Type 1 diabetes (T1D) is a chronic progressive autoimmune disorder with complex polygenic susceptibility, usually associated with certain HLA alleles (IDDM1 locus). Environmental factors, which are poorly defined, also contribute to the pathogenesis. T1D is characterized by lymphocyte infiltration into the islets of Langerhans in the pancreas, leading to inflammation and selective destruction of the insulin-producing $\beta$-cells, resulting in hyperglycemia (1). Patients with T1D fail to produce insulin and are dependent on exogenous insulin to maintain life. Although it is considerably less common than type 2 diabetes, the worldwide prevalence of T1D is increasing by approximately 3% per annum. The incidence varies widely among countries. In Americans under the age of 20 years, the prevalence of T1D rose by 23% between 2001 and 2009, and $>30,000$ people are diagnosed annually in the US with T1D. Progression to T1D is typically marked by the presence of islet-specific autoantibodies in the serum. In humans, autoantibodies are present months to years before disease onset, and a similar trend is seen in the nonobese diabetic (NOD) mouse model of autoimmune diabetes (2). The rate of T1D development varies among individuals, possibly due to non-HLA genetic factors and/or environmental factors beyond the initial trigger.

Most of the current information on the pathogenesis of T1D from the initial triggers to the final effector stages of $\beta$-cell destruction has been derived from animal models that mimic the human disease (3). $T$ lymphocytes are central determinants for $\beta$-cell destruction in T1D. Nevertheless, autoantibodies and B lymphocytes are components of some autoimmune diseases and may contribute to the pathogenesis of T1D. Multiple studies have documented the role of autoantibodies and $B$ lymphocytes in NOD mice. Observations include a low incidence of diabetes in NOD mice that are genetically deficient in $B$ lymphocytes and the prevention of diabetes by eliminating maternal transfer of autoantibodies from NOD mice to the progeny. A phase 2 clinical trial by the T1D TrialNet Anti-CD20 Study Group revealed that a single course of rituximab, an anti-CD20 monoclonal antibody that selectively depletes $B$ lymphocytes, slowed disease progression over 1 year in newly diagnosed T1D patients. Moreover, rituximab significantly reduced the amount of autoantibodies to insulin in the patients’ serum. However, it is not known if the effect of rituximab is mediated by reduced production of autoantibodies and/or by impairment of $B$-cell antigen-peptide presentation to CD4-positive $T$ cells. Thus, the potential role of autoantibodies in the pathogenesis of T1D in humans remains unresolved.

Patients with T1D commonly produce autoantibodies to islet cell cytoplasm (ICA), native insulin (insulin autoantibodies, or IAAs), the 65-kDa isoform of glutamic acid decarboxylase (GAD65), the insulinoma antigen 2 protein (IA-2), and variants of zinc transporter 8 (ZnT8). At least 1 autoantibody is present in $>95\%$ of individuals with T1D upon hyperglycemia detection (4, 5). ICA measured by indirect immunofluorescence in frozen sections of human pancreas are the most sensitive markers. However, the assays are cumbersome, labor intensive, and difficult to standardize and have been largely replaced by immunoassays. Most current autoantibody detection methods are based on quantitative radiobinding assays. Radiolabeled recombinant human insulin, GAD65, or IA-2 is incubated with patient’s serum, and the amount of radioactivity in the precipitated complex is proportional to the antibody concentration. Nonradioactive assays, such as ELISA-base formats, are limited for most islet cell antigens (currently available only for GAD65 and ZnT8).

Cross-sectional studies in relatives of patients with T1D have documented that the development of multiple autoantibodies augments the risk for progression to T1D. The risk of developing future T1D is highest in individuals with 2 or more autoantibodies. Multiple confounding factors have prevented formulation of a
The study evaluated the rate of progression to T1D in high-risk children (HLA DR/DQ genotype and 2 or more first-degree relatives with T1D) after islet autoantibody seroconversion (5). Data were combined from 3 prospective studies conducted in Colorado (Diabetes Autoimmunity Study in the Young, DAISY), Finland (T1D Prediction and Prevention, DIPP), and Germany (BABYDIAB and BABYDIET). Autoantibodies against insulin, GAD65, and IA-2 were measured in 13,377 children who were followed for 15 years. The frequencies of autoantibody analysis varied among the studies, but were conducted at least twice in the first 2 years after birth, then every 1 to 3 years (5). Because of its relatively recent discovery, ZnT8 autoantibodies were assessed only in children with autoantibodies in DAISY and BABYDIAB/BABYDIET studies. The key observations of this analysis (Fig. 1) are the following: (a) only 7.9% of the study population developed autoantibodies over 15 years; (b) 55% of these individuals expressed multiple autoantibodies; (c) the number of autoantibodies predicted development of T1D, ranging from 10% for 1 autoantibody to almost 100% if multiple autoantibodies were present [hazard ratios for progression to diabetes (compared to children with no antibodies) were 395.6 and 52.7 for multiple and single autoantibodies, respectively]; (d) within the subgroup of multiple autoantibodies, the combination of IAA with IA-2 had a greater risk of progression to T1D than the combination of either IAA/GAD65 or IA-2/GAD65; (e) autoantibodies were not a prerequisite for T1D, as a small group (0.2%) of autoantibody-negative children progressed to T1D; (f) faster progression to T1D after seroconversion was associated with younger age at seroconversion; and (g), the 3 studies performed in different parts of the world gave parallel time for the onset of T1D after seroconversion, suggesting that environmental factors after the initial trigger made no contribution to the progression of T1D. A notable omission from this study is the lack of measurement of antibody concentrations. Future reports should quantify autoantibodies to ascertain whether the concentration can be added as a factor for risk prediction. Notwithstanding this shortcoming, the study, which is the first longitudinal analysis from birth to development of T1D, substantially expands our knowledge of autoantibodies in T1D and has potential implications for the clinical laboratory community.

Measurement of autoantibodies in diabetes merits consideration. The paper does not provide details of autoantibody analysis (though cited references indicate that radiolabel was used for all autoantibody measurements), nor is the number of participating laboratories stated. Historically, autoantibody measurements have varied considerably among laboratories. The CDC and Immunology of Diabetes Society developed the Diabetes Autoantibody Standardization Program (DASP) to improve laboratory methods, evaluate laboratory performance, support development of sensitive and specific autoantibody measurement technologies, and develop reference methods. DASP provides serum from 50 patients with newly diagnosed T1D and from 100 control individuals (4). DASP was recently replaced by IASP (Islet Autoantibody Standardization Program). A WHO standard for both GAD65 and IA-2 has been established. Currently approximately 50 laboratories from approximately 20 countries participate in IASP. These laboratories demonstrated good concordance for GAD65, ZnT8, and IA-2, with the last improving with successive comparisons (4). By contrast, the IAA assay has poor performance. Few laboratories perform the ICA assay, which is no longer included in IASP. Implementation of autoantibody analysis for risk prediction of T1D will require resolution of several questions. For example, should assays be performed in all clinical laboratories or only specialized (reference) laboratories? Are clinical laboratories...
prepared to use radioactive materials for autoantibody assays? How many laboratories can be supported by IASP?

The concept of screening for islet autoantibodies is controversial. Some background information is required to evaluate what the Eisenbarth study has contributed to the debate. The objective of screening is to identify individuals likely to develop a disease for which treatment is available. Unfortunately, there is no therapy available to prevent, cure, or even delay the onset of T1D. Moreover, the vast majority (approximately 85%) of patients with newly diagnosed T1D do not have a first-degree relative with the disease, and approximately 20% of these patients produce a single autoantibody (4). Importantly, 1%–2% of healthy individuals have a single autoantibody against insulin, GAD65, IA-2, or ZnT8 and are at low risk of developing T1D. The low prevalence of T1D (approximately 0.3% in the general population) yields a very low positive predictive value if only a single autoantibody is detected. If screening is recommended, who should be screened, at what age, for how long, and how frequently? Eisenbarth and colleagues observed that T1D developed in almost all the children with genetic risk and multiple autoantibodies (5). These observations could imply that all high-risk children should be screened. Some experts maintain that screening for autoantibodies is superfluous because all patients are treated with insulin when hyperglycemic, while others counter that screening prevents development of diabetic ketoacidosis and allows early initiation of insulin. The time interval from seroconversion to onset of T1D in the Eisenbarth paper varied considerably, ranging from weeks to 18 years (5), thus not resolving the frequency or duration of analysis. On the basis of the available information, it seems reasonable to advocate that screening for autoantibodies should be performed in specialized laboratories for prospective clinical studies. The hope is that early identification of individuals at risk will yield effective therapies to protect the β-cells, thereby delaying or preventing the onset of T1D.

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References