Pathophysiological mechanisms involving aggressive islet cell destruction in fulminant type 1 diabetes

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Abstract. Fulminant type 1 diabetes is characterized by a rapid onset of severe hyperglycemia and ketoacidosis, with subsequent poor prognosis of diabetic complications. This review summarizes new findings related to the pathophysiology of accelerated β-cell failure in fulminant type 1 diabetes. Immunohistological examination revealed the presence of enterovirus in pancreatic islet cells and exocrine tissues and hyperexpression of pattern recognition receptors (PRRs) including melanoma differentiation-associated antigen 5 (MDA5), retinoic acid-inducible gene-I (RIG-I), Toll-like receptor (TLR)3 and TLR4, essential sensors of innate immunity, in islet cells and mononuclear cells (MNCs) infiltrating islets. Interferon (IFN)-α and IFN-β, products of PRR cascades, were expressed in both islet cells and infiltrating MNCs. Phenotypes of infiltrating cells around and/or into islets were mainly dendritic cells, macrophages and CD8+ T cells. Islet β-cells simultaneously expressed CXC chemokine ligand 10 (CXCL10), IFN-γ and interleukin-18, indicating that these chemokines/cytotoxic cytokines mutually amplify their cytoplasmic expression in the islet cells. These positive feedback systems might enhance adaptive immunity, leading to rapid and complete loss of β-cells in fulminant type 1 diabetes. In innate and adaptive/autoimmune immune processes, the mechanisms behind bystander activation/killing might further amplify β-cell destruction. In addition to intrinsic pathway of cell apoptosis, the Fas and Fas ligand pathway are also involved as an extrinsic pathway of cell apoptosis. A high prevalence of anti-amylase autoantibodies was recognized in patients with fulminant type 1 diabetes, which suggests that Th2 T-cell reactive immunity against amylase might contribute to β-cell destruction in fulminant type 1 diabetes.

Key words: Fulminant type 1 diabetes, Pattern recognition receptors, Melanoma differentiation-associated antigen 5, Retinoic acid-inducible gene-I, CXC chemokine ligand 10

FULMINANT TYPE 1 DIABETES is characterized by abrupt onset of severe hyperglycemia and ketoacidosis preceded by flu-like symptoms including fever, abdominal pain, and headache [1-3]. Due to rushed clinical course in most cases, patients with fulminant type 1 diabetes sometimes remain untreated until becoming comatose and/or entering a critical, life-threatening state [4]. Endogenous insulin secretion is completely abolished over time and diabetic microangiopathies develop within a short duration [5, 6]. Remarkable severe insulitis was recognized in autopsied pancreata of patients who died shortly after the onset of fulminant type 1 diabetes [3, 7-10]. The mechanisms underlying aggressive and rapid destruction of β-cells remain some of the key issues in question regarding this subtype of type 1 diabetes. This review presents in situ human data on affected pancreata and the possible mechanisms leading to aggressive islet destruction in fulminant type 1 diabetes.

Enterovirus in the pancreas

Enteroviruses are small RNA viruses that include coxsackie A and B, polioviruses and echoviruses, causing common and often asymptomatic infections. Immunoglobulin (Ig)A antibody titers to enterovirus were significantly higher in patients with recent-onset fulminant type 1 diabetes than in those with typical type 1A diabetes and controls [11], indicating that enterovirus infection might be one cause of fulminant type 1 diabetes. Indeed, we identified entroviral capsid protein (VP1) in autopsied pancreata from patients with
fulminant type 1 diabetes [7-9]. A proportion of islet cells and acinar cells were positive for VP1. Elevated levels of serum pancreatic enzymes and mononuclear cell (MNC) infiltration into the exocrine pancreas are characteristic findings in fulminant type 1 diabetes [1-3]. Enterovirus-associated involvement of the exocrine pancreas would be related to these findings of fulminant type 1 diabetes. Some VP1-positive cells showed shrunken and darkly staining nuclei suggestive of pyknosis, which has been reported in coxsackievirus-infected islets [12]. These degenerating pathological features might result from the direct effects of enterovirus infection and/or virus-initiated innate immune responses. It is reported that VP1 staining of the islet cells was recognized in patients with acute-onset type 1 diabetes [13], indicating that pancreatic infection by enterovirus would not be a specific finding for fulminant type 1 diabetes. Hence, in addition to enterovirus infection, some factors within the host might be needed for the development of fulminant diabetes. One candidate for such a host factor could be genetics. We reported that the HLA-DRB1*04:05-DQB1*04:01 haplotype is associated with Japanese fulminant type 1 diabetes in a homozygous manner [14, 15].

**Hyper-expression of pattern recognition receptors (PRRs) in affected pancreata of patients with fulminant type 1 diabetes**

Germline-encoded PRRs are responsible for sensing the presence of microorganisms [16]. This is achieved by recognizing structures conserved among microbial species including viruses, called pathogen-associated molecular patterns (PAMPs). Recent evidence indicates that PRRs are also responsible for recognizing endogenous molecules released from damaged cells, termed damage-associated molecular patterns or danger-associated molecular patterns (DAMPs). To date, five main classes of PRR families have been identified [16, 17]. These families include transmembrane proteins such as the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), as well as cytoplasmic proteins such as the retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and absent in melanoma-2 (AIM2)-like receptors (ALRs).

RLRs are expressed by most cells of the human organism and include three members of relevance: RIG-I; melanoma differentiation-associated antigen 5 (MDA5, also known as IFIH1); and laboratory of genetics and physiology 2 (LGP2) [18-21]. Studies of gene-deficient mice indicate that RLRs play an important role in antiviral response [22-24]. We have demonstrated significant hyper-expression of RLRs in islet cells of patients with fulminant type 1 diabetes [8, 9]. MDA5 was strongly expressed in islet cells (including β- and α-cells) of fulminant type 1 diabetic pancreata. In non-diabetic controls and patients with slowly progressive insulin-dependent (type 1) diabetes (SPIDDM) (a subtype of autoimmune type 1 diabetes [25-27]), weak MDA5 expression was observed in a few islet-cells. In contrast, significant expression of RIG-I was observed preferentially in islet β-cells in fulminant type 1 diabetes, although no expression was evident in SPIDDM patients or non-diabetic controls. LGP2 also was preferentially hyper-expressed in islet β-cells of pancreas with fulminant type 1 diabetic patients [9]. In non-diabetic and SPIDDM pancreata, weak basal expression of LGP2 was observed in β-cells. This hyper-expression of RLRs in islet cells suggests a crucial role of RLRs for sensing and responding to enterovirus infection in the pancreas of patients with fulminant type 1 diabetes (Fig. 1). Indeed, increased expression of MDA5, RIG-I and LGP2 is recognized in human islets infected with coxsackievirus B5 [28]. In addition, it is reported that polymorphisms of MDA5 (IFIH1) are associated with both reduced expression of the MDA5 protein and increased protection against type 1 diabetes [29].

TLRs are another type of PRRs for RNA virus detection. The human TLR multigene family comprises 10 members, with TLR2, -3, -4, -7, and -8 considered to be of importance in the recognition of structural components of RNA viruses, including viral double-stranded RNA, single-stranded RNA, and surface glycoproteins [30]. We showed that both TLR3 and TLR4 were expressed in MNCs that had infiltrated islets of fulminant type 1 diabetic pancreata, but not SPIDDM patients or non-diabetic controls [8, 9]. The frequencies of islets with infiltrating MNCs expressing TLR3 and TLR4 were 11.7% and 4.3%, respectively, although almost no islet with MNCs expressing TLR3 or TLR4 was recognized in pancreata of non-diabetic controls or SPIDDM patients. Professional immune cells also recognize enterovirus using TLRs and contribute to the innate immunity leading to adaptive immune responses in fulminant type 1 diabetes (Fig. 1).

As NLRs, NOD1 and NOD2 recognize the structures of bacterial peptidoglycans [16]. NOD2 also recog-
Islet destruction in fulminant T1DM

We studied the expression of NOD1 and NOD2, and found that they were expressed in the pancreata of patients with fulminant type 1 diabetes at levels similar to those in SPIDDM patients, individuals with type 2 diabetes and non-diabetic controls (Aida K et al., unpublished data). These findings suggest that NLRs might not contribute much to sensing of enterovirus in fulminant type 1 diabetes.

**Interferon-α and -β (IFNs-α/β)**

Sensing of enterovirus by PRRs markedly upregulates transcription of genes involved in inflammatory responses. These genes encode proinflammatory cytokines/chemokines, IFNs-α/β, and antimicrobial proteins [30]. Production of IFNs-α/β plays a central role in the induction of antiviral responses, as these trigger the transcription of many IFN-inducible genes that influ-
ence protein synthesis, growth regulation, and apoptosis. IFNs-α/β may also induce enhanced expression of major histocompatibility complex (MHC) class I, aberrant expression of MHC class II presentation, maturation of dendritic cells (DCs), cytotoxicity of natural killer (NK) cells, and the differentiation of virus-specific cytotoxic T lymphocytes, thus providing an important link between innate and adaptive immune responses [32, 33]. In pancreata of patients with fulminant type 1 diabetes, IFNs-α/β were strongly expressed in islet cells including β-cells, α-cells and other cells [8, 9] (Fig. 1). Some MNCs infiltrating around and into islets and exocrine cells (acinar cells and ductal cells) also expressed IFNs-α/β in fulminant type 1 diabetes. Interferon regulatory factor-7, a master transcription factor of IFN α/β-generating cascades, was strongly expressed in islet β- and α-cells and mostly stained around and within the nuclei of the islet cells [8]. MHC class II as well as class I was hyper-expressed in islets of fulminant type 1 diabetic pancreata [7, 8]. These findings indicate that islet cells are in activated state of innate immunity in response to enterovirus infection in patients with fulminant type 1 diabetes.

Inflammasomes in the islets

Some PRRs assemble into high-molecular weight, caspase-1-activating platforms called “inflammasomes” [34]. Currently, three distinct inflammasome complexes have been shown to be involved in antiviral immunity [17]: the NLRP3 (NLR family pyrin domain-containing protein 3) inflammasome; the RIG-I inflammasome and the AIM2 inflammasome. NLRP3, RIG-I and AIM2 belong to families of NLRs, RLRs and ALRs, respectively. In these inflammasome complexes, adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC) provides a link between NLRP3, RIG-I or AIM2 and the pro-caspase-1. All three types of inflammasome induce caspase-1 activation, leading to the production of interleukin (IL)-1β and IL-18 [17].

We examined the expressions of NLRP3, ASC, caspase-1 and IL-1β immunohistochemically. NLRP3 expression was weaker in the islets of fulminant type 1 diabetic patients than that in the SPIDDM patients, type 2 diabetic patients, or non-diabetic controls (Aida K., et al., unpublished data). While ASC was strongly expressed in the infiltrating cells, but weakly expressed in islet cells from the patients with fulminant type 1 diabetes, expression levels in the islet cells were similar with those in SPIDDM patients, type 2 diabetic patients, and non-diabetic controls. Caspase-1 and IL-1β were weakly detected in islets from fulminant type 1 diabetes, SPIDDM, type 2 diabetes, and non-diabetic controls. These finding suggests that inflammasomes might contribute little to islet destruction in fulminant type 1 diabetes.

Phenotypes of MNCs infiltrating pancreas

Predominant phenotypes of MNCs in islets with insulitis were DCs, macrophages and CD8+ T cells in fulminant type 1 diabetic patients [7, 8]. Immunohistochemical examinations indicated marked migration of CD11c+ DCs/macrophages to more than 90% of islets in fulminant type 1 diabetic pancreata. Most CD11c+ cells were also positive for CD1a and some for CD68, likely representing DCs and macrophages subsets, respectively. Some DCs and macrophages expressed MHC class II molecules. The frequency of islets with CD8+ T-cell infiltration was more than 75% in fulminant type 1 diabetic patients [7, 8]. B-lymphocytes, CD4+ T cells and NK cells were rare. Regulatory T cells (CD4+ Foxp3+ cells) were not detected in or around islets of fulminant type 1 diabetic pancreata [8]. This suggests that the extremely polarized local condition leading to a predominance of Th1 in response to enteroviral infection suppresses regulatory T-cell differentiation from naïve CD4+ T cells [35]. In turn, the regulatory T cells-depleted islet condition enhances the generation of Th1 cytokine (i.e. IFN-γ). Activated innate immune responses including virus sensing by PRRs with subsequent IFNs-α/β production and maturation of DCs and macrophages will not only protect against enteroviral infection, but also enhance the adaptive immunity leading to autoreactive CD8+ T-cell induction of islet cell destruction (Fig. 1). Although most patients with fulminant type 1 diabetes exhibit no islet-related autoantibodies including glutamic acid decarboxylase (GAD) autoantibodies, Shimada et al. [36] reported a fulminant diabetic patient with GAD-reactive CD4+ cells in the periphery. Furthermore, Kotani et al. [37] identified GAD-reactive Th1 cells and insulin-B9-23-reactive Th1 cells in 9 out of 13 and 3 out of 12 fulminant type 1 diabetic patients, respectively, using ELISPOT assay as in autoantibody-positive type 1 diabetic patients. This high prevalence of autoreactive T cells against β-cell self-antigens indi-
cates an autoimmune response against islet β-cells might contribute, at least in part, to the development of fulminant type 1 diabetes.

**Co-expressions of CXC chemokine ligand 10 (CXCL10), IFN-γ and IL-18 in islet cells**

We demonstrated CXCL10, a key chemoattractant for activated T cells and macrophages [38], is expressed in islet cells including β-cells, α-cells, and other subset cells in pancreata of fulminant type 1 diabetes [7, 8]. We also recognized MNCs bearing the receptor for CXCL10, named CXCR3, in and around the islet cells expressing CXCL10 [7, 8] (Fig. 1). Islet cells (β-cells, α-cells, other subset cells) of fulminant type 1 diabetic pancreata also expressed IFN-γ and IL-18 [7, 8]. IFN-γ stimulates CXCL10 production and IL-18 is a potent IFN-γ-inducing factor [38, 39]. Conversely, IL-18 can be induced by IFN-γ in combination with other cytokines in islet β-cells [40]. Wittmann et al. [41] reported that IL-18 alone or in combination with IFN-γ enhanced CXCL10 secretion from human keratinocytes. IFNs-α/β, as products of PRR cascades for enterovirus infection, also stimulate CXCL10 production [42]. These factors including CXCL10, IFN-γ, IL-18 and IFNs-α/β co-localized in most β-cells and non-β-cells and enhanced the production of each other (Fig. 1). This cytokine-/chemokine-positive feedback loop might amplify adaptive immune reactions, culminating in aggressive islet cell destruction. Serum levels of CXCL10 and IFN-γ are now recognized to be elevated in patients with fulminant type 1 diabetes [7, 8, 36] and these elevations of chemokines and cytokines would be derived, at least in part, from the cytokine-/chemokine-positive feedback loop in pancreatic islets.

**Bystander activation/killing of islet cells in fulminant type 1 diabetes**

In innate and adaptive immune processes, the mechanisms of bystander activation/killing might contribute to aggressive islet destruction. Viral infections lead to significant activation of antigen-presenting cells such as DCs. These activated antigen-presenting cells could potentially activate preprimed autoreactive T cells, which can then initiate autoimmune response (bystander activation of autoreactive immune T cells) [43]. In addition to this mode of bystander activation of autoreactive T cells, virus-specific T cells also might initiate bystander activation. Virus-specific T cells migrate to the pancreas and there encounter virus-infected cells that present viral peptides in the context of MHC class I molecules to virus-specific T cells. CD8+ T cells recognize these infected cells and release cytotoxic granules, resulting in the killing or death of the infected cells. Under these circumstances the dying cells, CD8+ T cells and inflammatory cells (macrophages) within the inflammatory focus release cytokines, lymphotoxin, and nitric oxide (NO), which can lead to bystander killing of uninfected neighboring cells [43]. This results in additional immunopathology at sites of infection [43-45]. In fulminant type 1 diabetes, enterovirus infection focused on pancreatic islets might lead to bystander activation of autoreactive T cells against islet β-cells. Hyper-expression of CXCL10 in islet cells would chemoattract and activate autoreactive T cells and macrophages. These cells elevate local concentration of cytokines such as IFN-γ and NO, leading to bystander killing of uninfected neighboring islet cells.

**Factors related apoptosis of β-cells in fulminant type 1 diabetes**

Apoptosis is likely to be the main form of β-cell death in immune-mediated diabetes mellitus. In mammals, a wide array of external signals may trigger two major apoptotic pathways, namely the extrinsic pathway (death receptor pathway) or the intrinsic pathway (the mitochondrial pathway) within a cell [46]. The extrinsic pathway is activated by apoptotic stimuli comprising extrinsic signals such as the binding of death inducing ligands to cell surface receptors. In other ways, apoptosis is initiated following intrinsic signals including DNA damage induced by irradiation or exposure to chemicals, growth factor deprivation or oxidative stress. In general intrinsic signals initiate apoptosis via the involvement of the mitochondria. The intrinsic pathway is the main pathway for endoplasmic reticulum stress and cytokine-induced beta cell apoptosis [47, 48].

The extrinsic pathway is mediated by cell surface death receptors, such as Fas, tumor necrosis factor receptor (TNF), or TNF-related apoptosis-inducing ligand (TRAIL) receptors. Death ligand stimulation results in oligomerization of the receptors and recruitment of the adaptor protein Fas-associated death domain (FADD) and caspase-8, forming a death-inducing signaling complex (DISC) [49]. Autoactivation
of caspase-8 at the DISC is followed by activation of effector caspases, including caspase-3, -6 and -7, which function as downstream effectors of the cell death program. The intrinsic pathway is mediated by diverse apoptotic stimuli, which converge at the mitochondria. Release of cytochrome c from the mitochondria to the cytoplasm initiates a caspase cascade. Cytosolic cytochrome c binds to apoptosis protease-activating factor 1 (Apaf-1) and procaspase-9, generating an intracellular DISC-like complex known as “apoptosome” [49]. Within the apoptosome, caspase-9 is activated, leading to processing of caspase-3. The two pathways of apoptosis, extrinsic/death receptor and intrinsic/mitochondrial, converge on caspase-3 and subsequently on other proteases and nucleases that drive the terminal events of programmed cell death.

In fulminant type 1 diabetic pancreata, elevated expression of Fas in islet cells coincided with marked MNC infiltration and Fas-ligand (FasL)-bearing MNCs infiltrated most islets [8]. The subsets of islet cells with Fas expression were predominantly β-cells. In islet cells of SPIDDM patients, Fas was not expressed. FasL-bearing MNC infiltration of islets was observed in SPIDDM patients, but not in non-diabetic controls.

Cleaved caspase 8, a marker of the extrinsic pathway, cleaved caspase 9, a marker of the intrinsic pathway, and activated caspase 3, a marker of the end-stage of apoptosis, were expressed specifically in islet β-cells of fulminant type 1 diabetic pancreata [8]. Taken together with the finding that MHC class I was strongly expressed in the affected islet cells, effector mechanisms for β-cell apoptosis in fulminant type 1 diabetes are likely mediated in part by MHC class I, by the extrinsic pathway and by the intrinsic pathway [50] (Fig. 1). Other apoptotic mechanisms acting through the IFN-γ-dependent JAK/STAT pathway [51] and innate immune pathway [52] would permissively result in β-cell destruction.

Autoimmunity against amylase in fulminant type 1 diabetes

We have reported a high prevalence (more than 80%) of autoantibodies against amylase in patients with fulminant type 1 diabetes or autoimmune pancreatitis [53]. Autoimmune pancreatitis has recently been reported as a unique form of chronic pancreatitis [54]. It is characterized by 1) irregular narrowing of the main pancreatic duct and swelling of the pancreas, both of which are due to abundant lymphoplasmacytic inflammation to the exocrine pancreas [55]; 2) increased serum level of IgG and IgG4 [56]; 3) positive autoantibodies such as lactoferrin autoantibody or carbonic anhydrase II (CAII) autoantibody [57]; and 4) a high prevalence of diabetes with complications [58]. We recently reported that pancreatic islets and exocrine pancreatic tissues were infiltrated by CD4+ or CD8+ T cells in autoimmune pancreatitis, just like fulminant type 1 diabetes [59], although the main lesions of inflammation in autoimmune pancreatitis are exocrine tissues rather than islets. With regard to the HLA genotype, Kawa et al. [60] demonstrated that the HLA-DRB1*04:05-DQB1*04:01 haplotype is closely associated with autoimmune pancreatitis in the Japanese population, and this HLA haplotype is also strongly associated with fulminant type 1 diabetes [15]. The similarity of histological findings and genetic background and the high prevalence of impaired glucose tolerance and autoantibodies against amylase suggest that autoimmune mechanisms against amylase might have some common effects on the destruction of pancreatic islets and exocrine tissues in both fulminant type 1 diabetes and autoimmune pancreatitis. Treatment with prednisolone improved insulin secretion and glycemic control and induced a rapid decrease of the titer of autoantibodies against amylase to normal levels in patients with autoimmune pancreatitis [53, 58]. As a result, immune modulatory treatment for autoimmunity against amylase may potentially contribute to improved endocrine function in fulminant type 1 diabetes.

Conclusions

Innate immune reaction against enterovirus infection would be an essential function in islet destruction in fulminant type 1 diabetes. Characteristically, islet β-cells express CXCL10, IFN-γ and IL-18 in fulminant type 1 diabetes, and these co-expressions of chemokines/cytokines enhance the expression of each other. This positive feedback system of chemokines/cytokines amplifies β-cell self-damage and enhances adaptive immune reaction, culminating in β-cell destruction. The aggressive innate and adaptive immune storm might induce bystander activation leading to further amplification of β-cell destruction in fulminant type 1 diabetes. Development of antagonists and neutralizing agents for an excessive immune reaction might represent one therapeutic opinion in fulminant type 1 diabetes.
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References


