Biopsy of the pancreas: the predictive value and therapeutic impact on autoimmune diabetes

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Diabetes mellitus is by definition an end-stage organ failure. Type 1 diabetes mellitus is an autoimmune disease. Autoinflammatory infiltrate appears to characterize the insulitis associated with type 1 diabetes mellitus. In recent times, a comprehensive composition of peri-islet capsules and their basement membrane (BM) has been described. Lymphocytic infiltration around the islets without invasion of the BM is the first step in disease induction (nondestructive insulitis phase). Invasion of the BM by leukocytic infiltration (destructive insulitis phase) occurs over a period of several years, offering good window for therapeutic intervention. Clinical symptoms appear only when 70–90% of β-cell mass are destroyed. These data emphasize on the importance of identification and classification of such pathologic features by performing a biopsy of the pancreas, along with histoimmunochemistry analysis at the prehyperglycemic stage in a high-risk, genetically predisposed, autoimmune-suspected patient, which may at least in part help in achieving new therapeutic approaches and halting the progression to end-stage pancreatic disease known as diabetes mellitus. In this review, we emphasize the predictive role biopsy of the pancreas can have, by building up a solid gold standard tool in the diagnosis, staging, and therapeutic follow-up of autoimmune diabetes mellitus.

Keywords:
autoimmune diabetes mellitus, biopsy of the pancreas, insulitis

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease, which is a diverse group of chronic illnesses, characterized by an immune response directed against islets β-cell mass. Moreover, autoinflammatory infiltrate appears to characterize the insulitis associated with type 2 DM. Furthermore, islet-reactive T cells responding to multiple islet proteins have been found in both T1DM patients and phenotypic type 2 DM patients with or without islet autoantibodies [1–3], emphasizing the need to implicate early immune-based therapeutic interventions in the treatment of prehyperglycemic stage of diabetic patients that is ideally effective and long-lasting, with minimal side effects and better cure rates.

The ability to predict the development of autoimmune diabetes has been improved markedly with the combined use of genetics, metabolic testing, islet autoantibodies, and assessment of β-cell mass [3]. Other parameters such as circulating microvesicles and exosomes appear to have a good predictive value in the near future.

However, T1DM has a strong genetic component, reflected by the observation that first-degree relatives have a higher risk compared with the general population. Three classes of class II HLA genes (DP, DQ, and DR) have the strongest association with T1DM. Certain genes such as HLA-DR3 and HLA-DR4 (DQ3.1 in particular) are highly susceptible antigens most associated with diabetes, and polymorphic variants of class II HLA genes determine 40–60% of genetic susceptibility [4].

Metabolic dysregulation precedes overt autoimmunity in T1DM [5]. The Finnish DIPP cohort study [6] showed that changes in serum metabolites were found only in the children who later developed T1DM. These changes included reduced serum succinate, lysophosphatidyl-choline (lysoPC), phospholipids, and ketoleucine, as well as elevated glutamic acid. These reactive lipid by-products are capable of activating proinflammatory molecules [7] that function as a natural adjuvant for the immune system [8].

Four biochemically characterized islet autoantibodies have been recognized – namely insulin autoantibodies (IAA), glutamic acid decarboxylase 65 (GAD65) antibody or (GADA), tyrosine phosphatases insulinoma antigen (IA)-2 and IA-2b (also known as ICA512), and the zinc transporter 8 (ZnT8) [9,10]. The presence of a single islet autoantibody is associated with relatively low risk on long-term follow-up (<5%), whereas the presence of two autoantibodies have a 68% risk and that of three autoantibodies have an estimate of more than 90% of developing T1DM within 5 years [9]. For T1DM prediction, a combination of GAD65 and IA-2 for primary

screening, followed by ICA and IAA testing, has been proposed [11]. However, autoantibodies can fluctuate or even completely disappear. In contrast, the American Diabetes Autoimmunity Study in the Young (DAISY) showed that about 95% of prediabetic children express anti-IAA, but only 50% express IAA at the time of diagnosis of T1DM [12]. This obviates the need for an adjuvant marker (e.g. biopsy) to facilitate the decision-making to start immunomodulatory therapy.

Currently, the monoclonal IgM antibody IC2, which specifically binds to the surface of β cells, might be the only reliable marker for noninvasive imaging and quantification of native β cells [13]. With sufficient amount of -β-cell mass at diagnosis, β-cell proliferating agents could be prescribed, whereas with significantly low amount of β-cell mass, other therapeutic options such as islets transplantation and stem cells transdifferentiation are more likely to be prescribed.

Histology of the pancreas

The pancreas is the main exocrine and endocrine gland of the digestive system. The exocrine part of the pancreas has closely packed serous acini. The secretions of the acini empty into ducts lined with a cuboidal epithelium, which are further transferred to stratified cuboidal in the larger ducts. The endocrine parts, islets of Langerhans, are clumps of secretory cells that contain its hormone-producing cells. Discovered in 1869 by German pathological anatomist Paul Langerhans at the age of 22 [14], the islets of Langerhans constitute ~1–2% of the mass of the pancreas. About one million islets are distributed throughout the pancreas of a healthy adult human, each of which measures about 0.2 mm in diameter; each islet is composed of 2000–4000 β cells [15]. The islets are supplied by up to three arterioles, which form a branching network of fenestrated capillaries, into which the hormones are secreted. The islet is drained by about six venules, which pass between the exocrine acini to the interlobular veins [16].

Hormones produced within the islets of Langerhans are secreted directly into the blood flow by (at least) five types of cells: α cells produce glucagon (15–20% of total islet cells), β cells produce insulin and amylin (65–80%), δ cells produce somatostatin (3 10%), pancreatic polypeptide cells (γ cells) produce pancreatic polypeptide (3–5%), and ε cells produce ghrelin (<1%). Islets can influence each other through paracrine and autocrine communication, and β cells are coupled electrically to other β cells (but not to other cell types). Electrical activity of pancreatic islets cells in intact islets differs significantly from the behavior of dispersed cells [16].

Immunohistochemistry of the extracellular matrix

The extracellular matrix (ECM) of the pancreatic islets separates the secretory cell compartment and provides specific signals to control the cell function and survival [17]. The ECM of the islet is formed mainly of two types: basement membrane (BM), which functions as a barrier limiting the transmembrane cross-movement of cells and molecules, and interstitial matrix (IM), which offers elasticity and flexibility to the islet cells. The BM is formed mainly of collagens, laminins, nidogens, and perlican. The nidogens stabilize the collagens and laminins, whereas the perlicans, which are a heparin-sulfate proteoglycans, by their large size (400–470 kDa) and side-chains, are known to act as a physical barrier to protect against the cell migration or cell invasion and can express adhesion ligands to prevent migrating leukocytes [18]. The IM layer is composed of fibrillar collagens, nonfibrillar collagens, and noncollagenous glycol proteins, such as fibronectin, tenascins, vitronectin, and chondroitin, or dermatan sulfate proteoglycans [19,20].

There has been some confusion about the existence of a peri-islet BM, in particular, due to reports of discontinuous staining of BM components around the islet periphery [21,22], incomplete analyses resulting from a limited range of BM-specific reagents, the close proximity of the acinar BM, and the presence of subendothelial BMs of the vasculature. The islet BM exists and, in the absence of enzymatic destructive insulitis, it is a continuous structure [16,23].

The proposed scenario of autoimmune diabetes

The initial step in the development of autoimmune diabetes is leukocytic extravasation and aggregation from the peri-islet vessels in a slowly progressive inflammatory process (Fig. 1). At this point clinical diabetes does not exist. Penetration of the islet BM by these leukocytes is crucial to proceed to destruction of the β cell, and as soon as the mass destruction approaches 70–90% of the islets, clinical diabetes supervenes [24]. Although leukocytic infiltration is widespread in the pancreatic tissue, few islets show BM destruction and not others, indicating that these are two different processes [24]. The lack of destruction of BMs of nearby acini and of intraislet capillaries, which have the same composition as the islet BM, suggests that destruction is site-specific and localized

to the immediate islet microenvironment [25]. Irving-Rodgers *et al.* [26] proposed that perlican, in particular, is essential for converting nondestructive autoimmunity to destructive autoimmunity and for the demise of islet β cells and the development of clinical symptoms of type 1 diabetes. Lymphocytic migration across a BM requires localized destruction by degradative enzymes [26]. No changes were observed in the composition of the peri-islet BM at or after the onset of type 1 diabetes, suggesting that it was not a change in composition that initiated or allowed leukocyte infiltration [27,28], but the composition of the islet BM that dictates the degradative enzymes needed to permit the migration of mononuclear cells across the islet BM [29]. These enzymes may include heparanases, which degrade heparansulphate, and metalloproteinase, which breakdown collagen [23,26]. Korpos É *et al.* [24] attributed this invasion to cathepsin expression associated with macrophages at the front of leukocyte penetrating the peri-islet BM of type 1 diabetes [23,25] and α-cells, glucagon secretors, and other pancreatic cells, which are a potential source of peri-islet BM components because of their tight association with the peri-islet BM in the reconstituted islets. Once inflammation had subsided, the peri-islet BM and underlying IM were shown to be reconstituted in mouse and human, indicating that the cells producing the peri-islet BM are not lost due to inflammation [24], which opens a new port for therapeutic modality to halt progression of autoimmune diabetes (Fig. 2).

Biopsy of the pancreas

Laparoscopic pancreatic biopsy has been reported to be a safe procedure in recent-onset type 1 diabetic patients [30,31]. T-cell–predominant infiltration to islets (insulitis) and hyperexpression of major histocompatibility complex class I antigens on islet cells were the two major findings observed in recent-onset type 1 diabetic patients. Anti-GAD and anti-IA-2 autoantibodies are significantly of high predictive value for abnormal histology in the islets [32,33]. The behavior of β-cell function could be predicted from the analysis of biopsy specimens [34,35]. A report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus referred to patients with insulitis and/or hyperexpression of major histocompatibility complex class I antigens in islets as those having (type 1A) autoimmune diabetes, and to patients without either of them as those having idiopathic (type 1B) [36,37].

Proposal and conclusion

In a genetically predisposed high-risk patient, an inciting factor(s) can be viral or bacterial infection either by itself or through an exosome from the distant infected cell [38], or through the B lymphocytes [39] reacting to the original infected cell. The β-islet cell introduces its antigenic epitope to the adaptive immune system, which in turn starts to form different types of anti-IAA. It is yet unclear whether the initial release of β-cell autoantigens is prompted by endogenous β-cell defects and/or an exogenous trigger, such as in hepatitis C viral infection [40].

Two important landmarks characterize the natural history of clinical diabetes: the nondestructive insulitis phase, which is characterized by leukocyte extravasation from the peri-islet blood vessels, and the destructive insulitis phase, which is characterized by leukocyte penetration of the islet BM. Between nondestructive and destructive phases, several years

Figure 1
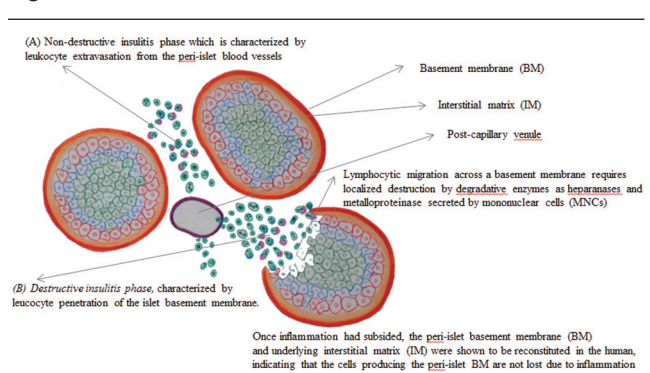
A flow diagram showing 'the proposed scenario of autoimmune diabetes'.

Figure 2
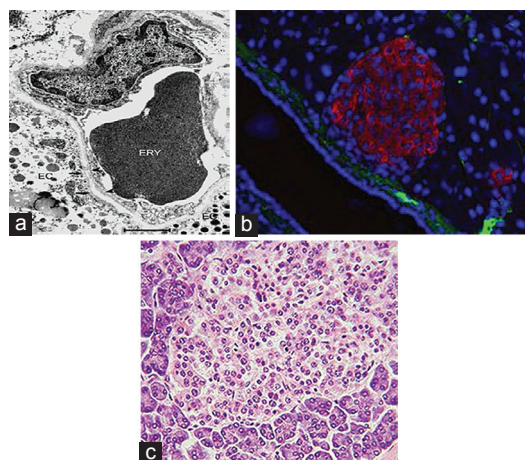
(a) Representative transmission electron microscopic (TEM) micrograph showing an erythrocyte (ERY) in a capillary blood vessel close to endocrine cells (EC). Arrowheads show the capillary basement membrane (BM), which is clearly distinct from BM (arrows) of the insulin-secreting and glucagon-secreting cells. Original magnification: ×20 000. Bar, 1 μm [23]. (b) Islet of Langerhans (mouse) in its typical proximity to a blood vessel; insulin in red, nuclei in blue [15]. (c) Islets of Langerhans [15].

could pass before the onset of hyperglycemia, giving a good window for therapeutic intervention [41]. Moreover, clinical diabetes will not present unless more than 90% of the islets have been destructed, which mostly takes months to occur. It sounds logical that only The detection of two or more of the islets' autoimmune antibodies can be considered diagnostic for autoimmune diabetes, and the reason for a fraction of patients having autoantibodies but not progressing to autoimmune diabetes can probably be attributed to the integrity of their immune system or because or the lack of other contributing factors to augment the action of these autoantibodies. It worthwhile to mention that the β cells are in direct contact not only with the islet BM but also with other four types of cells that may play an important role in the mechanism of BM destruction [42]. Some trials targeting the immune reaction either specifically, as with alum-formulated GAD (GAD-alum) vaccination, or nonspecifically, by targeting B cells such as anti-CD20 or T lymphocytes such as anti-CD3, have limited encouraging results possibly due to improper staging.

For lymphocytes to cross the BM, a localized degenerative destructive enzyme is required [23]. The composition of the islet BM dictates the degenerative enzymes needed to be produced by insulitis mononuclear cells to permit their migration across the islet BM. Leukocyte penetration of the peri-islet BM differs from leukocyte extravasation from blood vessels. This suggests that the ECM milieu influences the mode used by immune cells to infiltrate into tissues and raises novel possibilities for tissue-specific immunomodulatory therapies [43].

In conclusion, to date, none of the current predictive parameters of autoimmune diabetes are strong enough to start immunosuppressive drug therapy in a yet normal individual. Proper staging on a solid base, biopsy of the pancreas with immunohistochemistry assay, in a genetically predisposed high-risk patient with two or more autoantibodies will open up the gate for further histopathologic classification and hence allow better use of the already available therapeutic modalities and help in developing new ones and solving mysteries of autoimmune diabetes.

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