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REVIEW ARTICLE

Islet autoantibodies in disease prediction and pathogenesis

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Abstract
Type 1 diabetes (T1D) is now predictable by measuring specific islet autoantibodies (IAbs). Almost all children who developed multiple IAbs will progress to T1D with time, while individuals with single IAb have a very low risk although it is an important earlier biomarker. The poor prediction of single IAb has been found to be associated with IAb affinity. Majority of single IAb generated in current standard IAb radio-binding assay (RBA) are of low affinity, which have been demonstrated low risk in T1D development. New generation of nonradioactive IAb assay with electrochemiluminescence (ECL) technology has been shown to discriminate high-affinity from low-affinity IAbs and greatly improve sensitivity and disease specificity. With a high-affinity IAb assay, like ECL assay, single IAb will be expected to be a reliable biomarker for T1D early prediction. Although appearance of IAbs is most reliable biomarkers for T1D, there are no direct evidences that IAbs contribute to β-cell damage. With recent studies on ZnT8, a merging protein on β-cell surface membrane associated with insulin secretion, a subclass of ZnT8 autoantibodies directed to extra-cellular epitopes of ZnT8 on β-cell surface has recently been identified in T1D patients and these cell surface autoantibodies have been found to appear very early, before other IAbs. These findings lead us to a hypothesis that the immunogenic epitopes on β-cell surface might be early targets for autoimmune disease and IAbs to cell surface epitopes might be involved in β-cell destruction, which will change the paradigm of IAbs in T1D pathogenesis.

Keywords Autoantibodies · Type 1 diabetes · ECL assay · Prediction · Pathogenesis

The incidence of type 1 diabetes (T1D) is worldwide, increasing 3–5% annually [1] with rates doubling every 20 years [2, 3], especially in young children. Type 1 diabetes is a chronic autoimmune disease that causes an immune-mediated loss of functional pancreatic β-cell mass, which leads to symptomatic diabetes and lifelong insulin dependence [4–6]. Early prediction of T1D and finding ways to prevent the disease are great challenges to overcome. Islet autoantibodies (IAbs) are currently used as the most reliable biomarkers. T1D is characterized by, in peripheral blood, specific IAbs for insulin (IAA), glutamic acid decarboxylase (GADA), insulinoma-associated protein 2 (IA-2A), and zinc transporter 8 (ZnT8A). The IAbs usually appear years before overt clinical disease. Multiple national and international T1D clinical trials like the Environmental Determinants of Diabetes in the Young (TEDDY) study have been trying to identify environmental factors involved in initiating islet autoimmunity and multiple factors have been found in the study recently [7] including 25(OH) vitamin D [8]. In 2015, Juvenile Diabetes Research Foundation (JDRF) and American Diabetes Association (ADA) presented a scientific statement for staging presymptomatic T1D. Stage 1 is defined as the appearance of islet autoimmunity with normoglycemia. This represents individuals who have developed two or more T1D-associated IAbs, but are normoglycemic [9]. Stage 2 includes individuals with two or more IAbs, who have progressed to develop glucose intolerance, or dysglycemia, from further loss of functional β-cell mass. Stage 3 represents the manifestation of typical clinical symptoms and signs of diabetes. In the US, 1.4 million people have clinical T1D and many others have multiple IAbs or stage 1 T1D with normal glucose homeostasis. Of the latter, 84% will progress to clinical diabetes within 15 years, with a remarkable consistency across all populations [10].

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Screening IAbs for presymptomatic T1D, with normal glucose homeostasis, are necessary and important for prediction, prevention, better clinical treatments, and mechanistic studies for disease development. Highly sensitive and specific IAb assays play an essential role to mark the initiation of islet autoimmunity with accurate timing and provide high predictive values with disease specificity. The number of detectable IAbs correlates with the risk of diabetes. In a high-risk birth cohort, noted above, diabetes risk by 15 years of age was 12.7%, 61.6%, and 79.1% in children with one, two, and three IAbs, respectively [10]. In TEDDY study, the 5-year risk of developing symptomatic diabetes was 11%, 36%, and 47%, respectively, in those with one, two, and three IAbs [11]. However, with the progression of T1D, IAbs usually appear sequentially, not simultaneously [12], for the majority of children who were longitudinally followed from birth. They would begin with all IAbs negative, to a single IAb positive, then to two or more IAbs positive, before they finally progressed to clinical T1D in both prospective studies, Diabetes Autoimmunity Study in the Young (DAISY) and TEDDY. The TEDDY study depends on the sensitivity and validity of IAb assays used to pinpoint the "seroconversion" of islet autoimmunity. The efficacy of prevention studies to reverse or delay islet autoimmune progression to clinical T1D at its early stages, e.g., The Pathway to Prevention of TrialNet-T1D, relies on the disease specificity of IAb assays used to identify disease-relevant IAbs. The first positive IAb acts as a primary 'seroconversion' marker for the very beginning of islet autoimmunity to help identify potential environmental triggers and to plan early-stage prevention studies. Thus, a highly sensitive assay, with a high disease specificity that accurately detects T1D-relevant IAbs at the right time, is essential.

The current 'gold' standard assay, the radio-binding assay (RBA), for IAA, GADA, IA-2A and ZnT8A has been greatly improved through laboratory proficiency programs (IASP, Islet Autoantibody Standardization Program) and NIDDK-sponsored harmonization efforts. However, single IAb positivity (a single GADA or a single IAA in most cases) by RBA among relatives of patients with T1D and the general population has a very low predictive value. The majority of these single IAbs will disappear during follow-up and behave as a 'transient' positive. With such an uncertainty, single IAb positivity is difficult to be accepted as a reliable disease marker for early T1D staging and individuals with a single IAb are not creditable to be recruited into clinical trials for prevention studies. For the last several years, we have developed and extensively validated nonradioactive IAb assays using electrochemiluminescence (ECL) detection. ECL is superior to RBA, showing higher sensitivity and higher disease specificity in four independent clinical trial studies: DAISY, TEDDY, Type 1 Diabetes TrialNet, and Autoimmune Screening for Kids (ASK) [11–13]. These analyses have demonstrated that ECL-IAA is superior to RBA-IAA in detecting early seroconversion of islet autoimmunity. In DAISY [13], ECL-IAA detected IAA earlier than RBA-IAA by 2.3 years, on average (range 0.3–7.2 years). 25% of IAA positivity identified using the ECL-IAA assay during the pre-T1D period in young children was missed when using RBA. In TEDDY, ECL-IAA antedates RBA-IAA by 1.1 years (unpublished data). More importantly, both ECL-IAA and ECL-GADA assays were remarkably more disease specific and discriminated high-affinity, high-risk IAbs in pre-T1D subjects from low-affinity, low-risk IAbs that were detected only by RBAs. Over 60% of single IAb+, either GADA or IAA, in the relatives was shown to have low affinity and were not confirmed through ECL assays [13–15]. In the ASK study, screening the general population of children, near 80% of single IAb positivity generated by RBAs were low-affinity antibodies and were not confirmed through ECL assays (unpublished data). Multiple studies have demonstrated that individuals with low-affinity IAbs have low or no risk for progression to clinical T1D [14, 16]. In the TrialNet study, both the ECL-GADA and ECL-IAA demonstrated more disease specificity and they were able to remove low-affinity signals that were generated in the RBA. Both positive and negative predictive values for ECL-IAA and ECL-GADA assays were significantly higher than those for RBA-IAA and RBA-GADA [14] (Fig. 1). Furthermore, glucose metabolic changes with an OGTT (oral glucose tolerance test) for single IAb+ individuals were significantly different when divided by ECL positivity or negativity [17], as shown in Table 1. Subjects with a single IAb+, detected by RBA, and were negative using ECL showed no changes in the OGTT during follow-up and almost no progression to clinical T1D. On the other hand, subjects with a single IAb+, detected by RBA, and were positive using ECL behaved like high-risk multiple IAb+ subjects with a worsening OGTT during follow-up and progression to clinical T1D, in many cases. In the DAISY study, subjects that had a persistent single IAb+, with and without ECL confirmation after initial seroconversion, were followed for 10 years (unpublished data). Nearly 50% of the children, whose single IAb was confirmed by ECL ($n = 83$), progressed to T1D. In contrast, none of the 65 children, who were single IAb positive and ECL negative, progressed to diabetes. The presence of single IAb from current standard RBAs is causing a lot of confusion for T1D clinical studies and trials and this is due to imperfections in the current IAb assays that detect low-affinity signals that are not specific to the disease. Excluding these "low-risk autoantibodies" using a more specific assay, like the ECL assay, will greatly enhance the predictive value of single IAb. We expect that using a high-affinity assay for single IAb detection, we will be capable to use this as a reliable biomarker for early prediction of T1D and disease staging.

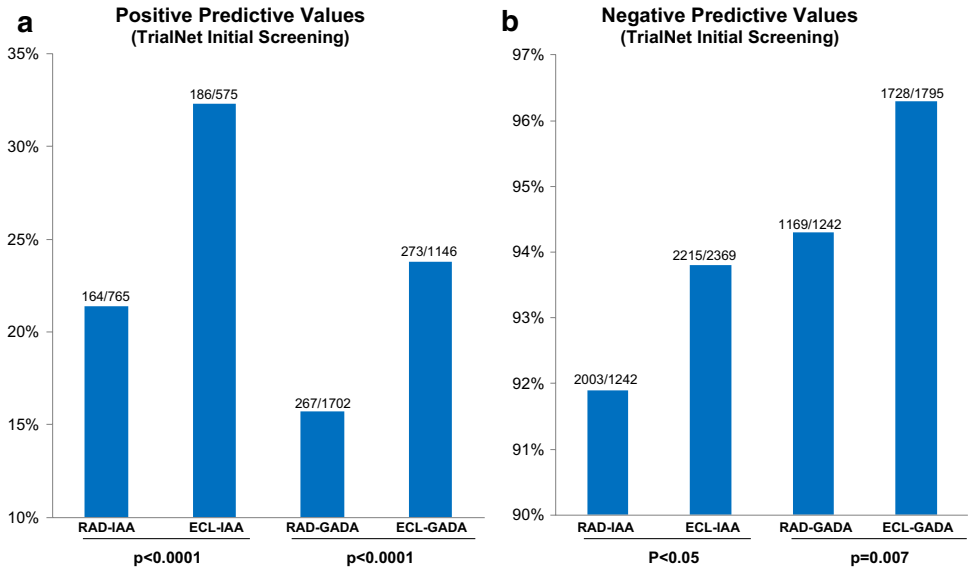

Fig. 1 Comparison of predictive values between ECL and RBA on 2,944 subjects for their very first initial screening samples in TrialNet Pathway to Prevention Study. **a** Comparison of positive predictive values between RBA-IAA and ECL-IAA ($p < 0.0001$), and between RBA-GADA and ECL-GADA ($p < 0.0001$). **b** Comparison of negative predictive values between RBA-IAA and ECL-IAA ($p < 0.05$), and between RBA-GADA and ECL-GADA ($p = 0.007$)

Table 1 OGTT and T1D follow-up for subjects with a single IAb* [17]

	Radioassay GADA+ only			Radioassay mIAA+ only		
	ECL+ ($n = 107$)	ECL− ($n = 78$)	p value	ECL+ ($n = 24$)	ECL− ($n = 63$)	p value
OGTT changes (mg/dl)	22.1 ± 90.1	− 18.8 ± 70.7	0.001	36.4 ± 75.4	− 9.4 ± 69.8	0.009
% Diagnosed with T1D	14.0% ($n = 15$)	1.3% ($n = 1$)	0.002	16.7% ($n = 4$)	0% ($n = 0$)	0.005

*For subjects with multiple Abs: 22.5 ± 95.8

Even though IAbs are proven to be related to the risk of T1D, there is no direct proof to indicate IAbs in β-cell pathogenesis. Severe loss of pancreatic β-cells in T1D is largely attributed to the targeting of islet cell autoantigens by autoreactive T cells [18]. All major biochemical autoantigens are targets of both IAbs and autoreactive T cells. This is shown in the presence of IAbs in patients and implies that the involvement of helper T cells is reactive to the same autoantigens. While diabetes-associated IAbs are strong predictors of ongoing autoimmunity, the general view is that IAbs are not the primary mediators of β-cell killing. In T1D, all four major biochemical autoantigens, insulin, GAD65, IA-2 and ZnT8, are generally thought to be intracellular autoantigens inside or bound/attached to secretory vesicles [19]. However, there are no IAbs routinely tested, so far, that react with epitopes on the cell surface and they are not thought to be cytotoxic. Recently, an abundant presence of ZnT8 was found on the surface of live β-cells (rat INS-1E cell line) [20] and the ZnT8 was shown to be trafficked to the surface of β-cells following insulin secretion. More importantly, we identified a subclass of ZnT8A directed to surface ZnT8 in human sera from patients with T1D [21]. The pathogenic potential of ZnT8A to β-cell surface is even more relevant in light of the increased surface exposure of ZnT8 following glucose-stimulated insulin secretion (GSIS) [20], that is, hyperglycemia with compensatory β-cell overactivity, caused by an initial β-cell loss, may feed into a vicious cycle of overburdening the remaining β-cells, leading to a positive enforcement of immune attack on the surfaced ZnT8. ZnT8 is a 6-spanning membrane protein containing a large transmembrane domain (TMD). The full-length ZnT8 (flZnT8) requires detergent solubilization, but it is highly unstable in detergent solutions. Only the C-terminal domain (CTD) and N-terminal domain (NTD) are soluble and can be readily purified as partial antigens for ZnT8A detection [22]. Until recently, almost all ZnT8A data in the literature, by default, indicated autoimmune reactivity toward the intracellular domain (ZnT8ic) at the CTD. This showed 60–80% positive immunoreactivity of sera from subjects with new onset T1D [23]. The NTD is a minor antigen, contributing to ~ 8% of positivity in T1D patients, with only 1% of the NTD positivity not corresponding with CTD positivity [24]. Since CTD + NTD encompasses less than half of the flZnT8 sequence, a significant portion of ZnT8 antigenicity is derived from TMD, which is not included in the CTD-based standard ZnT8A assay. Thus, the full predictive power of ZnT8A is not vested without the TMD antigen.

Recently, we found that the extra-cellular epitopes of ZnT8 (ZnT8ec) in the TMD domain are highly antigenic and recognized by the serum's anti-ZnT8ec autoantibodies

in patients with T1D. With a great effort, we adapted purified flZnT8, with its natural conformation, to a solution-based ECL assay platform. We found that 21% of the 96 newly diagnosed patients with T1D had autoantibodies for the surfaced portion of the ZnT8 molecule; this includes T1D patients with a negative ZnT8icA. While the surface display of TMD may expose ZnT8 to the immune system earlier than other intracellular autoantigens, such as GAD65, IA-2, and ZnT8ic, we have found that autoantibodies to ZnT8ec appear earlier than all other IAbs (unpublished data). These findings lead to the following hypothesis: the TMD of ZnT8 is a major immunogenic domain for surfaced-targeted serum ZnT8A, and its display on the cell surface might promote antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) involved in β-cell autoimmune destruction.

About 40 years ago, IAbs that were reactive to live islet cells were first observed in diabetic children [25]. A subclass of these IAbs was found to be β-cell specific [26] and preferentially lytic for β-cells [27]. However, the molecular identify of the targeted islet cell surface autoantigens (ICSAs) has remained controversial [28, 29]. Recent studies on ZnT8 in immuno-biochemistry and cell biology may clarify whether the surfaced ZnT8 is a sought-after pathogenic ICSA directly involved in β-cell autoimmune destruction through the pathways of ADCC and CDC. Such kind of studies will hopefully create new breakthroughs and help to shift the paradigm of humoral autoimmunity in T1D pathogenesis. Furthermore, the monoclonal antibodies capable to block these pathogenic ICSA would expand upon novel immunotherapy to slow down or break a dangerous cycle of β-cell autoimmune destruction.

In conclusion, IAbs are currently the best biomarkers for T1D and have greatly contributed to the prediction of T1D risk. The affinity of IAbs is found directly associated with disease specificity and only high-affinity IAbs are associated with the risk of T1D progression. Majority of single IAbs generated in current standard RBAs are of low affinity with low risk and it is the main reason why there is uncertainty for T1D specificity for a single IAb. A high-affinity IAb assay, like the ECL assay, will be very likely to overcome this problem. Furthermore, studies on ICSAs, like surfaced ZnT8-TMD, and their autoantibodies present in T1D patients may reveal a new role of IAbs on β-cell destruction, which will change the paradigm of IAbs in T1D pathogenesis.

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Author contributions XJ researched data and wrote the manuscript. YG researched data. HH researched data and edited the manuscript. LY designed the study, researched data, and wrote the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest Author Xiaofan Jia, Author Yong Gu, Author Hilary High, and Author Liping Yu declare that they have no conflict of interest.

Ethical approval This article does not contain any experimental studies with human or animal subjects.

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