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PATHOGENESIS OF TYPE 1 DIABETES (A PUGLIESE AND SJ RICHARDSON, SECTION EDITORS)

New Evidence of Exocrine Pancreatopathy in Pre-symptomatic and Symptomatic Type 1 Diabetes

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Abstract

Purpose of Review Type 1 diabetes (T1D) is one of the most frequent chronic autoimmune diseases in humans, characterized by the lack of insulin production resulting in high blood glucose levels and lifelong requirement of exogenous insulin administration for survival. It is now recognized that the autoimmune process begins years before the clinical onset, in a stage called pre-symptomatic T1D, in which the presence of β-cell-specific autoantibodies is detectable. Our aim is to review evidence for T1D as a "whole-pancreas disease," featured by both endocrine and exocrine pancreas alterations already at early disease stages.

Recent Findings In this review, we discuss a series of recent observations indicating that in genetically predisposed individuals, structural and functional abnormalities as well as immune cell infiltration of the exocrine pancreas are already present in the pre-symptomatic stages of the disease.

Summary Despite T1D being considered a β-cell-specific disease, numerous reports point to the presence of exocrine pancreas subclinical abnormalities occurring during disease development. These observations challenge the long-standing idea that T1D exocrine damage exists as a mere consequence of disease progression and provide further explanation of mechanisms underlying T1D pathogenesis.

Keywords T1D · Exocrine pancreas · Pancreatopathy · Pancreatitis · Acinar cells · Beta-cells · Inflammation

Introduction

Although often neglected, there is evidence of exocrine pancreas abnormalities in type 1 diabetes (T1D). The very first studies on human pancreas date back to the 1940s–1960s, when it was shown that in T1D, an autoimmune disease resulting in loss of insulin secretion, both the endocrine (i.e., pancreatic islet) and exocrine pancreas are involved [1, 2]. Insulin-producing cells, i.e., beta-cells, are localized in the pancreatic islet representing approximately 1% of the total pancreas volume [3]. The rest of the pancreas is mainly composed of exocrine tissue, namely, acinar, centro-acinar, and ductal cells, which are tightly organized into acini and lobules surrounded by connective tissue.

In 2016, Mohapatra and colleagues defined the moderate-to-severe subclinical pancreatic fibrosis combined with a modest exocrine dysfunction as "diabetic exocrine pancreatopathy" [4]. Nonetheless, the data on exocrine involvement in T1D pathogenesis are still scanty, and only few research groups had attempted to clarify whether these abnormalities in T1D are the trigger for beta-cell dysfunction or rather a consequence of β-cell loss. A wide-ranging review article describing the knowledge gained until 2015 on the role of exocrine pancreas in T1D has been recently published [5]. Thus, in this review, we will focus on the most recent evidence of T1D as a whole-pancreas disease, discussing compelling evidence of exocrine pancreas involvement already in pre-

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symptomatic phases of T1D (when autoantibodies are already detectable but hyperglycemia is not) and their possible implications in disease pathogenesis.

The Role of Genetics in Susceptibility to T1D as a "Whole-Pancreas" Disease

T1D is a complex disease; the development of which is determined by the interaction of environmental and genetic factors. It is hypothesized that one or more pancreatic insults—not yet identified—trigger T1D in individuals carrying a genetic predisposition [6]. Extensive literature coming from pathological and immunological studies, together with the evidence that variants of genes relevant for the immune system (such as HLA, IL2RA, CTLA4, and PTPN22) have been associated with T1D, outlines the crucial role of the immune system in the development of autoimmune diabetes [7, 8]. However, it is now evident that genes expressed in beta-cells controlling non-immune-related processes are linked to T1D risk as well, indicating the crucial role of immune cells in autoimmune diabetes.

It was reported that a single-base deletion of the CEL (carboxyl ester lipase) gene, expressed mainly in pancreatic acinar cells, is associated with the development of one form of monogenic diabetes, MODY8 (maturity onset diabetes of the young 8) [9]. This mutation leads to protein misfolding and degradation, resulting in cell death and exocrine pancreatic tissue degeneration. This syndrome features both endocrine and exocrine dysfunction, thus supporting the hypothesis that alterations of the exocrine pancreas can drive endocrine degeneration and dysfunction. It is also possible that in autoimmune diabetes, the subclinical damage of acinar cells may precede the destruction of pancreatic beta-cells. In the late 1990s, Panicot and colleagues identified antibodies recognizing CEL (or BSDL (bile salt–dependent lipase)) both in cohorts of T1D patients and first-degree relatives of T1D patients without diabetes [10]. Over 70% of T1D patients and 30% of pre-symptomatic individuals were positive for anti-BSDL antibodies. Moreover, 10% of the pre-symptomatic individuals were positive for anti-BSDL antibodies while antibodies anti-GAD65, IA-2, and insulin were still undetectable. These data suggest that besides being secondary to a gene mutation as in MODY8, degradation and recognition as "non-self" of molecules involved in exocrine pancreas function may occur in response to (unknown) triggers and that this event might precede endocrine damage in T1D [10]. Other autoantibodies targeting proteins of the exocrine pancreas have also been described in T1D [11–13] and in pre-symptomatic individuals [13], supporting the idea of a combined autoimmune exocrinopathy and endocrinopathy of the pancreas occurring during T1D development and suggesting that "non-self" recognition may occur for several acinar proteins. However, given the high heterogeneity of T1D, it is possible that the involvement of exocrine pancreas represents the mechanism contributing to T1D pathogenesis in only a subgroup of T1D patients endowed with a specific disease *endotype*, rather than of the entire T1D population.

The ability of beta-cells to resist external stressful conditions can play an important role in the context of T1D development, especially if whole-pancreas inflammation occurs. Thus, genetic variations conferring high susceptibility of beta-cells to local inflammation or oxidative stress need to be carefully considered in T1D. In 2016, Dooley and colleagues identified single nucleotide polymorphisms (SNPs) in genes that could promote an "intrinsic fragility" in beta-cells (i.e., GLIS3 and XRCC4, involved in protection from apoptosis and DNA repair mechanisms, respectively) [14]. Fragile beta-cells may undergo apoptosis, functional insufficiency, senescence, and de-differentiation when exposed to local inflammation triggered by environmental insults. Moreover, polymorphisms in the CLEC16A gene causing defects in mitophagia, an intra-cellular mechanism of autophagy required for mitochondrial quality control [15], have been associated with T1D [16]. Variants of this gene may be responsible for beta-cell fragility, by altering the functional robustness of beta-cells and their ability to survive.

All these reports suggest that (i) whole-pancreas inflammation in T1D may occur as a consequence of antigen recognition by T cells specific for both endocrine and exocrine antigens and that (ii) beta-cell damage in T1D may be the consequence of their extreme susceptibility to local inflammation. In case of whole-pancreas inflammation, which may characterize only a subgroup of T1D individuals, beta-cells in a stressful environment may be the first to die.

Structural Abnormalities of the Exocrine Pancreas in T1D

In physiological conditions, the human pancreas constantly grows, directly correlating with body weight, until the age of 30 [17, 18]. The first studies demonstrating that the pancreas volume is reduced up to 50% in T1D patients date back to the late 1960s and 1980s [2, 19, 20] and have been later confirmed by other authors with the advent of modern technologies such as computerized tomography (CT) and magnetic resonance imaging (MRI) [17, 21, 22••, 23••, 24–26].

The first attempt to evaluate pancreas size in T1D pre-symptomatic individuals was reported in 2012 and conducted on single-autoantibody-positive organ donors from nPOD (network for Pancreatic Organ donors with Diabetes). It was shown that pancreas weight is already reduced by 25% in T1D pre-symptomatic phases [18]. However, this study was performed on brain-dead organ donors, with all the limitations due to the use of autoptic samples.

Recently, to evaluate pancreas size during T1D progression, both Campbell et al. and Virostko et al. measured the pancreas volume by MRI in living children and adolescents newly diagnosed with T1D, autoantibody-negative and positive pre-symptomatic individuals, first-degree relatives (FDRs) and healthy controls. A significant reduction in pancreatic volume was measured both in pre-symptomatic subjects and T1D patients, corroborating the idea that a reduction in the pancreas size may already be present years before clinical onset, thus supporting the hypothesis that T1D may arise from a damaging process involving not only beta-cells but also the entire pancreatic parenchyma [22••, 23••]. Of note, in the study performed by Campbell and colleagues, a decreased pancreas volume was also seen in autoantibody-negative individuals, suggesting that other familial factors may influence pancreas size and that in those progressing to T1D, this abnormality may precede endocrine damage.

Although the loss of insulinotropic effects on acinar cells had long been considered the primary mechanism underlying exocrine pancreas abnormalities in T1D [17, 27], in a small cohort of patients, Virostko and colleagues reported that the amount of acinar atrophy found in T1D patients was independent of the presence of surviving beta-cells [22••]. Indeed, the authors observed no correlation between beta-cell function and pancreatic volume in both T1D patients and autoantibody-positive subjects, supporting the hypothesis that these exocrine alterations may not be justified entirely by the loss of a functional beta-cell mass. Moreover, although studies involving a larger number of subjects are necessary to corroborate these observations, by analyzing the microstructural changes of the pancreas using diffusion-weighted magnetic resonance imaging, it was reported that besides the volume, the composition of the pancreas is also altered already in pre-symptomatic phases of T1D, indicating the presence of microstructural alterations accompanying the volume reduction [22••].

An in-depth characterization of the human pancreas for a better delineation of anatomical abnormalities in T1D has been recently performed by Bonnet-Serrano et al. on $n = 75$ T1D and $n = 66$ control organ donors. By using an automated imaging method, they analyzed nPOD repository samples and calculated the area of endocrine, exocrine, and "non-exocrine-non-endocrine" tissues, the latter representing the sum of ducts, vessels, fatty degeneration, and fibrosis [28]. Decreased α-cell mass and early structural alterations of the exocrine pancreas emerged as T1D features, supporting, again, the idea that the entire pancreatic tissue is affected undergoing degeneration, remodeling, or transdifferentiation when T1D develops. However, autoantibody-positive organ donors were not included in this study. Therefore, it remains to be clarified whether, also at the microscopic level, autoantibody-positive subjects show similar alterations in the exocrine pancreas.

Altogether, these findings highlight how novel technologies have made it possible to detect several structural abnormalities of the exocrine pancreas occurring in T1D. We believe that advanced imaging technologies will play a key role in future studies allowing further characterization of the exocrine pancreas structure and of the entire pancreas abnormalities occurring already before T1D onset.

Functional Abnormalities of the Exocrine Pancreas in T1D

Besides the structural alterations and changes in volume, weight, and composition described above, anomalies of the exocrine pancreas associated with T1D include functional abnormalities.

Acinar cells contain zymogen granules in which several pro-enzymes, such as proteases, lipases, and amylases, are stored; after being cleaved and released into the pancreatic duct, they reach the duodenum in their active form [29]. This physiological process is apparently altered in T1D. In the early 1940s, Polland and colleagues reported evidence of exocrine pancreatic insufficiency in patients with T1D [1]; subsequent studies confirmed these data by measuring pancreatic enzymes in the duodenum after direct stimulation [30, 31]. These procedures were later replaced by much less invasive tests, such as assessment of fecal chymotrypsin activity and fecal elastase 1; studies in larger cohorts showed that approximately 50% of T1D patients are affected by exocrine pancreatic insufficiency, with a direct correlation with disease duration and glycemic control [32–35]. It has to be noted that the exocrine dysfunction in T1D is modest and that these subjects are asymptomatic, meaning this is a subclinical exocrine pancreatic dysfunction.

Only very recently researchers have attempted to investigate whether functional abnormalities of the exocrine pancreas are present prior to clinical onset of T1D. Li et al. showed that the serum level of trypsinogen—the inactive precursor of trypsin, a serine protease that hydrolyzes proteins at the carboxyl side of the amino acids lysine or arginine [36]—is reduced in pre-symptomatic individuals displaying elevated risk to develop T1D (i.e., positive for two or more T1D-related autoantibodies) [37••]. However, this study carries important limitations, such as the limited number of high-risk individuals studied and the influence that other factors, besides age, may have on trypsinogen levels. In line with this finding, our unpublished data reveal abnormal serum levels of the pancreatic enzyme amylases (a class of hydrolase enzymes that catalyze the hydrolysis of starch to glucose and maltose [3]) in subjects at risk of developing T1D. Levels of pancreatic enzymes in the circulation reflect exocrine pancreas function [38–40] and may represent a biomarker of T1D development. Thus, we believe that further investigation is needed to assess

exocrine pancreas function and its predictive value for the development of T1D in at-risk individuals.

In the field of T1D, little attention has been given to the nervous system. In physiological conditions, exocrine pancreas function is finely regulated by the autonomic nervous system, which is essential to guarantee proper enzyme secretion. In 2017, Lundberg and colleagues studied the parasympathetic axons by performing immunofluorescence analysis on pancreas tissues collected from non-diabetic brain-dead organ donors, living individuals with recent onset T1D recruited from the DiViD (Diabetes Virus Detection) study, and long-standing T1D organ donors [41•]. They demonstrated that parasympathetic axons innervating the exocrine pancreas—and not those near to the pancreatic islets—are decreased in patients with recent T1D onset, suggesting that exocrine abnormalities may be induced (or accompanied) by an imbalance in the parasympathetic axons innervating acinar cells. These results further support the idea of a role played by the exocrine pancreas in T1D development. Whether these abnormalities precede or follow beta-cell death remains to be elucidated.

In 2018, Stamatouli et al. reported a case series of diabetes induced by checkpoint inhibitor treatment, an anti-cancer therapy consisting of monoclonal antibodies that inhibit the checkpoint PDL-1/PD-1 [42]. Forty-two percent of the patients progressing to diabetes displayed pancreatitis (based on elevated serum lipase and amylase levels as well as increased pancreas volume) in the pre-diagnosis period. Interestingly, this subtype of diabetes, featured by a rapid disease course, shows similar exocrine abnormalities described in fulminant diabetes [43]. These data further support the hypothesis that beta-cell damage following acute or chronic inflammation of the entire pancreas is indeed possible. These forms of diabetes have both similarities and differences with T1D; whether similar mechanisms (at least in a subgroup of patients) may contribute to T1D development is not clear, and this deserves further exploration.

Immune Cell Infiltration of the Exocrine Pancreas in T1D

Abnormalities of exocrine pancreas in T1D patients also include alterations in acinar immune cell infiltration. It is generally accepted that T1D is mediated by autoreactive T cells, resulting in insulitis (i.e., islet infiltration by immune cells) and beta-cell disruption; however, several studies of T1D donors show that the exocrine pancreas is likewise affected by immune cell infiltration [44–46].

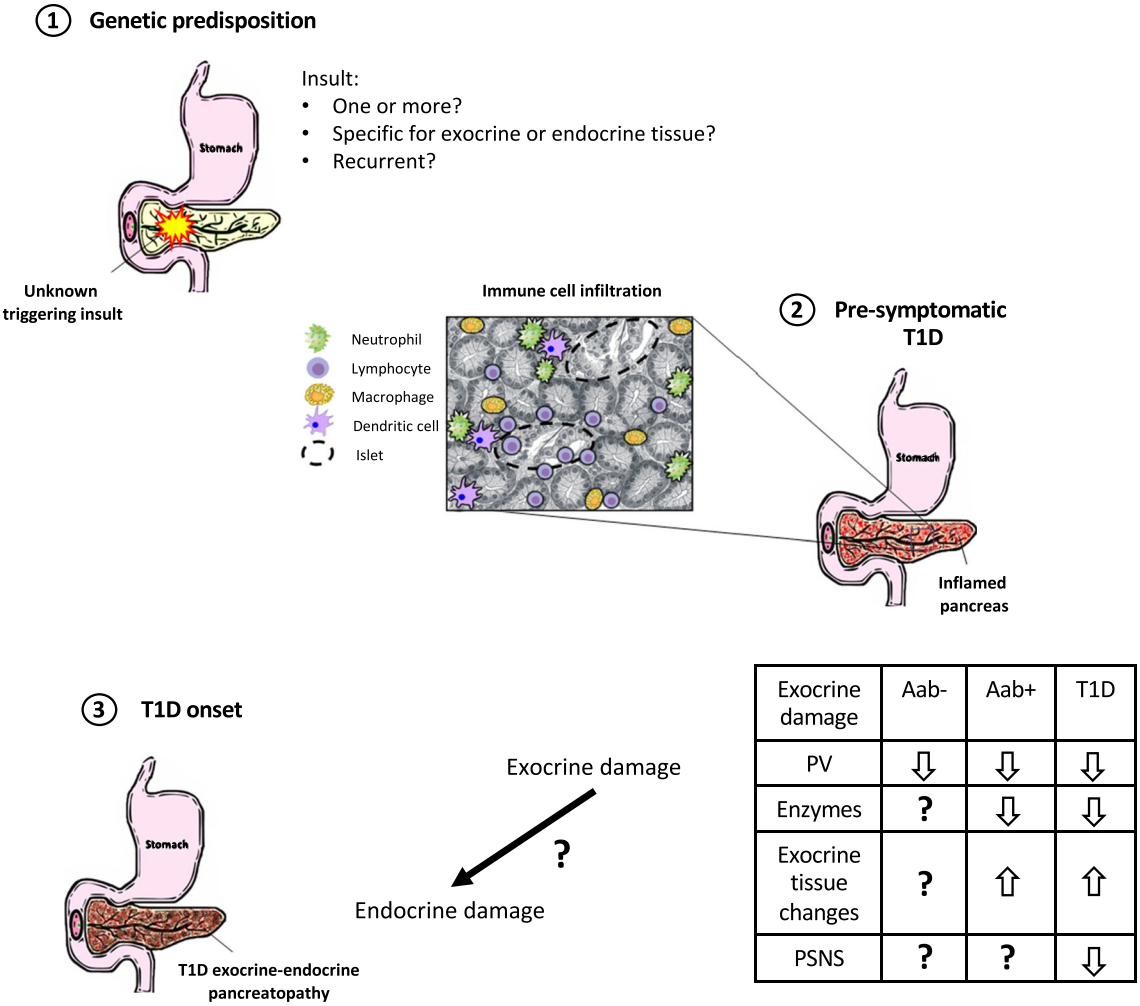
Increased presence of immune cells is evident in patients at risk of developing T1D before clinical onset, suggesting a pathogenic role for exocrine pancreas inflammation. In their work, Rodriguez-Calvo and colleagues profiled the level of CD11c+ (i.e., dendritic cells), CD4+, and CD8+ T cells in nPOD pancreata collected from organ donors without T1D and autoantibody-negative, donors without T1D but autoantibody-positive, and donors with T1D with short (< 5 years) and long (> 5 years) disease duration. A higher number of CD11c+ cells were found in the exocrine compartment at every disease stage as compared with control subjects, while CD4+ and CD8+ T cells were more abundant in short and long disease duration with respect to controls. Interestingly, beta-cell-derived factors are likely important in driving CD8+ T cell local infiltration; this is supported by the evidence that autoantibody-positive donors and donors with short disease duration of T1D (who all retain beta-cells) display enrichment of this cell subset both in exocrine and endocrine compartments. In contrast, donors with long disease duration who had lost most if not all of their beta-cells had elevated densities of CD8+ T cells in the exocrine tissue only. In keeping with this, elevated numbers of CD8+ T cells were found in insulin-containing islets compared with insulin-deficient islets, further corroborating the idea that beta-cell-derived factors, which may be or include the presence of insulin and other target antigens, may drive CD8+ T cell infiltration of the islets. Exocrine infiltration, instead, seems to be independent of insulin presence pointing to the fact that exocrine alterations are not entirely due to the decreased insulinotropic effect observed in T1D.

Evidence of immune cell infiltration in the exocrine pancreas has also been recently reported by our group [45, 46]. We demonstrate that the number of pancreas-residing neutrophils is increased not only in T1D patients but also in autoantibody-positive organ donors without T1D. Neutrophils have been identified throughout the pancreas, without a specific peri-islet localization, exhibiting a neutrophil extracellular trap (NET)–like morphology and associated markers. Our data suggest that in T1D, at early disease stages, neutrophils may promote or sustain inflammation of both exocrine and endocrine pancreata. Moreover, in both mice and humans, the presence of NETs in the exocrine pancreas can lead to the development of pancreatitis by inducing trypsin activation, inflammation, and tissue damage [47, 48]; this implies that T1D exocrine pancreatopathy might also be NET-driven. These data are in line with previous findings showing the presence of pathogenic neutrophils in a mouse model of autoimmune diabetes [49]. Moreover, our group was the first to report a mild reduction of neutrophil numbers in the circulation that precedes and accompanies T1D [46]—later confirmed by other groups [50–53]—and was shown to be associated with the presence of an interferon (IFN) signature in pre-symptomatic subjects. This supports on the one hand the idea that type I IFNs contribute to the development of T1D, as previously reported [54–57], and on the other hand that neutrophils might also play the role of "sensors" of this cytokine.

A recent report displayed a comprehensive mapping of immune cell localization in the pancreas from patients with

T1D using a novel technology called imaging mass cytometry [58••]. They showed that in a small number of patients older than 10 years of age at sampling, regardless of the disease stage (i.e., disease onset and long-standing T1D), cytotoxic, helper, and naïve T cells, as well as innate immune cells, such as macrophages and neutrophils, were found to be more abundant in the exocrine compartment compared with islets. However, it is also known that younger donors (< 10 years) show a more aggressive immune attack with elevated numbers of infiltrating cells in and around the islets (reviewed in [59]). Therefore, it is possible that the extent of immune infiltration in the exocrine and endocrine compartments depends on several factors, including age at diagnosis and disease duration. Interestingly, innate immune cells were found to be more abundant at late disease stages. Although counterintuitive and not in line with data reported in our recent report showing neutrophil infiltration already in autoantibody-positive organ donors, it has to be acknowledged that cross-sectional studies do not allow to assess the dynamics of T cell infiltration during T1D development. Therefore, it cannot be excluded that imaging mass cytometry performed at earlier disease stages would display a prevalent innate immune cell infiltration. We believe that further studies are required to assess the temporal dynamics of immune cell infiltration in the entire pancreas during T1D development.

Innate immune cell infiltration in exocrine tissues is also reported in Sjögren's syndrome (SS), a chronic autoimmune disease involving exocrine glands [60]. Similar to neutrophils



Exocrine damage	Aab-	Aab+	T1D
PV	⇩	⇩	⇩
Enzymes	?	⇩	⇩
Exocrine tissue changes	?	⇧	⇧
PSNS	?	?	⇩

Fig. 1 Proposed model of T1D development. In individuals genetically at risk of developing T1D, one or more unknown hits, likely recurrent over time, can induce whole-pancreas inflammation leading to the development of abnormalities involving both the exocrine and endocrine compartments. The long-standing idea that the exocrine tissue damage in T1D is a consequence of the lack of insulin (due to beta-cell death) has now been challenged by the evidence that functional and structural changes of the exocrine pancreas occur already in the pre-symptomatic phase of the disease. Therefore, in our model, we propose that exocrine changes may precede beta-cell loss. However, it cannot be excluded that alterations of both exocrine and endocrine compartments may even occur simultaneously. In this scenario, inflammation of the whole-pancreas may lead to the development of T1D via the death of "fragile beta-cells" and the exposure of beta-cell antigens to the local immune infiltrate. PV, pancreatic volume; PSNS, parasympathetic nervous system; Aab−, autoantibody-negative individuals; Aab+, autoantibody-positive individuals; T1D, type 1 diabetes

in T1D, in SS, plasmacytoid DC infiltrate the exocrine tissue, are reduced in the periphery, and show an IFN signature [61, 62]. However, it is unlikely that peripheral immune cell reduction in T1D and other autoimmune diseases is entirely due to the accumulation in the target tissue of the autoimmune process. In T1D, we hypothesize that active recruitment of neutrophils in the pancreas, together with the IFN rich environment, known to promote neutropenia [63–65], may participate in the reduction of circulating neutrophil count. However, further studies are needed to test this hypothesis.

Conclusions

It is currently accepted that both endocrine and exocrine compartments of the pancreas are damaged in T1D. Most recent data support the evidence that prior to T1D clinical onset, exocrine damage is present and that immune cell infiltration can be more abundant in the exocrine compartment rather than peri/intra-islet, in some donors. It is still unknown whether this damage occurs prior to or after beta-cell death. Another unanswered question is whether exocrine pancreas dysfunction occurs in all patients progressing to diabetes or, perhaps more likely, it contributes to the mechanism underlying T1D pathogenesis in a subgroup of patients with a specific disease *endotype*. Our proposed hypothesis is that an as yet unknown trigger recalls immune cells in the exocrine pancreas and that in genetically predisposed individuals, this may lead to structural and functional subclinical abnormalities of the whole pancreas, inducing beta-cell death, release of autoantigens, and development of autoimmunity (shown in Fig. 1). Unfortunately, the target site of autoimmunity in T1D cannot be accessed in humans (except for cadaveric donors), which is a limitation for the study of disease mechanisms. Newly developed imaging technologies have the potential to bring innovation in the field. Longitudinal trials involving subjects enrolled in natural history studies, such as TEDDY [66], or pathway to prevention studies, such as TrialNet [67], will help elucidating structural and functional features of the entire pancreas in at-risk subjects before and after seroconversion, thus clarifying the timing of appearance of exocrine pancreas damage and its contribution to the development of autoimmune diabetes.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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