ZnT8 and type 1 diabetes

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Abstract. Zinc is essential for the proper storage, secretion, and the action of insulin and is transported from cytoplasm to insulin secretory granules in the pancreatic β-cells by SLC30A zinc transporters (ZnT). ZnT8 is specifically expressed in the pancreatic β-cells and has been identified as a novel target autoantigen in patients with type 1 diabetes. Autoantibodies to ZnT8 (ZnT8A) are detected in 50-60% of Japanese patients with acute-onset and 20% with slow-onset type 1 diabetes. Furthermore, humoral autoreactivity to ZnT8 is unique in terms of a key determinant, which is not reported on other islet autoantigens such as insulin, glutamic acid decarboxylase, or the protein tyrosine phosphatase–related molecules IA-2. Type 2 diabetes-associated nonsynonymous single nucleotide polymorphism in SLC30A8 (the gene of ZnT8), rs13266634 (Arg325Trp), modulates ZnT8A specificities thereby indicating that this amino acid substitution has the critical role in antibody binding. The humoral autoreactivity to ZnT8 depends on the clinical phenotype, which may provide clues to understand the role of this protein in the pathogenesis of type 1 diabetes.

Key words: Type 1 diabetes, Autoantigen, Autoantibody, Zinc transporter 8

GROWING evidence has accumulated regarding the association between the zinc homeostasis and the development of diabetes. Hypozincemia is a common feature in diabetes [1, 2] and zinc supplementation has been shown to inhibit the development of experimental type 1 diabetes in mice [3]. Type 1 diabetes is characterized by a destruction of pancreatic β-cells, resulting in absolute insulin deficiency causing hyperglycemia. The etiology of type 1 diabetes is not fully clarified, but it is well-recognized that major form of disease is associated with autoimmune-mechanisms (type 1A) and both genetic and environmental factors affect the onset of the disease [4]. Evidence for the autoimmune basis of type 1 diabetes includes i) lymphocytic infiltration around and into the islets (insulitis), ii) the appearance of autoantibodies to multiple islet autoantigens, iii) the presence of both MHC-linked and non-MHC-linked disease susceptibility genes, and iv) the increased propensity to develop multiple organ-specific autoimmune diseases. Antibodies to islet autoantigens are detectable many years before clinical onset of type 1 diabetes and can be used to identify individuals at increased risk of diabetes.

Although β-cell destruction in type 1 diabetes caused by antigen-specific T cells is the hallmark of type 1 diabetes, it is difficult to detect the presence of pathogenic T cells in vivo. Therefore, autoantibodies against multiple islet autoantigens are generally utilized as well-established predictive and diagnostic markers for the development of the disease [4]. During the past decades a number of relevant islet autoantibodies were identified, including autoantibodies to insulin (IAA), the 65-kDa isoform of glutamic acid decarboxylase (GADA), and the protein tyrosine phosphatase–related molecules IA-2 (IA-2A) and IA-2β/Phogrin (IA-2β/PhogrinA). Recently, zinc transporter 8 (ZnT8) was identified as a novel autoantigen based on a bioinformatics analysis focused on discovery of β-cell-specific proteins associated with the regulatory pathway of secretion [5]. In this paper, we review the current basic and clinical research findings on ZnT8 in type 1 diabetes.

Zinc and insulin biosynthesis

Zinc is an important structural component of many proteins. In most species, the pancreatic islet β-cell is known to contain high levels of zinc. Free zinc is con-
532 Kawasaki porters. The ZnTs act to decrease intracellular zinc levels by transporting zinc ion from the cytoplasm to extracellular spaces or lumen of organelles, while the Zips increase cytoplasmic zinc by transporting zinc ion from extracellular spaces or lumen of organelles to the cytoplasm (Fig. 2). It has been reported that 10 members of the ZnT family (ZnT1 to ZnT10) and 14 members of the Zip family (Zip1 to Zip14) were currently identified in human [9]. Most ZnT transporters have six transmembrane domains, cytoplasmic amino- and carboxy-terminal tails, whereas ZIP transporters have eight transmembrane domains, extracytoplasmic amino- and carboxy-terminal tails. Furthermore, zinc binds to the histidine-rich intracellular loop and the transmembrane domains form the zinc transport pore in both zinc transporters. In pancreatic β-cells, Zip4 is expressed on cell membrane and transports zinc ion into the cytosol. Furthermore, ZnT-5 is localized to the membrane of the trans-Golgi network and secretory granules and is essential for the proper storage, secretion, and the action of insulin [6]. After cleavage of the signal sequence from preproinsulin, the proinsulin is assembled in the Golgi apparatus into two zinc ions containing hexameric proinsulin. Then hexameric proinsulin is converted into the insulin hexamer by excision of the C-peptide by the action of proteolytic enzymes, known as prohormone convertases (PC1/3 and PC2), as well as the exoprotease carboxypeptidase E (Fig. 1). Therefore, zinc is essential for structural stability of the storage form of insulin, and the transport of zinc into the cytoplasm and insulin secretory granules is accomplished by zinc transporter. When the exocytosis of insulin from the β-cell occurs, zinc is co-secreted with insulin into the islet extracellular space during insulin exocytosis. Zinc is released from insulin when it reaches the higher pH of blood, and these zinc ions provide an “off-switch” for glucagon release from the α-cell during glucose deprivation by closure of the α-cell K_{ATP} channel [7, 8].

Zinc transporters

Zinc homeostasis is regulated by the ZnT (SLC30A gene family) and Zip (SLC39A gene family) zinc transporters. The ZnTs act to decrease intracellular zinc levels by transporting zinc ion from the cytoplasm to extracellular spaces or lumen of organelles, while the Zips increase cytoplasmic zinc by transporting zinc ion from extracellular spaces or lumen of organelles to the cytoplasm (Fig. 2). It has been reported that 10 members of the ZnT family (ZnT1 to ZnT10) and 14 members of the Zip family (Zip1 to Zip14) were currently identified in human [9]. Most ZnT transporters have six transmembrane domains, cytoplasmic amino- and carboxy-terminal tails, whereas ZIP transporters have eight transmembrane domains, extracytoplasmic amino- and carboxy-terminal tails. Furthermore, zinc binds to the histidine-rich intracellular loop and the transmembrane domains form the zinc transport pore in both zinc transporters. In pancreatic β-cells, the Zip4 is expressed on cell membrane and transports zinc ion into the cytosol. Furthermore, ZnT-5 is localized to the membrane of the trans-Golgi network and secretory granules and is essential to buffer cytosolic zinc and to transport zinc to the Golgi apparatus. ZnT8 is localized to insulin containing secretory granule membrane and transports zinc ion from the cytosol into the vesicles [10]. In contrast, in pancreatic α-cells, Zip1, Zip10, and Zip14 are the most abundantly expressed Zips and ZnT4, ZnT5, and ZnT8 the dominant ZnTs [11]. Although several discrepancies

Fig. 1 A schematic representation of the biosynthesis and processing of insulin
After cleavage of the signal peptide from preproinsulin in the endoplasmic reticulum, the proinsulin is assembled in the Golgi apparatus into two zinc ions containing hexameric proinsulin. Then hexameric proinsulin is converted into the insulin hexamer by excision of the C-peptide by the action of proteolytic enzymes, known as prohormone convertases (PC1/3 and PC2), as well as the exoprotease carboxypeptidase E. PC1/3 and PC2, prohormone convertases 1/3 and 2; CPE, carboxypeptidase E; ER, endoplasmic reticulum.
ZnT8 and type 1 diabetes

On the metabolic phenotypes are present among ZnT8-knockout mice colonies, the dense core of Zn-insulin crystals is almost completely lost from β-cells and abnormalities in glucose tolerance, insulin processing and secretion are demonstrated, indicating the indispensable role of ZnT8 in β-cell function [12-15].

Human SLC30A8 gene is located to chromosome 8 at the position q24.11 which contains 8 exons, spanning 37kb and encoding a 369 amino acid ZnT8 protein. Human ZnT8 is reported to share 80 and 76% amino acid identity with mouse and rat ZnT8 and closely related to ZnT2, ZnT3, and ZnT4, all of which are involved in secretory/synaptic vesicles transport and lysosomal/endosomal zinc storage in different cells [16]. Recent genome-wide association studies analyzing the susceptible/protective loci for type 2 diabetes revealed that a nonsynonymous single nucleotide polymorphism (SNP) in SLC30A8 (rs13266634 C>T) which changes from arginine (R) to tryptophan (W) at position 325 is associated with type 2 diabetes. Furthermore, major epitope(s) for ZnT8A lie within the cytoplasmic domain of the molecule (aa268-369) and R325W is a key determinant of humoral autoreactivity to this protein.

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quent study reported the association between 325R and the increased plasma glucose and reduced insulin secretion [18]. However, this SLC30A8 SNP does not alter the risk of type 1 diabetes, even in patients diagnosed with type 1 diabetes at a very young age (<5 years) or by class II HLA genotype [19].

**ZnT8 and immune system**

Zinc modulates the expression of more than a hundred genes within the immune cells and it is an essential trace element for immune function [20]. Zinc has been shown to be required for the innate and the adaptive immune reactions, and it is especially important for the generation of proinflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor-α after lipopolysaccharide or other antigen stimulations and the development of T cells [21, 22]. Thus zinc deficiency induces thymic atrophy, lymphopenia, and suppression of cytolytic T cell responses, natural killer cell activity, and delayed-type hypersensitivity reactions [23]. Zinc also protects pancreatic β-cells from cytokine-induced destruction, which is observed in patients with type 1 diabetes, but also in type 2 diabetes. IL-1 β is a cytokine mainly derived from macrophage that participates in the regulation of inflammatory immune responses and is involved in the inhibition of glucose-stimulated insulin release as well as in islet cell destruction in both type 1 and type 2 diabetes. It has been reported that these effects are mediated by the activation of the transcription factor nuclear factor kappa B, which is negatively regulated directly by zinc or via induction of a zinc-finger protein A20 by zinc [3, 24].

In addition, ZnT8 is reported to be associated with β-cell survival. The overexpression of ZnT8 in β-cells increases the zinc content and protects β-cells from apoptosis related to zinc depletion [25]. However, Efegjord and coworkers reported that ZnT8 expression in β-cell is downregulated by IL-1 β and the overexpression of ZnT8 is more susceptible to IL-1 β-induced apoptosis [26]. The authors pointed out the possibility that ZnT8 might be expressed at an additional subcellular locale that plays a more active role in regulating cell viability.

**ZnT8 autoantibodies in three forms of type 1 diabetes**

It is well-known that there are at least three sub-types of type 1 diabetes in Japan, acute-onset, slow-onset, and fulminant type 1 diabetes [27]. The onset of type 1 diabetes frequently occurs before 20 years of age, but disease manifestation is also common in adults. In childhood and adolescent-onset type 1 diabetes, about 90% have the acute-onset form, and the remainders belong to the slow-onset form. Fulminant type 1 diabetes is rare in the childhood. Conversely, about two-thirds of adult-onset patients have the slow-onset form, and ~20% of those with ketosis or ketoacidosis fall into the category of fulminant type 1 diabetes [27]. In 2007, Hutton and coworkers found that ZnT8 is a major autoantigen in type 1 diabetes [5]. Previous studies have reported that autoantibodies to ZnT8 (ZnT8A) are detected in 60-80% of Caucasian patients with type 1 diabetes [28]. With radioligand binding assay using in vitro transcribed/translated 35S-labeled ZnT8 protein, we identified ZnT8A in 58% patients with acute-onset and in 20% with slow-onset type 1 diabetes in the Japanese population [28]. Furthermore, the prevalence of ZnT8A was inversely related to the onset age with the highest prevalence of 70% in patients aged <10 years (Fig. 4). Thus, ZnT8A identify heterogeneity in the age of diabetes onset and are good markers of childhood-onset type 1 diabetes. In contrast, none of sera from fulminant type 1 diabetes were reactive to ZnT8 construct, indicating that ZnT8A are not a diagnostic marker for fulminant type 1 diabetes (Table 1).
ZnT8 and type 1 diabetes

Patients are positive for ZnT8A among type 1 diabetic patients previously classified as autoantibody-negative on the basis of other anti-islet autoantibodies such as IAA, GADA, and IA-2A.

We and others recently reported that the amino acid encoded by a common polymorphism in human ZnT8 at aa325 (rs13266634C>T, R325W) is a key determinant of humoral autoreactivity to ZnT8 [29, 30]. Among patients with rs13266634CC genotype only 5% had ZnT8-325W-specific antibodies, 42% had ZnT8-325R-specific antibodies, and 32% had ZnT8-325R/ZnT8-325W dual reactivity. Conversely, 73% of patients with TT genotype had ZnT8-325W-specific antibodies, no patients had ZnT8-325R-specific antibodies, and 13% had ZnT8-325R/ZnT8-325W dual reactivity. Ninety percent with CT genotype reacted with either ZnT8-325R or ZnT8-325W constructs (Table 2). These results indicate that SLC30A8 genotype is an important determinant of ZnT8A specificity and it is critical to use both ZnT8 polymorphic variants for the ZnT8A determination.

Conclusion

Zinc is necessary for structural stability of the storage form of insulin as well as physiological functioning of the innate and adaptive immune system. In pancreatic β-cells, the transport of zinc into the cytoplasm and insulin secretory granules is accomplished by ZnT8, which is involved in the etiology of both type 1 diabetes and type 2 diabetes. The SLC30A8 locus confers susceptibility to type 2 diabetes and ZnT8 was recently identified as a major target of autoantibodies, but also an autoantigen of disease-associated autoreac-

Table 1 Prevalence of autoantibodies to multiple islet autoantigens in Japanese patients with classical and fulminant type 1 diabetes

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Classical type 1 diabetes (n=114)</th>
<th>Fulminant type 1 diabetes (n=85)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GADA</td>
<td>82%</td>
<td>9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IA-2A</td>
<td>58%</td>
<td>4%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IAA</td>
<td>55%</td>
<td>6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ZnT8A</td>
<td>50%</td>
<td>0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GADA only</td>
<td>14%</td>
<td>8%</td>
<td>NS</td>
</tr>
<tr>
<td>IA-2A only</td>
<td>2%</td>
<td>4%</td>
<td>NS</td>
</tr>
<tr>
<td>IAA only</td>
<td>2%</td>
<td>5%</td>
<td>NS</td>
</tr>
<tr>
<td>ZnT8A only</td>
<td>2%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>≥ 1Ab</td>
<td>94%</td>
<td>18%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥ 2Abs</td>
<td>75%</td>
<td>1%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥ 3Abs</td>
<td>54%</td>
<td>0%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

More than 90% of autoantibody-positive patients with fulminant type 1 diabetes are single autoantibody-positive. GADA, glutamic acid decarboxylase 65 autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; IAA, insulin autoantibodies; ZnT8A, zinc transporter 8 autoantibodies ≥ 1Ab, patients positive for one or more of GADA, IA-2A, IAA, or ZnT8A; NS, not significant

ZnT8 autoantibodies as a diagnostic tool for type 1A diabetes

Measurement of a combination of autoantibody markers has been suggested as a useful tool for determining type 1A (autoimmune) diabetes. With the combination analysis using four biochemically characterized anti-islet autoantibodies, 94% of Japanese patients with type 1 diabetes are classified as type 1A diabetes [28] (Table 1). However, the clinical utility of ZnT8A is limited over testing GADA, IA-2A, IAA, or ZnT8A; i.e. few

<table>
<thead>
<tr>
<th>SLC30A8 rs13266634 genotype</th>
<th>n</th>
<th>Positive for autoantibodies to</th>
<th>Both negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ZnT8-325R only</td>
<td>ZnT8-325W only</td>
</tr>
<tr>
<td>CC</td>
<td>19</td>
<td>8 (42%) 1 (5%)</td>
<td>6 (32%)</td>
</tr>
<tr>
<td>CT</td>
<td>10</td>
<td>3 (30%) 0 (0%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>TT</td>
<td>15</td>
<td>0 (0%) 11 (73%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&lt;0.05  &lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C carrier</td>
<td>29</td>
<td>11 (38%) 1 (3%)</td>
<td>12 (41%)</td>
</tr>
<tr>
<td>T carrier</td>
<td>25</td>
<td>3 (12%) 11 (44%)</td>
<td>8 (32%)</td>
</tr>
</tbody>
</table>

Data were analyzed in 44 patients with type 1 diabetes positive for ZnT8 autoantibodies. Statistics were based on a 3 x 2 chi-square test of genotypes; NS, not significant

Table 2 Autoantibody response to ZnT8 aa325 variants in patients with type 1 diabetes stratified by SLC30A8 rs13266634 genotype
tive T cells in type 1 diabetes [31]. The role of ZnT8 in the pathogenesis of type 1 diabetes is still unknown. Fine molecular analysis of antigenic determinants of ZnT8 should provide a strategy for autoantibody measurement in subjects to promote the early diagnosis of type 1A diabetes and for antigen-specific therapeutic intervention to arrest the progression of the disease.

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References


