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Autoantibodies in Diabetes MellitusEka Herawati^{1,2}, Ardian Susanto^{1,3}, Christina Noventy Sihombing^{1,3}¹Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Bandung, Indonesia²Prodia Diagnostic Line, Cikarang, Jawa Barat, Indonesia³Prodia Clinical Laboratory, Bandung, Indonesia

Based on American Diabetes Association (ADA), diabetes can be classified into the following general categories: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus (GDM) and specific types of diabetes due to other cause. Obesity is by far the main underlying factor causing T2D and its pathological potential lies in obesity-associated insulin resistance, activation of innate immunity and chronic low-grade inflammation. When tissue inflammation induced, tissue destruction occurs, 'self' antigens, which are generally not accessible to T cells, can be released from the affected tissues and promote autoimmune activation. The 4 major autoantibodies are islet-cell cytoplasmic autoantibodies (ICA), glutamid acid decarboxylase antibody (GADA), islet antigen-2 antibody (IA-2A) and insulin autoantibodies (IAA). In addition, ZnT8A has recently been found to predict T1D. ZnT8 is contained in the islets of Langerhans, with the highest expression is in β cells of the pancreas. ZnT8A measurements simultaneously with GADA, IA-2A and IAA achieve rates of 98% detection for onset level of autoimmune diabetes. Presence of antibodies in T2D also shows the potential serious complications compared with T2D without antibodies. The combination of GADA, IA-2A and ZnT8A can be suggested as the most powerful and cost-effective diagnostic approach in patients with T1D.

Keywords: autoantibody, autoimmune, diabetes mellitus, ICA, GADA, IA-2A, IAA, ZnT8A**Introduction**

Diabetes mellitus is a disease characterized by hyperglycemia and is caused by absolute or relative insulin deficiency, sometimes associated with insulin resistance.^{1,2} Diabetes also known as a complex chronic disease that requires ongoing medical care with a strategy of multifactorial risk reduction beyond glycemic control.³ Based on American Diabetes Association (ADA), diabetes can be classified into the following general categories: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus

(GDM), specific types of diabetes due to other causes, *e.g.*, monogenic diabetes syndromes, diseases of the exocrine pancreas, and drug- or chemical-induced diabetes.⁴

T1D known as juvenile diabetes, caused by a chronic autoimmune disorder where targeted immune response by both T and B cells.⁵ The pancreatic β -cells that produce insulin are the target of immune attack by both T and B cells leading to destruction of insulin production in the islets of the pancreas.^{6,7} T1D is an autoimmune disease that occurs when the body can no longer produce insulin due to

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the destruction of the insulin producing β -cells of the islet of Langerhans in the pancreas.⁸

Autoimmunity is a complex multifactorial process which are defined by the loss of self-tolerance and chronic excess of reactivity B and T cells due to release of the danger signals. The pathogenesis are caused by multiple factors, both genetic and environment. The involvement of autoimmune indicates by the persistent presence of the circulating autoantibodies or self-reactive T cells, the lymphocytic infiltration in the target organs.^{9,10}

Autoantibodies can be formed due to the breakdown in tolerance. For autoantibodies to appear, the autoantigen must become available to the immune system and the immunization event occurred. Immunization that results in an immunoglobulin (Ig)G autoantibody response requires switching of B-lymphocyte class, and therefore cluster of differentiation (CD)4 T cells must be involved in addition to naive mature B cells.¹¹

The autoantibodies can be detected before and at time of clinical diagnosis of disease. Although the role of autoantibodies in the pathogenesis is debated, their presence indicates a dysregulation of the humoral immune response.¹⁰ Mechanisms regulating autoantibodies in T1D are not well understood as well. Other than insulin and carboxypeptidase H, all islet antigens are intracellular and are not normally secreted or expressed on the surface of the cell. The release of intracellular antigens is possibly the result of cell mediated autoimmune damage to cells, which permits such otherwise sequestered self antigens access to naive mature B lymphocytes and naive CD4 T cells. However, if the cells were to die, β -cells death might have then led to subsequent islet autoantigen immunization.¹²

The pancreatic islets respond to insulin resistance by enhancing their cell mass and insulin secretory activity. However, when the functional expansion of islet β -cells fails to compensate for the degree of insulin resistance, insulin deficiency resulted and ultimately T2D develop.¹³

Obesity is by far the main underlying factor causing T2D and its pathological potential lies in obesity-associated insulin resistance, activation of innate immunity and chronic low-grade inflammation.^{14,15} Obesity and chronic overfeeding lead to metabolic and adipocyte stress. Stressed adipocytes secrete pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6 or tumor necrosis factor (TNF)- α , which activate T cells, B cells and macrophages. Chronic inflammatory state in T2D is characterized by an increased production of cytokines, most notably IL-1 β , which destroy β cells. When

tissue inflammation induced, tissue destruction occurs, 'self' antigens, which are generally not accessible to T cells, can be released from the affected tissues and promote autoimmune activation. Hyperglycaemia contributes to the increased of several β -cell antigens, such as glutamid acid decarboxylase antibody (GADA), increasing the vulnerability of β cells to autoantibodies such as anti-GAD, the most frequently detected autoantibodies in phenotypic T2D.⁹ Persistent GADA can be occurred between latent autoimmune diabetes in adult (LADA) and T2D.^{16,17}

The 4 major autoantibodies are islet-cell cytoplasmic autoantibodies (ICA), GADA, islet antigen-2 antibody (IA-2A) and insulin autoantibodies (IAA). ZnT8 islet autoantibody (ZnT8A) may further improve the value of islet autoantibody testing.¹⁸⁻²⁰ The presence of autoantibodies against islet cells and the enzymes of pancreatic β islet cells can also be used as predictors of the onset of T1D in patients with LADA.²¹

ICA

The availability of an epifluorescent light source was the technical breakthrough that permitted the development of indirect-immune-fluorescent (IIF) technique for ICA. Antibodies react with all endocrine islet cells.²² The lowest significant positive for ICA is 10 Juvenile Diabetes Foundation (JDF) units. ICA react against a sialoglycoconjugate, an insulinoma associated autoantigen, and GAD. ICA are the most difficult islet autoantibodies to measure because ICA assays are subject to variations in the pancreatic tissue and technical aspects including antibody conjugation, incubation time, humidity, and biological scientist interpretation.^{12,20,22}

GADA

GADA first identified through immunoprecipitation islet cell proteins labeled with ³⁵S Methionin dissolved in the serum of patients with T1D.¹⁷ GAD is an enzyme that catalyzes the formation of gamma-amino butyric acid (GABA) from glutamate.¹⁹ GABA is a major inhibitor neurotransmitter in the brain, it is stored in synaptic microvesicel. In the pancreatic islet β cell GABA is also produced in large number, stored in synaptic-like microvesicle, and have a paracrine or autocrine activity in pancreatic islet cells. GABA activity mechanisms in pancreatic β cells are not fully understood.²⁰ GABA is a ligand for a GABAergic receptor that located on

the surface of cell membrane. GABA receptor have three types of receptors, GABA-A and GABA-C receptors, which are the receptors of ligand-gated Cl⁻ ion channels, and GABA-B receptor is a ligand gated Ca₂⁺/K⁺ ion channel. GABA-A receptors (GABAAR) and GABA-B Receptors (GABABR) are expressed on the surface of pancreatic β cells.²² Activation of GABA receptors in pancreatic β cells resulting in an increased release of insulin, which has a protective and regenerative effect on pancreatic β cells, and decreases apoptosis of pancreatic β cells.²³

GAD has two isoforms, 67-Kd GAD (GAD67) and GAD65 Kd (GAD65).⁴ GAD67 is an enzyme that is always active and responsible in the basal concentration of GABA. GAD67 is encoded by genes located at chromosome 2q31 and is mostly found in neuronal cells. While GAD65 active transient, where activation depending on the needs extra GABA, GAD65 are encoded by genes located at chromosome 10p11. GAD65 has 585 amino acids and at most been recorded on pancreatic β cells.²⁴

The presence of GAD65 antibodies causes pancreatic β islet cells can not produce insulin adequately.²⁵ In patients with T1D, more than 90% found one or more antibodies directed against β islet cells of the pancreas, one of which is the GAD65 antibodies.²¹

IA-2A

Most patients with T1D have antibodies against tryptic fragments of 40- and/or 37-kDa derived from islet cell membrane protein which is not yet known and is a different protein from GAD.²⁶ The protein is IA-2, previously known as ICA-512. IA-2 are the main targets of islet cell autoantibodies. Protein is found also in these nerve tissue, encoded by a gene located at chromosome 2q35.²⁷ This gene encodes a 979 amino acid, enzymatic trans-membrane protein that is inactive, and the protein tyrosine phosphatase (PTP)-like-molecule. This antigen has no enzymatic activity for several substitutions in highly conserved sites and expressed mainly in neuroendocrine cells such as the central nervous system as well as in the islets of Langerhans cells. The residue intracellular antigen is anchored on the membrane of the insulin secretory granule, and the dominant antibody will attack the intracellular domain (amino acids 601-979) and this only occurs when there is a damage cells. The function of the antigen is not known with certainty. Experiments on mice showed the role of IA-2 in insulin secretion.²⁸

PTP-like molecule called IA-2 β or phogrine resemblance in many respects to IA-2, especially in the location and the intracellular domain (74% identical). Immunoprecipitation of cell lysates insulinoma with T1D sera produce a 64kDa protein, and after treatment with trypsin, fragments of 37 and 40 kDa can be resulted. Additional studies revealed that IA-2 is the precursor of the 40kDa and IA-2 β of the 37kDa molecule.²⁹

IA-2A can be the complement of GADA test, since more than 90% of children have antibodies to at least one of these proteins at diabetes onset. IA-2 and GAD make the predominant, but not exclusive, contribution to ICA-reactivity. The presence of IA-2A in particular, which is often associated with other antibodies, confers a higher risk of rapid progression toward clinical onset than multiple antibodies.³⁰ It is suggested that the production of IA-2A coincides with a critical switch in disease progression, where the intracellular domain of IA-2 may only become visible to the immune system at the outer cell surface in the case of β cell damage or dysfunction.³¹

IAA

IAA may appear in the beginning of life, IAA is the first autoantibodies that often arise during the prediabetic phase and they are usually detected in young children in diagnosis of T1D, so that it can be used to predict the onset of T1D.³⁰⁻³³ But not all IAA will develop into T1D. Children who do not develop autoantibodies to several β cell antigen, rarely will develop into T1D. For the initial risk assessment, therefore, it would be useful to identify children with IAA-positive who will develop to diabetes.³²

Knowing affinity can be identified IAA-positive children who are more likely to become diabetic. Studies have been conducted to determine if an affinity useful as a marker of risk stratification of T1D in IAA-positive children from the general population. IAA affinity was determined by competitive binding with increased concentrations of ¹²⁵I insulin and insulin cool with cold proinsulin in sera from 46 IAA-positive children identified in Karlsburg Risk Type 1 Diabetes Study of normal school children population in the north-east German. The result showed higher affinity IAA on 24 children who develop autoantibodies island or diabetes compared to 22 children who did not develop autoantibodies island or diabetes. In conclusion IAA affinity measurements provide strong identification of IAA and associated with a higher risk of diabetes. Research shows high-affinity IAA

and proinsulin reactivity characteristics of the IAA in first-degree relatives who developed autoantibodies islet and T1D.³²

IAA level from time to time is not affected by oral insulin. The level of autoantibodies is only one aspect of the autoimmune response. Factors such as IgG subtypes, IAA affinity and T cell response to insulin are also important factors. For that we need further evaluation.³³

IAA level inversely correlated with age. The highest level seen in diabetic children under the age of 5 years. In patients with T1D, insulin antibody comprises mainly IgG1 antibodies, whereas IgG3 antibodies were more common prior to the initiation of exogenous insulin. In children of parents with T1D, IgG1 was mostly detected while IgG4 was detected in some cases. The presence of IgG4 was not associated with protection from clinical disease. There are some heterogeneity in the response of IAA isotype, but mostly the response IgG1-IAA is the first to appear, while IgG4-IAA show up late.³⁴

ZnT8A

In addition to ICA and biochemical autoantibodies (BAA) to insulin IAA, GAD65A, and IA-2A, ZnT8A has recently been found to predict T1D.³⁵⁻⁴¹ ZnT8 is contained in the islets of Langerhans, with the highest expression is in β cells of the pancreas. ZnT8A expression may not occur until damage to β cells enough to make ZnT8 immunological visible.^{21,28} ZnT8 is a trans-membrane protein 369 amino acids, encoded by the SLC30A8 at chromosome 8q14.11. ZnT8 contains six trans-membrane domain, amino and carboxy-terminal cytoplasmic tail. ZnT8 more specifically expressed in insulin secretion granules, containing than GAD65 and IA-2. ZnT8 serves to maintain intracellular zinc homeostasis, which modulates insulin biosynthesis process, storage, and secretion. Therefore, antibodies to ZnT8 may affect some of the important processes.³⁹⁻⁴³

More than 60% of new cases were diagnosed positive for ZnT8A, and 4% of the cases were positive only on ZnT8A. ZnT8A was detected in about 70% of patients at diabetes onset.³⁷ ZnT8A then can be used as markers and prediction of T1D. The percentage of newly diagnosed cases of T1D and positive ZnT8A (according to the size titer) was positively correlated with older age at diagnosis of T1D in children.³⁵ ZnT8A prevalence tends to be higher in children than teenagers, but the difference was not statistically significant. The relationship between ZnT8A

and actual age controversial because on the Chinese children, the prevalence ZnT8A lower in older patients, while in a very large study in Finland, ZnT8A positivity was associated with older age at diagnosis. Prevalence ZnT8A remained substantially stable in patients up to four years of clinical diagnosis, but then showed a significant decrease, and ZnT8A finally fixed at about 34% of 5 years-diagnosed patients.⁴³

The ZnT8A are appear between 60-80% of Caucasian patients with T1D at onset and together with IAA, GAD65, and IA-2A, can confirm the diagnosis of autoimmune diabetes in more 94% of subjects. Genetic risk in first-degree relatives T1D cases followed prospectively, ZnT8A appear at an average age of 3-4 years and generally persist until the onset of clinical disease.⁴⁴ ZnT8A are positively detected in 530 children (73%). Positive for ZnT8A at diagnosis appears to reflect a more aggressive disease process before and after the diagnosis.³⁸

In families with T1D were positive for one or more of the standards relating to diabetes BAA (IAA, GAD65A, or IA-2A). ZnT8A test can be useful information about the risk of diabetes. A direct link between the number of positive autoantibodies and the risk of diabetes, that ZnT8A gradually adding risk compared to standard BAA and ICA.³⁶

ZnT8A measurements simultaneously with GADA, IA-2A and IAA achieve rates of 98% detection for onset level of autoimmune diabetes. This approach is needed to detect pre-diabetes in children. ZnT8A associated with other autoantibodies, can improve diagnostic performance as a whole and a positive predictive value of laboratory.⁴³ When introducing ZnT8A as a complement of the current biomarker, the overall diagnostic performance significantly improved the serological test. The combination of GADA, IA-2A and ZnT8A as the most powerful and cost-effective diagnostic approach in patients with T1D.⁴³

The relation between autoantibody and T2D

Islet autoimmunity may be more common than previously thought and may be an important contributor to the progressive decline in β -cells function observed in phenotypic T2D patient.¹⁰ The identification of these autoantibodies in elderly patients with slowly progressive manifestation of diabetes led to the introduction of a distinct clinical entity termed LADA, which combines features of both T1D and T2D. The autoantibody cluster differs in patients with LADA from patients with T1D, but their

presence indicates steady progression towards β -cell death and subsequent need for initiation of insulin treatment in a shorter period of time compared to autoantibody-negative T2D patients. Autoimmune aspects in T2D are not solely restricted to autoantibodies and thus LADA. They include the self-reactive T cells or defects in regulatory T cells (Tregs), which have been detected in autoantibody-negative T2D patients as well.⁹

T2D is the consequence of a defective insulin response to increased insulin needs that result from insulin resistance, but evolve with time to progressive loss of insulin secretion. The respective part of a functional defect and that of a decrease in the β -cells mass remains an open issue in T2D.⁴⁴ Autoimmune diabetes may masquerade as T2D in adults as well. It has been known since the early days of ICA measurements that diabetic patients who failed oral hypoglycemic agents often had ICAs. Many features of insulin release and metabolic phenotypes in LADA patients distinguish them from both T1D and T2D patients.³¹ It is also known that islet autoimmunity progresses more rapidly in the context of “overload” of the β -cell due to increased insulin resistance.⁴⁵ However, the type of autoantibody in T1D and T2D are the same. The antibody in patients with T2D make consideration whether the future will require exogenous insulin as in T1D.⁴⁶ Presence of antibodies in T2D also shows the potential serious complications compared with T2D without antibodies. In addition, adiposity is a risk factor in the development of autoimmune T2D.⁴⁷

Autoantibodies in GDM

The prevalence of gestational diabetes in the world is about 7% of the entire population of pregnant women in the world.⁴⁸ Some of them have such antibodies associated T1D.⁴⁹ Pregnancy itself is a risk of the outbreak of T1D. Where, the pregnancy is the combination of physiologic changes of pregnancy-related in metabolize glucose and immune system regulation.⁵⁰⁻⁵¹ Women with GDM autoimmune generally do not have such characteristic in T2D, they typically do not obesity, occurs at a young age (<30 years).⁴⁵ They looks like be in need of treatment with insulin beyond diet modification.⁴⁹

Antibodies against GAD and IA-2 are antibodies most widely reported. Then antibodies against ZnT8 also found in some women with GDM.⁵² Several studies have reported that the frequency of antibody ZnT8 almost as high as GAD antibodies in clinical onset T1D, but often in combination

with GAD and/or IA-2 antibodies.³⁷ Autoimmune antibody screening is recommended in women who are at risk of GDM. It is certainly aimed to anticipate the development of GDM, whether toward T2D or T1D.⁵³

Conclusion

The assumption that the pathogenesis of T2D also encompasses autoimmune aspects is recognized increasingly, based on the presence of circulating autoantibodies against β cells, self-reactive T cells, also on the glucose-lowering efficacy of some immunomodulatory therapies in T2D. The combination of GADA, IA-2A and ZnT8A can be suggested as the most powerful and cost-effective diagnostic approach in patients with T1D.

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