Molecular Manipulation of Autoantibody Testing in Type 1 Diabetes: Two for One

Diabetes mellitus is an epidemic that, according to estimates, will have a prevalence of 5.4% of the global population by the year 2025 (1). The number of adults with diabetes in the world is expected to rise from 135 million in 1995 to 300 million by 2025. Although the majority of the escalation will be attributable to type 2 diabetes, the prevalence of type 1 diabetes is also increasing. Data pooled from 27 countries reveal that the incidence of type 1 diabetes is rising by 3% per year (2), predominantly in children and young adults. In addition, several studies report that ~10% of type 2 diabetes patients have islet autoantibody markers of type 1 diabetes, which predict insulin dependency (3, 4). Because the number of patients classified with type 2 diabetes is 10- to 20-fold greater than those deemed to have type 1 diabetes, this group of possible slow-onset type 1 diabetes is likely to have a significant impact on the severity of the diabetes epidemic.

Despite advances in diabetes treatment and monitoring that permit improved glycemic control, insulin therapy is inadequate to prevent patients from developing eye, kidney, nerve, and cardiovascular disease. Moreover, tight control, which cannot be achieved by all patients, is associated with a substantially increased risk of hypoglycemia (5). A desirable goal in the management of diabetes is to prevent the disease before clinical onset. Although several strategies aimed at prevention are under evaluation (6, 7), this will be attained only if type 1 diabetes can be diagnosed before the appearance of clinical manifestations of the disease.

Type 1 diabetes is an autoimmune disease of variable phenotypes. The classification of the disease is complicated by the fact that it is based on clinical symptoms rather than etiology. Both environment and genetics contribute to the pathophysiology. There are strong indications that environmental factors are important, and the list of viruses reported to be associated with the disease is growing. The genetic etiology is complex. Most patients have the HLA haplotypes DR3-DQ2, DR4-DQ8, or both, but depending on ethnicity, other HLA genetic factors are also associated with type 1 diabetes. The HLA factors are necessary, but far from sufficient, for the development of disease. The majority of the β cells of the pancreatic islets are destroyed at the time of clinical diagnosis. The loss of β cells is associated with multiple immunopathologic phenomena, best reflected by the appearance of autoantibodies to one or more of the following islet autoantigens: the 65-kDa isoform of glutamic acid decarboxylase (GAD), an insulinoma antigen-2 (IA-2 or ICA512), or insulin itself (8). The simultaneous presence of autoantibodies to all three antigens strongly predicts type 1 diabetes in first-degree relatives of individuals with the disease (9). The appearance of GAD65 autoantibodies in patients classified with type 2 diabetes seems to be the best predictor for the progression to insulin dependency (3, 10).

GAD65, IA-2, and insulin autoantibody tests are currently being used to identify individuals at risk for type 1 diabetes in several large screening programs involving newborns, schoolchildren, first-degree relatives, and the general population. The ultimate objective of these trials is to ascertain the diagnostic sensitivity, specificity, and predictive values of these serum markers for type 1 diabetes. Two major studies of islet autoantibody screening, the Diabetes Prevention Trial type 1 (DPT-1) (11) and the European Nicotinamide Prevention Trial (ENDIT) (12), are approaching completion. These trials, which enrolled subjects following antibody screening of >70,000 first-degree relatives in each study, should yield valuable information on the ability of islet autoantibody screening to detect individuals at risk of type 1 diabetes. Moreover, the results are likely to reveal whether our current knowledge of the disease is adequate to permit the institution of measures to prevent the clinical onset of diabetes by use of therapy with insulin or nicotinamide.

In this context, two reports that describe the concurrent analysis of GAD65 and IA-2 in a single assay (13, 14) represent an advance. The strategy adopted apparently independently by the two groups was inventive. Briefly, fusion proteins consisting of both GAD65 and portions of IA-2 were constructed by PCR and cloning. The IA-2/GAD65 chimera were radiolabeled with [35S]methionine by a standard in vitro transcription-translation method using reticulocyte lysate as first described for measurement of GAD65 (15). The radioactive protein was incubated overnight with serum samples, the immune complexes were separated, and bound radioactivity was quantified. Rickert et al. (14) evaluated their assay in 101 patients at clinical onset of type 1 diabetes and 245 healthy subjects. ROC analysis identified 4% antibody binding as the optimal cutoff, yielding a sensitivity of 80.2% and a specificity of 98.4% for type 1 diabetes. Comparison of the combined assay with analysis of GAD65 and IA-2 separately by standard assays revealed essentially identical performance between the two approaches. Analogous results were obtained by Zavialov et al. (13), although their population of 34 patients was younger and had diabetes for 7 to 9 years.

This novel approach, which allows simultaneous detection of two antibodies with a single antigen, appears to have a diagnostic efficacy similar to that obtained with separate analysis of the autoantibodies. This methodology is likely to reduce the time and costs of analysis. Much needs to be done, however, before the assays are suitable for incorporation in high-throughput, automated analysis in clinical laboratories. For example, it is not clear that an automated immunoassay can be developed using this strategy. Moreover, both methods require an overnight incubation. Whereas Rickert et al. (14) did not indicate the
reproducibility of their assay, Zavialov et al. (13) observed twofold variation in the incorporation of radioactivity in the transcription-translation step. Nevertheless, the assays indicate a continuing positive trend in the transition of analysis from research laboratories to a clinical setting.

An important deficiency in the measurement of diabetes-associated autoantibodies that requires resolution is standardization. Currently, each laboratory establishes its own cutoff values for GAD65 and IA-2, usually set at the 99th percentile or the mean plus 3 SD of values derived from analysis of 100 or more healthy subjects. This situation renders interlaboratory comparisons difficult. Efforts to achieve standardization have been slow. A World Health Organization standard has recently become available (16), and it is hoped that this serum will accelerate the progress of standardization and spark the interest of clinical chemists. The Centers for Disease Control is working with the Immunology of Diabetes Society to develop the Diabetes Autoantibody Standardization Program (DASP). A limited pilot proficiency testing program using samples obtained from patients with type 1 diabetes was initiated recently, and the results of the first serum exchange workshop are expected to be published soon. The acquisition of adequate amounts of proficiency material is difficult, however, and it is not yet clear whether this program will become generally available.

What is the role of measurement of autoantibodies in diabetes in 2001? There are two major areas, one is research, the other clinical. Large-scale autoantibody screening of newborns has been initiated in several countries, and first-degree relatives of individuals with type 1 diabetes continue to be recruited for a variety of screening and intervention trials. The emerging clinical use is the screening of a subset of patients with type 2 diabetes for autoantibodies, particularly GAD65 and islet cell antibodies, to identify those with possible slow-onset type 1 diabetes. Oral hypoglycemic agents often fail in autoantibody-positive patients, and early initiation of insulin therapy is likely to enhance metabolic control. Although additional data are required before this strategy gains widespread adoption in clinical practice, the interest is escalating because of the potential benefit to these patients.

Notwithstanding the challenges described above, these studies are emblematic of the progress being realized in the field of the autoantibodies associated with diabetes. We eagerly await the results of ongoing work that promises to maintain the advances in the diagnosis and possible prevention of this debilitating disease.

References


David B. Sacks1*
Ake Lernmark2

1 Department of Pathology
Brigham and Women’s Hospital
Harvard Medical School
Boston, MA 02115

2 Department of Medicine
University of Washington
Seattle, WA 98195

*Address correspondence to this author at: Brigham and Women’s Hospital, Thorn 530, 75 Francis St., Boston, MA 02115.
Fax 617-278-6921; e-mail dsacks@rics.bwh.harvard.edu.