⁷. The above-mentioned proteins are pancreatic factors that might

Table 1 | Clinical characteristics of fulminant type 1 diabetes

Drastic onset of ketoacidosis occurs within a few days after the development of hyperglycemic symptoms
Almost all β -cells have been destroyed at disease onset
Islet-related autoantibodies are usually negative
Digestive or flu-like symptoms frequently precede disease onset, which suggests viral infection
Elevation of blood amylase and/or lipase level is observed
HLA <i>DRB1*04:05-DQB1*04:01</i> is the susceptible haplotype
Recently, SNPs of <i>CSAD/Inc-ITGB7-1</i> have been reported to be associated
Most patients without ICI therapy are distributed in Asia
Recently, patients after ICI therapy are reported both from Asian and Western countries

HLA, human leukocyte antigen; ICI, immune checkpoint inhibitor; SNPs, single-nucleotide antigen.

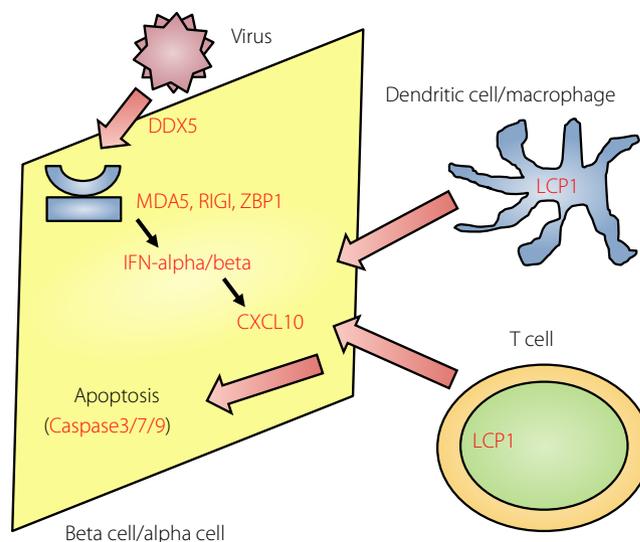


Figure 1 | Schema of pathogenesis of fulminant type 1 diabetes. Virus infection to the pancreas triggers onset and intracytoplasmic pattern recognition receptors, melanoma differentiation associated protein 5, retinoic acid inducible gene I and Z-DNA-binding protein 1, sense infected virus. DEAD box helicase 5 is associated with virus replication. Sensing of virus infection induces interferon alpha and beta production. These promote migration of dendritic cells and macrophage and production of C-X-C motif chemokine 10. lymphocyte cytosolic protein 1 is involved in migration of immune cells. Infiltration of T cells induces β -cell apoptosis. HLA, human leukocyte antigen. CXCL10, C-X-C motif chemokine 10; DDX5, DEAD box helicase 5; IFN, interferon; LCPI, lymphocyte cytosolic protein 1; MDA5, melanoma differentiation associated protein 5; RIGI, retinoic acid inducible gene I; ZBP1, Z-DNA-binding protein 1.

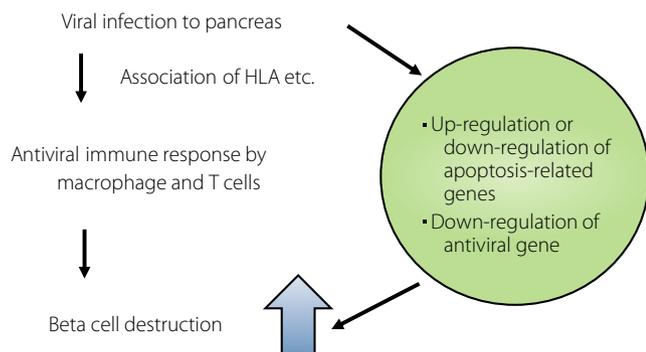


Figure 2 | Viral infection of the pancreatic islet cells induces infiltration of macrophages and T cells to the islets, causing β -cell destruction concurrent with the immune reaction to the invading virus. Class II human leukocyte antigen (HLA) is reported to be involved with the onset of fulminant type 1 diabetes. Upregulation or downregulation of apoptosis-related genes or antiviral genes in β -cells might cause accelerated β -cell destruction.

be associated with the pathogenesis of fulminant type 1 diabetes. In terms of immune cells, macrophages, dendritic cells and CD8-positive T cells infiltrated the intra- and peri-islets of patients^{17–19}. Nishida *et al.*²⁰ carried out proteomic analysis of islets in autopsy samples obtained from patients with fulminant type 1 diabetes by using laser capture microdissection coupled with liquid chromatography-tandem mass spectrometry. They detected new proteins associated with cell migration (lymphocyte cytosolic protein 1), virus replication (adenosine triphosphate-dependent RNA helicase DEAD box helicase 5) and anti-viral activity (SAM domain and HD domain-containing protein 1). These lines of evidence suggest that viral infection of pancreatic islet cells could induce infiltration of immune cells to the islets, resulting in β -cell destruction through immune reactions to the virus. However, this would not completely explain the rapid, drastic and complete loss of β -cells in patients with fulminant type 1 diabetes (Figure 2).

IMMUNE CHECKPOINT INHIBITORS AND TYPE 1 DIABETES

Recently, immune checkpoint inhibitors have been widely used for the treatment of many types of cancer. Immune checkpoint inhibitors include the following drugs: ipilimumab and tremelimumab (cytotoxic T-lymphocyte antigen 4 [CTLA-4] antibodies), nivolumab and pembrolizumab (programmed cell death 1 [PD-1] antibodies), and atezolizumab, avelumab and duravalumab (programmed cell death ligand 1 [PD-L1] antibodies). Dendritic cells present tumor antigen to naïve and regulatory T cells through the binding of CD80/CD86 to CD28, initiating anti-tumor immunity²¹. In contrast, binding of CD80/86 to CTLA-4 induces inhibition of the immune response²¹. CTLA-4 antibodies block the binding of CD80/86 to CTLA-4, restoring a normal immune response against the cancer. As for the PD-1/PD-L1 pathway, PD-L1 expressed on tumor cells binds to

PD-1 expressed on cytotoxic T cells, leading to suppression of the anti-tumor effect of T cells²². Antibodies to PD-1 or PD-L1 block the binding between PD-1 and PD-L1, maintaining anti-tumor immunity. Thus, immune checkpoint inhibitors block the negative signaling of T cells and accelerate the immune response to cancer cells.

Treatment with immune checkpoint inhibitors has also been reported to induce immune-related adverse events (irAE), including multiple endocrine diseases. Hypophysitis, hyper- and hypothyroidism, hypoparathyroidism, adrenal insufficiency, and type 1 diabetes were all reported as irAE²³. Type 1 diabetes, including fulminant type 1 diabetes, was also reported as an irAE²⁴ (Table 1). The incidence of type 1 diabetes was 0.33% in patients receiving nivolumab and 0.14% with pembrolizumab, whereas the fulminant type 1 diabetes incidences were 0.13% and 0.03%, respectively^{25,26}. These frequencies were high compared with the overall incidence of fulminant type 1 diabetes, which was estimated to be approximately 0.01% in Japan²⁷. We carried out a nationwide surveillance on the incidence of type 1 diabetes after treatment with PD-1 antibodies in Japan for the consultation of the Japan Diabetes Society Committee on Type 1 Diabetes Mellitus Research²⁸. We collected 22 patients who developed type 1 diabetes after immune checkpoint inhibitor treatment. Of these, 11 patients (50.0%) fulfilled the criteria for fulminant type 1 diabetes, whereas only one patient was positive for islet-related antibodies. The mean timespan between initial treatment with PD-1 antibody and onset of type 1 diabetes was 155 days (range 13–504 days), with 17 out of 20 patients (85.0%) developing ketosis, and seven of 18 patients (38.9%) developing ketoacidosis.

The pathogenesis of type 1 diabetes, including fulminant type 1 diabetes, after treatment with PD-1 and PD-L1 antibodies, has not been fully clarified. In terms of immune cells, we reported that the expression of CTLA-4 on CD4-positive T cells from patients with fulminant type 1 diabetes was significantly lower than from either patients with autoimmune type 1 diabetes or healthy individuals²⁹. We also reported that PD-1 expression in CD4-positive T cells was significantly lower in patients with autoimmune type 1 diabetes than in either patients with type 2 diabetes or in healthy individuals, but was not significantly different when comparing patients with fulminant type 1 diabetes with those with type 2 diabetes or healthy individuals³⁰. However, Iijima *et al.*³¹ recently reported that PD-1⁺CD-4⁺ and PD-1⁺CD-8⁺ T cells were reduced at the time of onset of fulminant type 1 diabetes, and this reduction was reversed after treatment. Therefore, we suppose that fulminant type 1 diabetes onset after treatment with PD-1 and PD-L1 antibodies proceeds according to the following steps. First, the CTLA-4 signal is blocked either by decreasing CTLA-4 expression on T cells or by treating with CTLA-4 antibodies. Second, treatment with PD-1 and PD-L1 antibodies induces blocking of the PD-1 signal. The combined suppression of these two immune regulators is thought to consequently activate T cells to attack not only cancer cells, but also pancreatic β -cells (Figure 3).

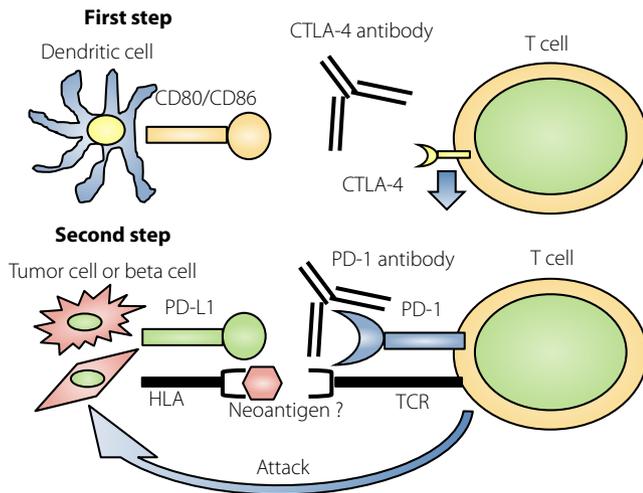


Figure 3 | Our hypothesis on fulminant type 1 diabetes onset after treatment with programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) antibodies is as follows: First, the CTLA-4 signal is blocked by a decrease of cytotoxic T-lymphocyte antigen 4 (CTLA-4) expression on T cells or treatment with CTLA4 antibodies. Second, treatment with PD-1 and PD-L1 antibodies blocks the PD-1 signal, and the combined suppression of these two immune regulators activates T cells to attack not only cancer cells, but also pancreatic β -cells. HLA, human leukocyte antigen.

In terms of β -cells, the PD-L1 expression of β -cells in patients who developed irAE was unreported. In contrast, patients who expressed high PD-L1 in tumor cells presented with a high response rate to treatment with PD-1 antibodies³², and patients who developed severe irAE also showed a high response rate to PD-1 antibodies³³. A higher non-synonymous mutation burden in non-small cell lung cancer presented as an improved response to pembrolizumab³⁴. Higher non-synonymous mutation in tumors correlates with higher neoantigen burden, and it has been suggested that PD-1 antibodies enhance neoantigen-specific T-cell reactivity. If these neoantigens were to have an amino acid sequence that mirrored pancreatic β -cells, neoantigen-specific T cells would also react to β -cells and might induce their destruction. It is unique that blockade of only one signal; that is, the PD-1 and PD-L1 pathway, induces type 1 diabetes. Revealing the pathogenic mechanism underlying the induction of type 1 diabetes by immune checkpoint inhibitors might also help to reveal other mechanisms underlying type 1 diabetes, unrelated to immune checkpoint inhibitors (Figure 3).

IPSCS OF FULMINANT TYPE 1 DIABETES

As previously discussed, many studies on the pathogenesis of fulminant type 1 diabetes have been reported, which used patient pancreatic samples^{15–20}. However, the availability of autopsy samples is finite, and the number of residual pancreatic β -cells was very low in samples from patients who developed

hyperglycemia and ketoacidosis several months earlier. It is also difficult to study the course of β -cell destruction by using autopsy samples. In contrast, many recent studies have generated iPSCs from patients with intractable disorders as models with which to study disease mechanisms^{35–37}. Research using patient-derived iPSCs has many advantages; the cells maintain the genetic background of the patient while also allowing the study of target organs or cells differentiated from iPSCs unlimitedly *in vitro*. We attempted to generate iPSCs from patients with Fulminant type 1 diabetes to differentiate patient-derived iPSCs into insulin-producing cells and to reveal the pathogenesis of the disease using an iPSC disease model³⁸. We generated iPSCs from three Japanese patients who fulfilled the inclusion criteria for fulminant type 1 diabetes patients (fulminant type 1 diabetes-iPSCs), and successfully differentiated fulminant type 1 diabetes-iPSCs into the pancreatic endocrine lineage: sex-determining region Y-box 17-positive definitive endoderm cells, pancreatic and duodenal homeobox 1-positive pancreatic progenitor cells and insulin (INS)-positive cells. The insulin-secreting functions of insulin-producing cells differentiated from fulminant type 1 diabetes-iPSCs could be induced by KCl, but not by glucose. As cytokine-induced apoptosis is assumed to be one of the mechanisms of β -cell destruction in fulminant type 1 diabetes^{17,19,39,40}, we examined the effect of cytokines on iPSCs. Consequently, treatment with TNF- α , interleukin-1 β and IFN-gamma induced a significantly higher proportion of cleaved caspase-3-positive cells among the induced INS-positive cells in fulminant type 1 diabetes-iPSCs than in control-iPSCs derived from healthy individuals. This result suggests that β -like insulin-producing cells from fulminant type 1 diabetes-iPSCs might be more vulnerable to apoptosis-inducing stimuli compared with those from control-iPSCs. To reveal the cytokine-induced apoptosis-related genes involved in β -cell destruction in fulminant type 1 diabetes, we isolated INS-positive cells derived from fulminant type 1 diabetes- and control-iPSCs after treatment with the three cytokines by fluorescence-activated cell sorting, and carried out RNA sequencing analysis. RNA sequencing identified several apoptosis-related genes, including poly (adenosine diphosphate-ribose) polymerase 3 (PARP3), coiled-coil-helix-coiled-coil-helix domain containing 2 (CHCHD2), inositol 1,4,5-trisphosphate receptor type 2 (ITPR2) and the antiviral gene cholesterol 25-hydroxylase (CH25H). PARP3 and CHCHD2, which were known as anti-apoptosis genes, and CH25H, which was known as an antiviral-related gene, were decreased, and ITPR2, which enhances apoptosis, was increased in the RNA sequencing. These might be candidate genes related to the pathogenesis of β -cell destruction in fulminant type 1 diabetes (Figure 2).

In this way, we introduced one case of application for iPSCs derived from patients with fulminant type 1 diabetes, and these patient-derived iPSCs have potential in not only regenerative therapy, but also in revealing the pathogenesis of the disease (Figure 4). For example, among the candidate genes that were extracted by the RNA sequencing analysis of INS-positive cells

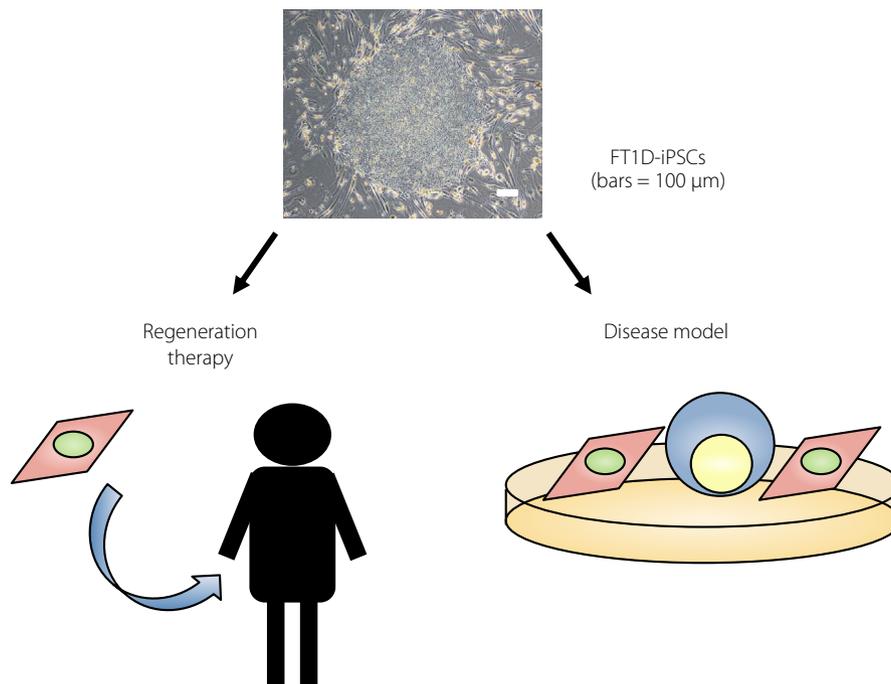


Figure 4 | Induced pluripotent stem cells (iPSCs) derived from patients with fulminant type 1 diabetes (FT1D-iPSCs) have potential not only in regenerative therapy, but also in disease models, including the co-culture of β -cells and immune cells both differentiated from FT1D-iPSCs.

derived from fulminant type 1 diabetes- and control-iPSCs after treatment with three cytokines, *CH25H* plays a very unique role *in vivo*. *CH25H* encodes cholesterol 25-hydroxylase and alters cholesterol to 25-hydroxycholesterol. *CH25H* also acts as an interferon-stimulated gene, and 25-hydroxycholesterol has multiple antiviral effects⁴¹. Therefore, downregulation of *CH25H* in pancreatic β -cells could decrease the reactivity to viruses in patients with fulminant type 1 diabetes, and might accelerate β -cell destruction.

In addition, our previous disease model of fulminant type 1 diabetes-iPSCs focused only on iPSCs-derived β -like cells, and took no account of immune cells. Now, however, iPSCs differentiate into macrophages or T cells. A co-culture system using β -cells and immune cells differentiated from fulminant type 1 diabetes-iPSCs could reproduce the pathogenic process that leads to β -cell destruction. This could serve as a disease model in the near future after overcoming problems, such as generating T cells from iPSCs, which present T-cell receptor-reactive islet-specific autoantigen. These iPSC techniques could provide us with unlimited organs or cells *in vitro*, not only in fulminant type 1 diabetes, but also in autoimmune type 1 diabetes, and help elucidate the pathogenesis of type 1 diabetes.

Our study using β -cells differentiated from patient-derived iPSCs represents a significant step forward compared with past studies, and provides a pathway to overcome the low cell number limitations inherent in autopsy samples. Currently, β -cells differentiated from iPSCs are immature compared with β -cells

in adults. However, recently reported protocols show efficient *in vitro* generation of functional β -cells with glucose-stimulated insulin secretion potential^{42,43}. Adaptation of such a differentiation protocol for iPSCs to produce β -cells would help reveal the pathogenic mechanism of β -cell destruction in more detail.

CONCLUSION

Pathogenesis of fulminant type 1 diabetes has been clarified step-by-step. Detailed analysis of the immune checkpoint inhibitor-induced subtype and/or patient-derived iPSCs would provide us with further insights into this field.

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