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Mini Review

Islet Autoantibodies, Assay Specificity and Disease Specificity

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Introduction

Type 1 Diabetes (T1D) is one of the most common chronic diseases in childhood, which is caused by destruction of insulin-producing pancreatic beta cells. Its incidence increases 3-5% annually and doubles every 20 years [1,2]. On one hand, acute and chronic complications of T1D seriously affect the quality of life and even life span of patients. On the other hand, prognosis can greatly be improved when the disease prediction and closely monitoring are applied, leading to earlier diagnosis and treatment [3]. Islet Autoantibodies (IAbs), as most reliable biomarkers at present for islet autoimmunity, precede clinical T1D by years and play an essential role in prediction and clinical diagnosis of T1D [4,5].

Radio-Binding Assay (RBA) is currently 'gold' standard method for measurement of IAbs while great efforts from multiple laboratories have being applied trying to improve the method of IAb assay with different technology such as ELISA, LIPS assay, PCR-based ADAP, ECL, etc. [6-9]. The sensitivity and specificity are two most important indicators to evaluate the assay. In traditional concept, assay specificity is referenced as a direct measure for disease specificity. Conventional antibody workshops determine truly present or absent of antibodies in the samples while often ignore the qualities or categories of antibodies like binding affinity, IgG subclasses, etc. and the later could be very important for disease pathogenesis and disease prediction. Assay specificity of antibody measurement is commonly defined as the ability of an assay to score a positive result when the serum sample contains an antibody that can bind and/or neutralize the target molecule. Disease specificity, in terms of pre-clinical disease screening, refers to truly disease predictive values of antibodies detected in the assay. Recently, low- or non-disease relevant autoantibodies are especially paid attention for IAbs detected in screening of non-diabetic populations. These positive signals were detected by the assay with a high assay specificity, but in terms of clinical disease, they have an extremely low predictive value. Thus, an IAb assay with a high assay specificity is not necessary to represent a high disease specificity and it will depend on the quality of autoantibodies detected.

Study from three prospective cohort studies performed in Colorado (DAISY), Finland (DIPP) and Germany (BABYDIAB9 and BABYDIET) followed children from the birth in either relatives of T1D patients or general population with T1D-susceptible HLA genotypes [10]. Children with seroconversion of multiple IAbs by RBA were revealed 80% of risk of progression to clinical T1D in 15 years. The incidence rates of T1D development in all three studies were shown remarkably linear increase with years of follow-up and almost all children positive for multiple IAbs are believed eventually to progress to clinical T1D with time. In contrast, children with single IAb had an extremely low predictive value, only 14.5% in 15-year follow-up. Similar observations were also found in multiple national and international clinical trials like TrialNet Type-1 and The Environmental Determinants of Diabetes in the Young (TEDDY) studies. The methodologies of RBA performed in these laboratories that served in these clinical studies were reported to have the high assay specificities, usually with 98 to 99% [11]. The positive signals of single IAb generated under such a high assay specificity but with extremely low predictive value have created a lot of confusions in past a few decades. People started to question for these low risk IAbs detected in screening and to investigate the underlying mechanisms of these low predictive signals, especially observed in single IAb positivity.

At initial screening either in first degree relatives of patients with T1D or in general population, most of IAb positivity detected are those isolated as a single IAb, usually single GADA or single IAA and less often to see the cases with single ZnT8A or single IA-2A. Unlike the positivity of multiple IAbs that were usually persistent during disease progression until the time of overt clinical onset, majority of single IAb were found disappeared during the follow-up with years, even months, behaving as 'transient positivity', and people with these single 'IAb' were never progressed to the disease. With the current standard RBA, single IAb unfortunately represented majority of IAb positivity in non-diabetic population screening and it has drawn a lot of attention and paid great efforts for the follow-up studies on these large proportion with single IAb positivity in clinical trials. Previous studies have found that the IAbs with low affinity are at low risk with less or non-disease relevance and this is consistent and well documented from multiple clinical studies [12-18]. These low-affinity IAbs detected by RBA, no doubt, are truly positive biochemically and the positive signals can completely be absorbed with native antigen molecules. Routine procedure of RBA is not able to distinguish between high and low affinity autoantibodies unless absorption assay is performed, which is much costed with both labor and reagents. We have developed and extensively validated a nonradioactive IAb assay using ECL detection in recent years and high affinity antibody detection is its great advantage [9]. It can discriminate high-affinity autoantibodies from low-affinity autoantibodies and remove the positivity from low affinity signals generated by RBA. Prediction of disease risk for each IAb by ECL assay has greatly been improved

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[19] while without decreasing the sensitivity for detection of high-risk cases with multiple IAbs or truly pre-T1D who were followed to clinical disease. In DAISY, over 50% of the children, whose single IAb was confirmed by ECL (n=83), progressed to T1D in 10 years. In contrast, none of the 65 children, who were single IAb positive by RBA but negative by ECL, progressed to diabetes (unpublished data). In an ancillary study of TrialNet, overall positive predictive values of both GADA and IAA for clinical T1D were significantly increased over 50%, compared with RBA, from 15.7% to 23.8% ($p<0.0001$) and 21.4% to 32.3% ($P<0.0001$), respectively [19]. In this same cohort, the negative predictive values (reflex of the assay sensitivity) of ECL assay for both GADA and IAA were also shown significantly increased. In another ancillary study of TrialNet [20], subjects who were positive for a single IAb by RBA but negative by ECL showed no worsening of glycemia, similar to subjects negative for all IAbs, during a median follow-up of 4.7 years. In contrast, glycemia were worsened significantly in the subjects with single IAb confirmed by ECL, comparably with the worsening in subjects with multiple IAbs; the latter group had a higher progression to T1D (30%). The ECL assay can substantially refine the selection of single IAb positive individuals potentially for participation in T1D prevention trials. Unlike childhood, T1D who were often seen multiple IAbs at disease onset, large proportion of adult-onset patients with T1D were featured single positivity of GADA while the disease specificity and clinical value of GADA by current standard RBA remains questionable. GADA using N-terminus truncated GAD65 in RBA was reported to improve the disease specificity by removing low-affinity signals from antibodies binding to N-terminus [21,22]. In our recent study of two adult-onset T1D cohorts, Action LADA and Diabetes in Young Adults (DiYA) study, near 40% of single GADA positivity by RBA were shown negative by ECL assay with low affinity and their clinical phenotypes were more similar to T2D (Diabetologia, in press). High-affinity ECL assay showed a greater clinical utility in screening adult-onset diabetes, by allowing for more accurate clinical diagnosis to the benefit of clinical care. General population screening in children has recently being initiated in Europe and USA [23,24]. In an ongoing Autoimmunity Screening for Kids (ASK) study of general population children screening in Colorado of USA, as high as 80% of single IAb positivity generated by RBA were found ECL negative with low-affinity (unpublished data). High affinity IAbs confirmed by ECL assay at their very first initial positive visit will stay high affinity, consistent over time [25]. Similarly, those who were negative by ECL and showed low affinity at initial screening will stay low over time. No converting events from low to high or high to low affinity were seen over time. These results implicated that high disease specific IAbs are capable to be pre-identified on the early stage of initial screening using a high affinity assay.

To improve the performance of immunoassays measuring IAbs and to harmonize the results between laboratories, the Islet Autoantibody Standardization Program (IASP, previous DASP) put a great effort and has been successfully making a great progress to organize international IAb workshop [11,26] for interlaboratory comparison studies every 18 months in which blinded T1D patient and control serum samples were distributed to each participating laboratory and tested for IAbs. IASP is so far the only official, laboratories voluntarily participating and internationally accepted workshop for IAbs supported by the Immunology and Diabetes Society (IDS) and NIH/NIDDK. Centralized collection and analysis of the results by the IASP Committee provide participants with an unbiased comparison of assay performance whereas assay sensitivity and assay specificity are accessed. All samples recruited in IASP workshop are unexceptionally limited to cases of T1D and controls of normal from which only diagnostic values of IAbs for clinical patient's vs healthy people can be estimated, while the predictive values of IAbs detected in non-diabetic population are not able to be evaluated. To search for more disease specific assays superior to current standard RBA, an IAb workshop using the samples from non-diabetic subjects in comparison of T1D predictive values of IAbs will be highly expected.

Conclusion

In conclusion, islet autoimmunity precedes clinical T1D by years and IAbs play an essential role in prediction and diagnosis of T1D. The current 'gold' standard RBA, with its high assay sensitivity and specificity, has met a great challenge with disease specificity for the risk prediction in non-diabetic population screening. Majority of single IAb detected by RBA, which represent a large proportion of IAb positivity in screening, were found of low affinity with low disease risk and it resulted in overall low predictive value. A high-affinity IAb assay like ECL assay is needed to discriminate highly disease specific IAbs with high-affinity from those low-affinity with low-risk signals. This will greatly improve the disease prediction of IAbs detected, especially in people with single IAb.

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References

1. Harjutsalo V, Sjoberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. Lancet. 2008; 371: 1777-1782.
2. Vehik K, Hamman RF, Lezotte D, et al. Increasing incidence of type 1 diabetes in 0-17-year-old Colorado youth. Diabetes Care. 2007; 30: 503-509.
3. Elding Larsson H, Vehik K, Bell R, et al. TEDDY Study Group; SEARCH Study Group; Swediabkids Study Group; DPV Study Group; Finnish Diabetes Registry Study Group. Reduced prevalence of diabetic ketoacidosis at diagnosis of type 1 diabetes in young children participating in longitudinal follow-up. Diabetes Care. 2011; 34: 2347-2352.
4. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014; 383: 69-82.
5. Regnell SE, Lernmark Å. Early prediction of autoimmune (type 1) diabetes. Diabetologia. 2017; 60: 1370-1381.
6. Ziegler AG, Haupt F, Scholz M, et al. 3 Screen ELISA for High-Throughput Detection of Beta Cell Autoantibodies in Capillary Blood. Diabetes Technol Ther. 2016; 18: 687-693.
7. Liberati D, Wyatt RC, Brigatti C, et al. A novel LIPS assay for insulin autoantibodies. Acta Diabetol. 2018; 55: 263-270.
8. Tsai CT, Robinson PV, Spencer CA, et al. Ultrasensitive Antibody Detection by Agglutination-PCR (ADAP). ACS Cent Sci. 2016; 2: 139-147.
9. Gu Y, Zhao Z, High H, et al. Islet Autoantibody Detection by Electrochemiluminescence (ECL) Assay. J Clin Cell Immunol. 2017; 8: 1000531.

10. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013; 309: 2473-2479.

11. Lampasona V, Pittman DL, Williams AJ, et al. Islet Autoantibody Standardization Program 2018 Workshop: Interlaboratory Comparison of Glutamic Acid Decarboxylase Autoantibody Assay Performance. Clin Chem. 2019; 65: 1141-1152.

12. Achenbach P, Koczwara K, Knopff A, et al. Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. J Clin Invest. 2004; 114: 589-597.

13. Schlosser M, Koczwara K, Kenk H, et al. In insulin-autoantibody-positive children from the general population, antibody affinity identifies those at high and low risk. Diabetologia. 2005; 48: 1830-1832.

14. Mayr A, Schlosser M, Grober N, et al. GAD autoantibody affinity and epitope specificity identify distinct immunization profiles in children at risk for type 1 diabetes. Diabetes. 2007; 56: 1527-1533.

15. Siljander H, Harkonen T, Hermann R, et al. Role of insulin autoantibody affinity as a predictive marker for type 1 diabetes in young children with HLA-conferred disease susceptibility. Diabetes Metab Res Rev. 2009; 25: 615-622.

16. Curnock RM, Reed CR, Rokni S, et al. Insulin autoantibody affinity measurement using a single concentration of unlabeled insulin competitor discriminates risk in relatives of patients with type 1 diabetes. Clin Exp Immunol. 2012; 167: 67-72.

17. Yu L, Miao D, Scrimgeour L, et al. Distinguishing persistent insulin autoantibodies with differential risk: non-radioactive bivalent proinsulin/insulin autoantibody assay. Diabetes. 2012; 61: 179-186.

18. Miao D, Guyer KM, Jiang L, et al. GAD65 autoantibodies detected by electrochemiluminescence (ECL) assay identify high risk for type 1 diabetes. Diabetes. 2013; 62: 4174-4178.

19. Miao D, Steck A, Zhang L, et al, and the type 1 diabetes TrialNet study group. Electrochemiluminescence Assays for Insulin and GAD Autoantibodies Improve Prediction of Type 1 Diabetes Risk. Diabetes Tech & Therapeutics. 2015; 17: 119-127.

20. Sosenko JM, Yu L, Skyler JS, et al. Type 1 Diabetes TrialNet Study Group. The use of Electrochemiluminescence assays to predict glycemic and autoantibody progression to type 1 diabetes in individuals with single autoantibodies. Diabetes Technol Ther. 2017; 19: 183-187.

21. Williams AJ, Lampasona V, Wyatt R, et al. Reactivity to N-terminally truncated GAD65(96-585) identifies GAD autoantibodies that are more closely associated with diabetes progression in relatives of patients with type 1 diabetes. Diabetes. 2015; 64: 3247-3252.

22. Achenbach P, Hawa MI, Krause S, et al. Action LADA consortium. Autoantibodies to N-terminally truncated GAD improve clinical phenotyping of individuals with adult-onset diabetes: Action LADA 12. Diabetologia. 2018; 61: 1644-1649.

23. Raab J, Haupt F, Scholz M, et al; Fr1da Study Group. Capillary blood islet autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. BMJ Open. 2016; 6: e011144.

24. McQueen RB, Geno Rasmussen C, Waugh K, et al. Cost and Cost-effectiveness of Large-scale Screening for Type 1 Diabetes in Colorado. Diabetes Care. 2020; 43: 1496-1503.

25. Steck AK, Fouts A, Miao D, et al; TrialNet Study Group. ECL-IAA and ECL-GADA Can Identify High-Risk Single Autoantibody-Positive Relatives in the TrialNet Pathway to Prevention Study. Diabetes Technol Ther. 2016; 18: 410-414.

26. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: First, assay proficiency evaluation. Diabetes. 2003; 52: 1128-1136.