Islet autoantibodies have been associated with type 1 diabetes for more than a quarter of a century (1, 2). Detection of islet cell antibodies (ICA) before clinical onset of diabetes provided evidence for the long disease prodrome (3) and allowed these antibodies to be used in disease prediction (4). ICA also identified a subset of patients treated with oral hypoglycemic agents that progressed more rapidly to insulin treatment (5). Since then, the major islet autoantigens to which ICA are directed have been identified (6–9), and others have been found (10–12). In addition, assays have been improved, standardized, and made applicable to high throughput. Testing strategies have also been refined, and the length of follow-up of individuals with autoantibodies has increased. The major clinical applications for antibody testing in diabetes, however, remain essentially unchanged. This review will therefore focus on issues related to the use of diabetes autoantibodies for prediction of disease and insulin requirement.

Autoantibodies, Assays, and Thresholds

ICA are detected by immunofluorescence on human group O pancreas, a technique that is technically demanding and difficult to standardize. Antibodies to the islet antigens glutamate decarboxylase 65 (GADA) and the tyrosine phosphatase-related proteins islet antigen 2 (IA-2A/ICA512) and IA-2β (phogrin) were subsequently found to contribute to ICA staining (6–9, 12), and autoantibodies

| Abbreviations: DKA, Diabetic ketoacidosis; GADA, glutamate decarboxylase 65; GDM, gestational diabetes; HbA1c, glycosylated hemoglobin; IA-2, islet antigen 2; IA-2A, IA-2 antibodies; IAA, insulin autoantibodies; ICA, islet cell antibodies; ZnT8, zinc transporter 8; ZnT8A, ZnT8 antibodies. |
radiobinding assays for antibodies to GADA and IA-2A are most commonly measured using between the U.S. Centers for Disease Control and Prevention's Diabetes Autoantibody Standardization Program (DASP), a collaboration that has historically performed less well, but recent workshops, whereas some laboratories can consistently achieve high sensitivity and specificity, these are relatively few, and many assays continue to have problems detecting low levels of IAA (Mueller, P., personal communication). IAA ELISA appears less able to detect disease-associated autoantibodies than radiobinding assays (23), and none has achieved high levels of sensitivity and specificity in the DASP workshops (15). GADA testing is generally available to clinicians in North America and Europe, but ease of access and cost vary widely across the world. Fewer routine clinical laboratories offer IA-2A testing (although this may change with the improved IA-2A assay kits now available), and reliable IAA measurement is available only in specialist laboratories.

In interpreting islet autoantibody results, it is important to appreciate that antibody level represents a continuous variable—just as much as blood pressure, serum sodium, or pulse—and the concept that antibodies are present (or “positive”) or absent (or “negative”) is at best a gross oversimplification and, at worst, misleading because the distributions of antibody levels in health and disease overlap. Antibody cutoffs are used for convenience but are essentially arbitrary; by using a higher threshold, the chance of mislabeling a healthy person is reduced, and by using a lower threshold, the chance of mislabeling a person with disease is reduced. It is therefore appropriate to use different thresholds for different purposes in the same way that you might define “unacceptably high” blood pressure differently in patients with and without nephropathy.

Cutoffs are commonly based on a percentile of a control population (generally the 99th), although some investigators have used q-q probability plots to identify the point of inflection between populations of cases and controls (24). It is therefore apparent that assay performance, choice of control population, its size, and the definition of positivity used are all crucial in interpreting results and that comparisons between studies need to be viewed with caution.

**Using Autoantibodies to Predict Type 1 Diabetes**

**How can risk be assessed?**

Prospective studies in relatives of patients with type 1 diabetes have shown that development of multiple islet autoantibodies is a critical step in pathogenesis and that this provides a robust and early marker of risk of progression to diabetes. It has been known for more than a decade that detection of two or more islet autoantibodies is associated with a much higher risk of type 1 diabetes than a single autoantibody (25–28). This observation was confirmed in the large prevention trials, the European Nicotinamide Diabetes Intervention Trial (ENDIT) (29) and the
Diabetes Prevention Trial-Type 1 (DPT-1) (30). In ENDIT, baseline samples from all participants were tested for ICA, IAA, GADA, and IA-2A. Those with ICA alone had a 2.2% cumulative risk of diabetes within 5 yr, whereas the risks with one, two, or three additional antibodies were 17, 39, and 70%, respectively. Multiple antibodies are not only specific but also sensitive; 91% of the ENDIT relatives who progressed to diabetes had two or three markers in addition to ICA. This message is strikingly consistent between studies, irrespective of the autoantibodies tested, although those using only GADA, IA-2A, and IAA and omitting ICA have generally found higher rates of progression in single antibody-positive relatives (26, 27). Additional testing for the recently described ZnT8A, however, reclassifies some relatives previously “single antibody positive” as “multiple antibody positive” and is associated with increased risk, thus bringing results into closer alignment with other studies (11, 31).

Islet autoantibodies appear early in life in both offspring of parents with diabetes and children at high genetic risk in the general population (24, 32–34), and in those who develop diabetes, the highest risk antibody combinations are generally detectable before age 2 or 3 yr (32). The maximum time lag between autoantibody detection and the clinical onset of diabetes is essentially as long as the longest follow-up. In the Bart’s-Windsor family study, established in 1978, one sibling who was found to have ICA, IAA, and GADA in a sample collected at the age of 10 developed diabetes at the age of 35 (Bingley PJ, unpublished observations). Long-term follow-up of individuals with multiple antibodies, albeit in relatively small numbers, suggests that all may eventually progress to diabetes (35), although larger studies are needed to confirm this.

In the research setting, definition of autoantibody characteristics such as epitope specificity and antibody titer and isotype can further refine risk assessment (36). Autoantibody affinity may be particularly useful in distinguishing disease-associated IAA (37), and antibodies to IA-2β identify a subgroup of multiple autoantibody-positive relatives at particularly high risk of progression to diabetes (38). In summary, therefore, autoantibody testing of a single blood sample collected at early age (with the caveat that the assays used must be sensitive, specific, and reproducible) has the potential to indicate whether or not that child will develop type 1 diabetes at some point in his/her life. The majority of studies have been performed in relatives of children with type 1 diabetes, but more recent data suggest that the strategy of multiple antibody testing is also effective in children in the general population (39–41). The risks that can be assigned (up to 70% within 10 yr for highest risk combinations) are much higher than any estimates based on genotype and, at the same time, identify a larger proportion of future cases. We have therefore reached the point at which diabetes antibody testing strategies provide powerful tools for the prediction of disease.

**Should we screen for type 1 diabetes risk?**

The general criteria for implementation of screening in any condition are clear-cut (42). Type 1 diabetes is certainly an important health condition that incurs high costs for the individual and society, and simulation modeling suggests that an intervention that delays clinical onset by only 2–3 yr will be cost effective when compared with the costs of health care (43). The natural history of the condition is increasingly well understood, and there is a long preclinical prodrome. Autoantibody testing strategies can achieve high levels of sensitivity and specificity and are relatively cheap. On the other hand, the condition does not fulfil what is probably the most important criterion in that no agent has yet been shown to be effective in the prevention of type 1 diabetes, although some agents have achieved short-term preservation of residual β-cell function after diagnosis (44–47). Despite promising pilot data in animals and humans, large randomized controlled trials of nicotinamide, parenteral, oral, and nasal insulin showed no overall reduction in risk of progression to diabetes (39, 48–51). The only suggestion of efficacy was found on post hoc analysis of the DPT-1 oral insulin trial. This showed that, among participants with high levels of IAA (≥80 nU/ml), 6.2% per year of those in the oral insulin group developed diabetes compared with 10.4% in the placebo group (hazard ratio, 0.566; 95% confidence interval, 0.361–0.888; P < 0.015) (50). This is currently being reexamined in the TrialNet oral insulin study (52).

In contrast to type 2 diabetes, no lifestyle changes have been shown to impact on progression to type 1 diabetes. In general, routine screening can therefore only bring anxiety (53), implementation of unproven behavior changes, or exposure to agents not known to alter the course of disease. Guidelines for screening for risk of type 1 diabetes proposed by the Immunology of Diabetes Society in 2001 and current American Diabetes Association standards for medical care in diabetes recommend that testing for immune or genetic markers of risk of type 1 diabetes should usually be undertaken only in high-risk individuals in the context of clinical research studies and that “widespread clinical testing of asymptomatic low-risk individuals cannot currently be recommended” (54, 55).

Experience in prospective studies nonetheless indicates that knowing a child’s risk status may confer some benefits because the majority of participants under follow-up in the Diabetes Autoimmunity Study in the Young (DAISY) or in DPT-1 were asymptomatic at the time of diagnosis of diabetes, and less than 4% presented with diabetic keto-
acidosis (DKA) (56, 57). In DAISY, this was 10-fold lower than in contemporary controls not participating in the study, even after adjusting for family history. Before using these data to advocate routine autoantibody testing, even in family members of children with diabetes, however, it is important to look at the implications. In these studies, antibody-positive participants had 6-monthly oral glucose tolerance tests, and it has been calculated that 9–20 high-risk individuals would need 6-monthly testing to prevent one case of DKA (58). Another benefit is that C-peptide levels at diagnosis were higher, which may be important if interventions that can preserve C-peptide secretion and translate into long-term reduction in severe hypoglycemia or microvascular complications become available (59). At present, however, antibody testing and regular monitoring cannot be recommended as routine clinical practice (53, 58).

**Using Autoantibodies to Classify Diabetes**

Soon after they were described, ICA were noted to be associated with a different clinical course in patients initially thought to have non-insulin-dependent diabetes. ICA-positive patients were leaner, progressed more rapidly to insulin requirement, and had lower C-peptide secretion (5, 60). The term latent autoimmune diabetes in adults (LADA), defined in terms of GADA positivity, was coined to describe this slowly progressive autoimmune diabetes that has also been called “type 1.5 diabetes” and “slowly progressive insulin-dependent diabetes” (61, 62). In the UK Prospective Diabetes Study (UKPDS), 12% of patients with type 2 diabetes were found to have ICA or GADA at diagnosis, and 4% had both. The phenotype of patients with both antibodies was similar to that of classic type 1 diabetes and, at different ages, 59–94% required insulin within 6 yr, compared with 5–14% in those with neither ICA nor GADA. Both ICA and GADA (in isolation or together) were associated with an intermediate phenotype—lower body mass index, higher glycosylated hemoglobin (HbA1c), and lower β-cell function—compared with antibody-negative patients; the positive predictive value for requiring insulin was also intermediate (63). Other studies have similarly reported patient phenotypes converging with those of typical type 1 with detection of other islet autoantibodies or higher levels of GADA (64–69).

**Autoantibody testing in type 2 diabetes?**

We therefore have the potential to use islet antibodies to identify a subgroup of patients with type 2 diabetes who appear likely to progress to insulin requirement more rapidly, but should we be doing this in routine clinical practice? Is a diagnosis of LADA of clinical value to an individual patient? We are well aware of the need to aim for tight glycemic control in type 2 diabetes to minimize the risk of microvascular complications, and that this may include early insulin treatment if targets are not achieved (70). Some clinicians would argue that better classification benefits both health care providers and patients. I, however, feel it is important to determine whether there is any evidence that knowing a patient’s antibody status can alter management and improve outcome when compared with careful monitoring of glycemic control. There are a number of issues here. First, it has been pointed out that, despite the impressive-looking positive predictive values given, GADA were actually of limited value in predicting insulin requirement in UKPDS; of 237 patients who required insulin within 6 yr, 38% had GADA; of 117 with GADA, only 51% overall went on to insulin—35% in those diagnosed over age 45 (63, 71). Second, evidence is lacking that any clinical management is superior in patients with LADA. In UKPDS, autoantibody status did not influence the HbA1c response to sulfonylurea or insulin in autoantibody-positive participants over the first 10 yr after diagnosis (72). A recent Cochrane Review suggested that there was “some evidence that sulfonylurea may bring about earlier insulin dependence,” but this was based in part on examination of published survival curves of these UKPDS data showing rates of progression of about 42% at 10 yr without apparent statistical analysis and on preliminary results from the only randomized trial comparing insulin and sulfonylurea treatment in LADA—the Tokyo study (72, 73). The final results of this open-label study have subsequently been published (74). Progression to an insulin-dependent state (defined on the basis of C-peptide secretion in the oral glucose tolerance test) was compared in GADA-positive patients with non-insulin-dependent diabetes of less than 5-yr duration randomized to sulfonylurea or insulin (30 per group). C-peptide secretion did fall less rapidly with insulin treatment than sulfonylurea in those with GADA, particularly those with preserved C-peptide at baseline. There are, however, caveats to these results; the same trend was seen in those with high and low levels of GADA (P = 0.04 and P = 0.09, respectively), suggesting that the effect may be independent of islet autoimmunity. It would therefore have been of interest also to assess the effects of these agents in antibody-negative patients, ideally matched for baseline C-peptide. In addition, although HbA1c did not differ significantly between the two groups, the power to detect a difference in glycemic control, a potentially important determinant of β-cell preservation, was limited and fasting blood glucose was lower in the insulin-treated group. Al-

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Autoantibody testing in diabetes of uncertain type

The incidence of postpartum diabetes in women with GDM seems to be increasing (86), and islet autoantibodies can also be useful in this situation. Although antibodies are found in only 5–18% of women with GDM (86, 87), the associated risk of postpartum diabetes is very high.

Ketosis-prone diabetes

Ketosis-prone diabetes that is not easily classified is becoming more common, particularly in non-Caucasian populations (81, 82). In a multiethnic cohort, autoantibody testing alone gave low sensitivity and specificity for predicting long-term preservation of C-peptide secretion in patients presenting in DKA, but a classification combining antibodies with a measure of β-cell function performed well (83). Among patients with preserved β-cell function 2 wk after resolution of an episode of DKA, lack of autoantibodies identified a group with long-term preservation of β-cell function. In the majority of these, exogenous insulin could be stopped.

Monogenic diabetes

In current guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young, absence of islet autoantibodies is one of the criteria for testing for HNF1α and HNF4α mutations in children and young adults with diabetes and a strong family history of diabetes (84). A diagnosis of one of these forms of maturity-onset diabetes of the young may allow patients previously thought to have type 1 diabetes to come off insulin (85).

Gestational diabetes (GDM)

The incidence of gestational diabetes (GDM) is increasing (86), and islet autoantibodies can also be useful in this situation. Although antibodies are found in only 5–18% of women with GDM (86, 87), the associated risk of gestational diabetes is very high.
(87–89). In one study, the 8-yr risk of diabetes among women with GADA and/or IA-2A was 97% (90). As in other situations, risk increases with the number of antibodies detected, and in the same cohort women with two or more antibodies had at least 60% risk of developing type 1 diabetes within 2 yr (87).

Conclusion

In summary, islet autoantibodies are very robust tools for the prediction of type 1 diabetes and, providing high-quality assays are used, antibody testing in early childhood can potentially identify individuals destined to develop the disease. Autoantibodies have many applications in the research setting—as the basis for recruitment into prevention trials and immunointervention trials at diagnosis, and providing the outcome measure in studies of the etiology and early natural history of islet autoimmunity and in trials aiming to prevent the initiation of autoimmunity (91, 92). The place of autoantibody-based risk assessment in routine clinical practice is, however, currently limited because no interventions have yet been shown to prevent disease, and the benefits of identifying and following high-risk subjects are uncertain. Type 1 diabetes prevention is nonetheless a very active area of research, and the situation may change. If and when clear and proven therapeutic options are available for people at high risk of progression to type 1 diabetes, the means of pinpointing such individuals are in place. Autoantibody testing in adults with clinically diagnosed type 2 diabetes picks out a subset with features and clinical course approximating to those of typical type 1 diabetes, but translating this observation into measures that can usefully influence individual patient management is far from simple. An antibody-positive patient’s risk of progression to insulin requirement is modulated by age and clinical features as well as by the extent and intensity of islet autoimmunity detected. A middle-aged or elderly patient with type 2 diabetes who has one antibody is probably at no greater risk of early insulin requirement than a patient of the same age who lacks antibodies, whereas a young person with multiple autoantibodies is almost certain to need insulin soon. These factors need to be taken into consideration in counseling patients. A further major limitation is that the clinical benefits to an individual patient of a diagnosis of LADA—as opposed to careful monitoring and treatment of hyperglycemia—are unclear. There is some evidence that in antibody-positive patients, sulfonylureas might lead to more rapid reduction in β-cell function than insulin therapy, but larger and more complete studies, including assessment in antibody-negative patients, are needed before clinical practice is changed. In some situations, such as atypical diabetes, it is useful to know that a patient is antibody negative, but again the result must be taken in context. A test applied to exclude autoimmune diabetes will be most useful if the pretest probability is low and may be misleading if the pretest probability is high. Although my research life has been largely focused on the topic, my overall interpretation of the available evidence is that the current indications for diabetes antibody testing in routine clinical practice are actually quite limited. Just because we can does not necessarily mean we should—yet.

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