Islet Autoantibody Testing: An End to the Trials and Tribulations?

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Islet autoantibodies have taught us almost all we know about the disease process leading up to type 1 diabetes (T1D). In the absence of direct access to target organ tissue prior to diagnosis, they have provided the best available window on islet autoimmunity in humans. Detection of islet cell antibodies (ICA) in unaffected relatives identified for the first time the prodrome preceding clinical onset (1), and autoantibodies to insulin (IAA), glutamate decarboxylase (GADA), islet antigen-2, and zinc transporter 8 now provide the foundations for studies of the natural history of the condition. In prospective studies from birth, such as Diabetes Autoimmunity Study of the Young (DAISY), BABYDIAB, Diabetes Prediction and Prevention (DIPP), and more recently The Environmental Determinants of Diabetes in the Young (TEDDY), appearance of islet autoantibodies has been used to define the onset of autoimmunity (2–5). This can then be related to genetic characteristics and environmental exposures of potential etiological relevance. Autoantibodies also form the basis of disease prediction, allowing the sensitive, specific, and quantified assessment of risk that has made possible intervention studies to delay or prevent clinical onset of T1D (6). Islet autoantibodies are, however, difficult to measure.

Islet cell antibodies have been largely abandoned because the indirect immunofluorescence assays are complex, labor-intensive, and hard to standardize. Even for antibodies directed against the four identified islet autoantigens—and in spite of international workshop programs—assay performance varies markedly between laboratories and a relatively small number achieve high levels of sensitivity and specificity (7,8) Current assays pick up both disease-relevant and nondisease-associated (disease-irrelevant) signals. While this can be largely overcome by using combined testing for multiple antibodies, it introduces additional complexity and expense. An additional issue is that radioimmunoassays (RIAs), generally the method of choice for measuring islet antibodies (9), are unpopular with clinical laboratories because they use time-limited labels and have onerous regulatory requirements—a further incentive to develop alternative assay technologies. The Holy Grail for islet autoantibody measurement is therefore a low-cost nonradioactive assay that detects only disease-relevant signals, can do this as soon as autoimmunity is initiated, and is simple enough for high-throughput general use.

In this issue, Miao et al. (10) take a step nearer to this goal, describing a novel electrochemiluminescence (ECL) assay for GADA that builds on the group's recently published ECL assays for IAA (11–12). These assays take advantage of bivalent binding by IgG; the autoantibody acting as a bridge between the antigen anchored to the plate and antigen carrying a luminescent label. Only when both components are bound by the autoantibody will a signal be produced. The authors have compared the performance of the ECL-GADA assay with that of a very well-validated RIA and shown that, in patients with newly diagnosed T1D and in samples collected prior to diagnosis, the two assays achieved equivalent sensitivity but the ECL-GADA assay was more specific. Similarly, among relatives and children at high genetic risk with GADA detectable by RIA who were followed up prospectively, 95% of those who subsequently developed diabetes or progressed to the high-risk pattern of multiple antibody positivity had ECL-GADA, compared with 23% of those who remained persistently positive for GADA alone and were thus at low risk of disease. These findings imply that, like the ECL-IAA assay, the new ECL-GADA assay detected fewer disease-irrelevant signals than the RIA. In addition, both ECL assays appear able to distinguish children with disease-relevant islet autoimmunity earlier in infancy—before additional autoantibodies have appeared (11,12).

Miao et al. (10) also explored the potential mechanisms underlying the superiority of their assay. As shown in Fig. 1, a number of antibody characteristics related to the breadth and maturity of the autoimmune response, including epitope specificity and antibody affinity have been found to be useful in distinguishing disease-relevant islet autoantibodies associated with high risk of progression to disease (13–18). Using serial competition assays, Miao and colleagues (10,11) have shown that the ECL-IAA and ECL-GADA appear to detect high-affinity antibodies while ignoring low-affinity antibodies less associated with progression to disease.

Access to samples from a broad range of diabetic and at-risk populations is a particular strength of the report by Miao et al. (10). They were able to evaluate performance of the assay in young children at high genetic risk, and progressors and nonprogressors in prospective studies, as well at clinical onset of T1D. The study therefore covered all the key points of interest in the pathogenesis of T1D of relevance for research into the etiology and natural history of the condition, as well as for identifying high-risk individuals for trials of preventative therapies. The most obvious weakness is that the results come from a single laboratory, and we need confirmation that the technology can be transferred.

The ECL-GADA and ECL-IAA assays address many limitations of RIAs, but they do have some drawbacks that are likely to be relevant for general implementation. First,

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DOI: 10.2337/db13-1445

See accompanying original article, p. 4174.

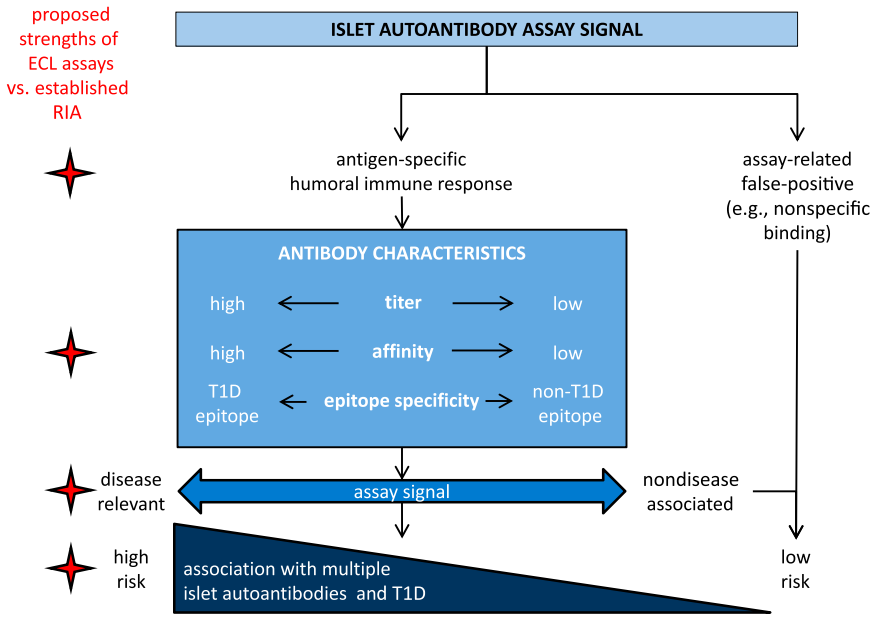

FIG. 1. Discrimination of disease-relevant autoantibodies from those conferring low risk of T1D. Autoantibody characteristics associated with high risk of disease include high titer and affinity, specificity for T1D-associated epitopes, as well as positivity for multiple islet autoantibodies. Autoantibody assay signals associated with low risk of T1D may derive from assay nonspecific binding or by detection of autoantibodies not relevant to β-cell destruction. The importance of each antibody characteristic will vary according to antibody and assay type, with a balance to be struck between improving specificity and loss of sensitivity. The reported ECL assays for IAA and GADA appear to detect predominantly high-affinity antibodies that are strongly associated with disease and to achieve higher specificity than established RIAs without loss of sensitivity.

the machine and consumables are currently more costly than those required for RIA, rely on a single supplier, and are available in few laboratories. The volume of serum needed is also double that for GADA RIAs. The acidification step involved in the ECL-IAA assay increases the technical complexity. It also impacts on the ability of the ECL assay to measure several analytes simultaneously in a single sample (multiplexing); a potentially important benefit of the assay format that could reduce cost and increase throughput. From a research perspective, the ECL assays' requirement for purified proteins makes them less flexible than RIAs for which new or mutated antigens are produced rapidly from plasmid DNA using in vitro transcription translation labeling kits.

There are alternative nonradioactive assays that share some of the benefits of the ECL assays (19,20), including GADA-bridging ELISAs. These commercially available ELISAs are technically straightforward and offer sensitivity and specificity comparable to the ECL assay, though their performance in distinguishing disease-relevant autoantibodies in infancy and prediabetes has not been studied. The relatively high cost and serum volume required have limited their use in research, but in experienced hands they have shown excellent reproducibility in autoantibody workshops (7).

Before ECL-based islet autoantibody assays can be adopted more widely, the promising results obtained by Miao et al. (10) need to be confirmed in other laboratories and in samples not prescreened with RIA. Additional work to enhance the signal-to-noise ratio and develop epitope-specific labels may further improve the discrimination achieved and, if multiplex measurement of multiple islet autoantibodies proves successful, the disease sensitivity and specificity offered by combined islet autoantibody testing may be achieved with a single assay. The ECL assay format therefore shows great promise as the basis of a test that could be applied for general population screening if and when preventive therapy becomes available.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

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