Immunological Markers in the Diagnosis and Prediction of Autoimmune Type 1a Diabetes

William E. Winter, MD; Neil Harris, MD; and Desmond Schatz, MD

Diabetes mellitus encompasses a family of disorders of carbohydrate metabolism that are characterized by hyperglycemia and the development of long-term macrovascular, microvascular, and neuropathic complications. In 1997, the American Diabetes Association revised the classification of diabetes. Diabetes is now classified as type 1 diabetes, type 2 diabetes, other specific types of diabetes (e.g., secondary diabetes), or gestational diabetes. Type 1 diabetes is primary insulinopenic diabetes that is subclassified as autoimmune type 1 diabetes (type 1a) or idiopathic type 1 diabetes (type 1b). The most important factor differentiating type 1a from type 1b diabetes is the presence of islet autoantibodies.

Our goal is to address two key questions concerning autoimmune type 1 diabetes: 1) Which autoantibodies serve as diagnostic markers of autoimmune type 1 diabetes?, and 2) Can islet autoantibodies that are used to diagnose type 1 diabetes predict the development of type 1 diabetes in nondiabetic individuals?

ISLET AUTOANTIBODIES AS MARKERS OF AUTOIMMUNE DIABETES

Many autoantibodies are detected at the onset of type 1 diabetes. Islet cell (cytoplasmic) autoantibodies (ICAs) and islet cell surface autoantibodies (ICSAs) were initially described in the 1970s. In the 1980s, insulin autoantibodies (IAAs), 64-kDa autoantibodies (64KAs), insulin receptor autoantibodies, carboxypeptidase-H autoantibodies, and heat shock protein (HSP) autoantibodies were recognized. In the very early 1990s, the nature of the 64KA autoantigen was revealed as glutamic acid decarboxylase (GADs) leading to the recognition of glutamic acid decarboxylase autoantibodies (GADAs). Subsequently, a cascade of various islet autoantibodies were identified including 51-kDa aromatic-1-amino-acid decarboxylase autoantibodies, chymotrypsinogen-related 30-kD pancreatic autoantibodies, DNA topoisomerase II autoantibodies, glima 38 autoantibodies, GLUT2 autoantibodies, glycolipid autoantibodies, GM2-1 islet ganglioside autoantibodies, IA-2 autoantibodies (IA-2As), IA-2/H9252 autoantibodies (IA-2/H9252As), ICA69 autoantibodies, islet cell–specific 38-kD autoantibodies, proinsulin autoantibodies, and 52-kDa RIN (rat insulinoma) autoantibody (Rubella-related autoantibody). New autoantibodies associated with type 1 diabetes continue to be discovered.

Four autoantibodies have emerged as the most useful autoimmune markers of type 1 diabetes: ICAs, IAAs, GADAs, and IA-2As. IA-2As include ICA512 and IA-2c autoantibodies.

ICAs

ICAs are detected by indirect immunofluorescence using blood group O human pancreas as substrate. The ICA assay is labor intensive and non-automated and requires a very high level of quality assurance and quality control to produce accurate and precise results. ICAs are polyclonal autoantibodies that react with all cells of the islet (e.g., α, β, γ, δ, and PP cells). Lipid and protein autoantigens that are recognized by ICAs include sialoglycoconjugate, GAD, and IA-2. ICAs are not etiological markers of β-cell destruction but do serve as important markers of β-cell autoimmunity. At onset of type 1 diabetes, 70% or more of Caucasians are ICA-positive. Only 4 in 10 African Americans with new-onset type 1 diabetes are ICA-positive. This suggests that a considerable proportion of African Americans with new-onset insulin-requiring diabetes do not have autoimmune diabetes. ICA frequency declines following diagnosis, and no more than 5–10% of type 1 diabetic patients remain ICA-positive after 10 years.

The general population frequency of ICAs is low. In the University of Florida Pasco County School study, ~1 in 250 normal schoolchildren were ICA-posi-
is distinct from that of classic type 2 diabetes. The pathophysiology of LADA is an indication for islet autoantibody bodies. The onset of type 2 diabetes in based on the presence of islet autoantibodies from antibodies arising from insulin immunization, which indicates the slow, persistent progress of β-cell damage in LADA.

ICA-positive individuals initially presenting with phenotypic type 2 diabetes display lower C-peptide levels and higher frequencies of the HLA alleles DR3 and DR4, which are associated with type 1 diabetes. Between 4 and 17% of type 2 diabetic patients have LADA based on the presence of islet autoantibodies. The onset of type 2 diabetes in lean individuals also suggests LADA and is an indication for islet autoantibody testing. The pathophysiology of LADA is distinct from that of classic type 2 diabetes. ICAs are usually absent in cases of pediatric type 2 diabetes.

**Insulin Autoantibodies**
The first islet autoantigen and β-cell-specific autoantigen reported was insulin. Autoantibodies to insulin should be sought before the administration of exogenous insulin (either animal or human) because after 5–7 days of exogenous insulin treatment, insulin antibodies will arise. The immunoprecipitation assay for insulin autoantibodies does not distinguish spontaneous autoantibodies from antibodies arising from insulin immunization, which occurs as a consequence of exogenous insulin therapy. IAs may occur more commonly in individuals who carry HLA-DR4. Techniques that detect insulin autoantibodies by an enzyme-linked immunosorbent assay should not be used because IAs so detected do not correlate with autoimmune diabetes.

At onset of type 1 diabetes, IAs occur in 35–60% of children but are decidedly less common in adults. A new IAA microassay has been developed that requires less serum than the traditional IAA assay. At the onset of type 1 diabetes in Australians, Feeney et al. found that IAs were present in 90% of children <5 years old, 71% of children 5–10 years old, and 50% of children 10–15 years old. Bingley et al. reported IAs in 83% of children <10 years old and 56% of children 10 years old or older.

IAs occur in many other autoimmune diseases including autoimmune thyroid disease. IAs were found in 13–44% of Graves’ disease patients and in 16–23% of Hashimoto’s thyroiditis patients. IAs have also been detected in Addison’s disease (40%), chronic hepatitis (36%), pernicious anemia (40%), systemic lupus erythematosus (29%), and rheumatoid arthritis (25%). Their significance is unknown in the latter conditions.

**GADAs**
GAD is neither β-cell nor islet specific. GAD is expressed predominantly in the nervous system. Other tissues that express GAD include the testes, ovary, adrenal, pituitary, thyroid, and kidney.

Because GADAs are more persistent than IAs after the diagnosis of type 1 diabetes, GADAs may be more often positive than IAs in LADA. Because the frequency of LADA is ~5–15% based on ICA studies, using GADAs as the autoimmune marker, LADA prevalence in phenotypic type 2 diabetes might be even greater. GADAs have been a major focus of diabetes research for more than 10 years. GADAs are detected in 60% or more of new-onset cases of type 1 diabetes and 3–5% of relatives.

**IA-2As**
IA-2 is a member of the protein tyrosine phosphatase (PTP) family. Similar to GAD, IA-2 is found in nervous tissue and other endocrine tissues including the pituitary. Some IA-2 autoantibody assays detect the entire molecule, whereas others detect portions of the molecule (e.g., IA-2c and ICA512 assays).

When testing for autoantibodies against ICA512 or IA-2A in natural history studies of pre-diabetes, some investigators suggest that ICA512 autoantibodies develop later than GADAs and thus may serve as markers of higher short-term risk for the clinical onset of type 1 diabetes. Like GADAs, IA-2As and related PTP autoantibodies have been a major focus of recent diabetes research.

IA-2As and IA-2βAs are detected in ~60% or more of cases of new-onset type 1 diabetes. The general population frequencies for these autoantibodies are similar to those of GADAs at 2–3%.

A review of 10 representative studies spanning 1995–1998 that include Americans, Australians, Britains, Germans, Italians, Japanese, and Swedes reveals at the onset of type 1 diabetes ICA frequencies of 70–90%, IAA frequencies of 43–69%, GADA frequencies of 52–77%, and IA-2A frequencies of 55–75% (Table 1).

**ISLET AUTOANTIBODIES THAT PREDICT AUTOIMMUNE TYPE 1 DIABETES IN NONDIABETIC INDIVIDUALS**
To understand the predictive role of islet autoantibody testing for type 1 diabetes, it is helpful to review the natural history of type 1 diabetes before diagnosis. β-Cell destruction can be viewed as roughly passing through five phases: 1) genetic predisposition, 2) autoantibody positivity, 3) abnormal insulin responses during intravenous glucose tolerance test (IVGTT), 4) glucose intolerance on oral glucose tolerance test (OGTT), and 5) clinical diabetes (Figure 1). The period of time to develop type 1 diabetes may take months (young children) to years (adults).

Most cases of type 1 diabetes occur sporadically, e.g., in the absence of a family history of type 1 diabetes in a
Table 1. Islet Autoantibody Frequency Comparisons in New-Onset Type 1 Diabetes (Single Studies)

<table>
<thead>
<tr>
<th></th>
<th>Swedes (^{77})</th>
<th>Italians (^{71})</th>
<th>Germans (^{83})</th>
<th>Swedes (^{88})</th>
<th>Australians (^{82})</th>
<th>Britains (^{60})</th>
<th>Germans (^{84})</th>
<th>Americans (^{81})</th>
<th>Italians (^{85})</th>
<th>Japanese (^{86})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>84</td>
<td>88</td>
<td>75</td>
<td>87</td>
<td>70</td>
<td>88</td>
<td>69</td>
<td>90</td>
<td>85</td>
<td>—</td>
</tr>
<tr>
<td>IAA</td>
<td>56</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>65</td>
<td>69</td>
<td>43</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GADA</td>
<td>70</td>
<td>70</td>
<td>75</td>
<td>66</td>
<td>69</td>
<td>74</td>
<td>52</td>
<td>72</td>
<td>77</td>
<td>70</td>
</tr>
<tr>
<td>IA-2A</td>
<td>—</td>
<td>54</td>
<td>73</td>
<td>68</td>
<td>60</td>
<td>75</td>
<td>55</td>
<td>63</td>
<td>62</td>
<td>58</td>
</tr>
</tbody>
</table>

Data are % positive.

Because concordance for type 1 diabetes is 50% at most, environmental factors must play a significant role in the development of type 1 diabetes. Many environmental factors have been explored as possible triggers of β-cell autoimmunity including viral infections, diet (e.g., nitrosamines in smoked meat), and breast-feeding and early exposure to cow’s milk. The specific environmental factor or factors that precipitate β-cell autoimmunity in type 1 diabetes remain elusive.

In genetically at-risk individuals, sometime after exposure to the proposed environmental trigger or triggers, β-cell autoimmunity is first recognized by the detection of islet autoantibodies. Cell-mediated autoimmunity is believed to mediate β-cell destruction. Although it is extremely difficult to reproducibly measure cellular autoimmunity, the assays for humoral autoimmunity are reasonably accurate and precise. Islet autoantibodies can appear throughout childhood in siblings of type 1 diabetic probands.

The cell-mediated immune attack targeted against β-cells is histologically termed “insulitis.” Insulitis has been observed in animal models of autoimmune diabetes including in the BioBreeding rat, in the nonobese diabetic mouse, and as streptozotocin-induced diabetes in mice. It has also been observed in 80% of individuals with clinical type 1 diabetes who come to autopsy within 6 months of diagnosis. Using a pancreatic biopsy technique in living human subjects, Japanese investigators have observed insulitis before the later development of type 1 diabetes. In prospective studies
of initially nondiabetic individuals, ICAs, IAAs, GADAs, and IA-2As have all been detected before the diagnosis of type 1 diabetes.\(^{123}\)

The first biochemical evidence of β-cell dysfunction can be detected by a loss of first-phase insulin response to the administration of intravenous glucose.\(^{124-127}\) IVGTT measures the β-cell’s content of preformed insulin and its ability to release that insulin in response to an acute intravenous glucose challenge. Usually, first-phase insulin response is measured as the sum of the plasma insulin concentration at 1 plus 3 minutes after the acute glucose bolus injection.

In an OGTT, there are three forms of β-cell stimulation: autonomic nervous system stimulation of insulin secretion, rising systemic glucose levels, and release of glucagon-like peptide-1 from the intestine. On the other hand, in the IVGTT, there is only one type of insulin stimulus (i.e., rising plasma glucose), and thus with fewer β-cell stimuli, the IVGTT is apparently more sensitive than the OGTT in indicating β-cell dysfunction. In fact, β-cell insulin response to intravenous amino acids (e.g., arginine) is preserved longer than the insulin response to intravenous glucose.\(^{128}\)

There is a predictable rate of decline in insulin secretion in those people with ICAs that ultimately go onto develop type 1 diabetes.\(^{129}\) Nevertheless, the course of the development of type 1 diabetes is often most properly described as a waxing-waning pattern of insulin secretion that is typical of many autoimmune diseases (e.g., systemic lupus erythematosus). In this phase of the destructive process, the OGTT is normal and thus there are no detectable abnormalities of glucose tolerance excluding the IVGTT. It is estimated that by the time the first-phase insulin response during IVGTT is low, there has been a 50% decline in β-cell mass.

When more β-cell mass has been lost (substantially <50% but >10% still remaining), the OGTT may display abnormalities at fasting (impaired fasting glucose, 110–125 mg/dl) or after a glucose challenge (impaired glucose tolerance, 2-hour glucose 140–199 mg/dl), which is more likely in younger children. Such a degree of β-cell impairment is similar to the β-cell dysfunction observed in individuals with type 2 diabetes. Individuals with LADA experience a very gradual decline in β-cell function. However, in individuals who eventually present with typical acute-onset type 1 diabetes, frank clinical diabetes develops within 1–2 years of the onset of oral glucose intolerance. By the time an insulin-dependent state occurs, manifested in acute symptoms of type 1 diabetes, β-cell mass is estimated to have declined by ~90% or more from baseline.

The potential of islet autoantibodies to predict type 1 diabetes is discussed below.\(^{130,131}\)

ICAs: Prediction of Type 1 Diabetes

In a prospective study of first-degree relatives of type 1 diabetic patients from the University of Florida,\(^{132}\) ICA titers >40 Juvenile Diabetes Foundation (JDF) units predicted a 70% 7-year risk for type 1 diabetes, ICAs of 20–40 JDF units predicted a 20% risk, and ICAs of 10 JDF units predicted a 10% risk. Other studies have also found that a higher ICA titer is associated with a higher risk for type 1 diabetes.\(^{133}\)

A question of great relevance is whether ICAs predict type 1 diabetes in individuals who do not have a family history of type 1 diabetes. This is an important issue because 85% of people who develop type 1 diabetes are from the general population, i.e., lacking a family history of type 1 diabetes. This question was addressed in the Pasco County, Fla., prospective study of schoolchildren.\(^{134}\) Approximately 10,000 normal schoolchildren were screened for ICAs. Those with ICAs (0.59% of the all children screened) were entered into a prospective study and followed for the development of type 1 diabetes. The 7-year risk for type 1 diabetes in these otherwise normal ICA-positive children was 45% compared to 43% in a group of age-matched relatives of type 1 diabetic patients (\(P = 0.3\)). Thus, ICAs appear to be as valuable for the prediction of type 1 diabetes in the general population as they are in relatives of type 1 diabetic patients.

Age at the time of detection of ICAs influences the risk for type 1 diabetes. In the University of Florida prospective family studies, the 7-year risk for type 1 diabetes in ICA-positive individuals <10 years old was ~80% versus 20% in those ICA-positive individuals >10 years of age.\(^{132,134}\) The risk in children <2 years old was ~90%. In the Bart’s-Windsor, Bart’s-Oxford prospective family studies,\(^{133}\) the risk for type 1 diabetes in the youngest ICA-positive quartile (<13.2 years old) was 62% versus 4% in the oldest quartile (>40.7 years old).

β-Cell function assessment in addition to the presence of islet autoantibodies influences the prediction of type 1 diabetes. The presence of decreased first-phase insulin responses during IVGTT in ICA-positive children predicts a 5-year risk for type 1 diabetes of 50–65%. During the Diabetes Prevention Trial–Type 1, these data were borne out in a 60% risk for type 1 diabetes.

IAAs, GADAs, and IA-2As: Prediction of Type 1 Diabetes

Most current studies involve investigations of multiple islet autoantibodies. The ability of a single autoantibody by itself to predict type 1 diabetes is limited. In the Florida school study, none of the IAA-positive, ICA-negative children developed type 1 diabetes.\(^{38}\) However, IAAs in combination with ICAs increased the risk of developing type 1 diabetes. In a study from the Joslin group,\(^{135}\) the predicted 5-year risk for progression to type 1 diabetes in ICA-negative/IAA-positive relatives was 17 versus 42% for ICA-positive/IAA-negative relatives and 77% for ICA-positive/IAA-positive relatives.

As noted above, some serial autoantibody studies in infants indicate that IAAs may, however, be the first islet
autoantibody to develop during progression to type 1 diabetes.136-139 The presence of IAAs do influence diabetogenesis.140 In the Seattle Family Study, which included first-degree relatives of type 1 diabetic probands, GADAs predicted a 50% 5-year risk for type 1 diabetes.141 In the Munich, Germany, family study,84 GADA-positive relatives had a 56% 5-year risk for developing type 1 diabetes versus a 24% risk in GADA-negative relatives. Surprisingly, Yu et al.142 found that very high levels of GADAs are less likely to predict type 1 diabetes. In the Munich, Germany, family study,84 IA-2A–positive relatives had a 64% 5-year risk for type 1 diabetes versus a 13% risk in IA-2–negative relatives.

As with any assay, the cutpoint chosen to define positivity influences the sensitivity and specificity of the assay. Raising the cutoff increases the predictive power of autoantibodies, although the number of autoantibody-positive people declines as the cutoff increases.

**Multiple Islet Autoantibodies: Prediction of Type 1 Diabetes**

Nondiabetic individuals who express combinations of islet autoantibodies have a much higher risk for type 1 diabetes than individuals who express fewer types of islet autoantibodies.141-143 Furthermore, the total number of types of islet autoantibodies is usually more important than the specific combination of positive islet autoantibodies. For example, the addition of any positive islet autoantibody to ICA positivity in the Bart’s-Windsor, Bart’s-Oxford prospective family studies144 raised the 15-year risk for type 1 diabetes from 47 to 66%. ICAs alone provided a 6% 10-year risk versus a 27% 10-year risk for ICAs plus one other islet autoantibody and an 88% 10-year risk for ICAs plus two other islet autoantibodies. Of the islet autoantibody–positive cohort, 36% were positive for ICAs alone, 38% were positive for ICAs and one other islet autoantibody, and 27% were positive for ICAs and two other islet autoantibodies.

From the Barbara Davis Center in Denver, Colo., first-degree relatives without any islet autoantibodies had a 5-year risk for type 1 diabetes of only 0.2%.145 When testing for GADAs, ICA512bdg autoantibodies (an IA-2A assay based on immunoprecipitation of the ICA512 autoantigen), and/or IAAs, the risk for type 1 diabetes was 15% with one autoantibody, 44% with two autoantibodies, and 100% with three autoantibodies.

In the Milan, Italy, family studies,146 the 6-year risk for type 1 diabetes was 26% for ICA positivity, 18.2% for GADA positivity, 17.9% for IA-2A positivity, and only 5.6% for IAA positivity. Whereas the 6-year risk with no islet autoantibodies was 0% and the 6-year risk with any one islet autoantibody was 2.9%, when two or more islet autoantibodies were present, the 6-year risk rose ~10-fold over the risk from any one islet autoantibody to 31.4%.

In the University of Florida study of 15,224 nondiabetic relatives of type 1 diabetic probands,147 ICAs were the most sensitive marker for the 5-year prediction of type 1 diabetes. ICAs carried a 74% risk for type 1 diabetes, GAD65A (autoantibody to GAD65) carried a 60% risk, IA-2As carried a 54% risk, IAAs carried a 50% risk, and IA-2Bs carried a 34% risk.147 The 5-year risk data demonstrate that as the number of islet autoantibodies increase in any individual, so does the risk for type 1 diabetes (Table 2).

**CONCLUSIONS**

Islet autoantibodies are very common at the time of onset of type 1 diabetes. Islet autoantibody testing can confirm autoimmunity in cases of new-onset diabetes and can differentiate type 1 from type 2 diabetes: islet autoantibody-positive individuals should be classified as having type 1a diabetes. The absence of islet autoantibodies, however, does not exclude type 1 diabetes.123 The appearance of islet autoantibodies in pancreas transplant recipients predicts recurrence of type 1 diabetes.148 Type 1 diabetes can occur after organ donation, and thus living kidney donors from families with histories of type 1 diabetes should be screened for islet autoantibodies.149 Individuals with phenotypic type 2 diabetes who express islet autoantibodies are affected with LADA. Islet autoantibodies predict type 1 diabetes in relatives of type 1 diabetic probands as well as in the general population.150 Combinations of autoantibodies enhance predictability.

Once therapies are developed that can prevent type 1 diabetes when applied in the prediabetic phases of β-cell autoimmunity,151 large screening programs for islet autoantibodies should be undertaken in children. Screening on at least two occasions appears warranted: before age 5 and before puberty. This is because diabetes appears first near age 5 and then peaks with puberty.152 Combinations of islet autoantibody assays, e.g., the simultaneous detection of GADAs and IA-2As,153,154 will likely supplant ICAs testing in future screening programs.

It is potentially valuable to predict type 1 diabetes because early treatment of type 1 diabetes with tight glycemic control preserves β-cell function, prolonging the honeymoon peri-

### Table 2. University of Florida Study of Nondiabetic Relatives of Type 1 Diabetic Probands

<table>
<thead>
<tr>
<th>Islet autoantibody status</th>
<th>5-year risk of developing type 1 diabetes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA-negative</td>
<td>3.2</td>
</tr>
<tr>
<td>ICA alone</td>
<td>5.3</td>
</tr>
<tr>
<td>IAA alone</td>
<td>9.1</td>
</tr>
<tr>
<td>Any two islet autoantibodies</td>
<td>28.2</td>
</tr>
<tr>
<td>ICA-positive + one other islet autoantibody</td>
<td>50.3</td>
</tr>
<tr>
<td>IAA-positive + one other islet autoantibody</td>
<td>55.7</td>
</tr>
<tr>
<td>Any three or four islet autoantibodies</td>
<td>66.2</td>
</tr>
</tbody>
</table>
2) early diagnosis and treatment of type 1 diabetes should prevent the development of diabetic ketoacidosis; and 3) prediction of type 1 diabetes provides an opportunity for entrance into trials to prevent type 1 diabetes.

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