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GAD-65 AUTOANTIBODIES AND THEIR ROLE AS BIOMARKERS OF TYPE 1 DIABETES AND LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA)

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SUMMARY

One of the hallmarks of autoimmune diabetes is the presence of adaptive responses directed against neuroendocrine proteins, such as glutamic acid decarboxylase (GAD). While GAD is widely distributed in neuroendocrine tissues, its specific significance in diabetes has paralleled the advances in understanding humoral and cellular immunity in type 1 diabetes (T1D) and in a subset of type 2 diabetes (T2D), going from the seminal discoveries of islet autoantibodies to the development and standardization of bioassays as diagnostic tools, to studies on the structure of GAD and its antigenic determinants. Autoantibodies targeting glutamate decarboxylase 2 (GAD-65) can accurately predict T1D development in combination with other surrogate humoral biomarkers and they are considered the most sensitive and specific biomarker to identify a subset of clinically diagnosed T2D termed latent autoimmune diabetes in adults (LADA). We and others provided evidence indicating that GAD-65 autoantibody detection should be part of the diagnostic assessment for clinically diagnosed T2DM, mainly because it predicts the rate of progression to insulin requirement in patients affected by LADA. More recently, GAD has been used as a tolerogenic vaccine to preserve beta cell function in autoimmune diabetes. While the results of phase III trials did not substantiate the earlier promise of phase I and II data, there are still many unanswered questions and approaches that need to be investigated in the applications of GAD in the therapy of T1D and LADA.

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INTRODUCTION

The plasticity of the interest in glutamic acid decarboxylase (GAD) across time is only matched by its relevance in human health and by the interest it continues to elicit as a tool to predict or possibly treat diabetes. It is difficult to conceive another molecule that has such critical biochemical relevance in a major pathway of neural physiology, with encoding genes that are necessary for normal development, tissue distribution that encompasses regulation of major parts of physiology in nerve function and metabolic regulation, and with a role as a self-antigen in type 1 diabetes (T1D) that allows detection of at-risk populations, as well as the development of potential therapeutic approaches.

What is GAD?

In basic biochemical terms, L-glutamic acid decarboxylase is the major enzyme in the synthesis of γ-aminobutyric acid (GABA), which is a potent inhibitory neurotransmitter and a critical component of neurophysiological function. It requires a cofactor, pyridoxal 5′-phosphate (i.e., PLP or activated vitamin B_6), to catalyze this reaction. GAD and GABA are mainly present in GABAergic nerve cells, but interestingly, they are also detected in certain non-neural cells and organs such as the pancreas (1-3), where GABA is stored in synaptic-like vesicles in islet beta cells (4). Although its functional relevance is presently unclear, it may be related to paracrine effects in the modulation of glucagon secretion in beta cells (5).

It is noteworthy that the term GAD basically denotes an enzymatic activity that is part of a critical synthetic pathway. In terms of gene and protein structure and function, GAD involves two major protein isoforms that catalyze GABA synthesis. One isoform has a molecular size of 67 kDa and is termed glutamate decarboxylase 1 (GAD-67), while the second one, 65 kDa in size, is called glutamate decarboxylase 2 (GAD-65). The proteins are also the product of separate genes (6) residing on different chromosomes. GAD-67 is the product of the *GAD1* gene, which is located on chromosome 10 (7), and GAD-65 is encoded by *GAD2*, located on chromosome 2 (8). However, in spite of these differences, the two isoforms share 65% homology in their amino acid sequence, with GAD-67 composed of 594 amino acids and GAD-65 of 585 (2, 3). The main sequence vari-

ation occurs in the isoforms' *N*-terminal region, which also appears to account for their different intracellular distribution (9). In the pancreas, GAD-65 is mostly detected in synaptic-like vesicles (SNLVs), while GAD-67 exhibits a cytosolic localization in the beta cells. Interestingly, both of these localizations are separate from the large dense-core insulin-secretory granules and other T1D islet cell autoantigens (2, 4). Their synthetic path in neural tissue and beta cells shows similarities; both isoforms are initially synthesized in the cytosol rather than the endoplasmic reticulum, and importantly, GAD proteins exhibit different translational regulation than proinsulin and other insulin-secretory granule autoantigens (10-12). In a thought-provoking report relevant to the hyperglycemic conditions of diabetes, medium- to longer-term exposure of isolated islets to glucose was shown to specifically increase GAD-65 transcription above the overall level of protein synthesis in the cells (13). However, there are still areas in need of elucidation in terms of the physiological effects of GAD-65. A key example is that in spite of the importance of the tissue localizations of GAD and the relevance of the synthetic path it modulates, research in animal models has yielded puzzling results, such as with the GAD-deficient mouse model in which islet cell function does not appear to be impaired (2, 3).

Historical perspective in diabetes

The earlier indications of the relevance of GAD as an important biomarker of T1D arose from immunoprecipitation studies (14) involving incubation of rat islets with radiolabeled methionine, followed by exposure of solubilized membranes to sera from patients with newly diagnosed T1D or controls. The results of these experiments showed that the sera from the patients precipitated a ligand with a molecular weight of 64 kDa. Moreover, as a critical indication of the potential of GAD as a predictor of T1D risk, the immunoreactivity to the 64-kDa antigen was observed both in approximately 80% of patients with new-onset T1D and also in individuals before they developed diabetes. The nature of the 64-kDa antigen was later elucidated by the work of Solimena et al., showing autoantibodies to GABAergic neurons and pancreatic beta cells in a rare condition termed stiff man syndrome (15). This finding facilitated the identification of GAD as the 64-kDa autoantigen in T1D by Baekkeskov et al. (16). Subsequent research on GAD-65 humoral autoimmunity in diabetes has led to the development of assays using reticulocyte-transcribed/translated GAD-65 cDNA to incorporate radiolabeled methionine, which is followed by exposure to sera and the subsequent immunoprecipitation of the labeled antigen, to determine whether the sera contains GAD-65-specific autoantibodies (17). This is now the standard procedure used throughout the world. The ability to assess humoral GAD-65-directed autoimmunity and the relative simplicity of the assay have established GAD-65 as the primary diagnostic endpoint to assess T1D-associated autoimmunity and T1D risk in a wide diversity of research goals and conditions, such as in different age-defined populations (18), in diverse ethnic groups (19), in the characterization of novel subpopulations of diabetes (20-23) and in the development of potential immunotherapies to prevent or treat T1D, to name but a few of the more relevant instances. As illustrated in Figure 1, the story of GAD and its applications in diabetes is a textbook example of evolution and progress in research, with landmark early studies, an abundant contemporary body of research and an exciting future, with significant potential for future avenues of basic and clinical studies.

ANTIGENICITY OF GAD

In T1D autoimmunity, only GAD-65 is antigenic despite the higher expression of GAD-67 in islet beta cells (2, 3, 24). Interestingly, in stiff man syndrome, the other well-documented case of GAD antigenicity, GAD-65 is also the major autoantigen (15). The differences in the antigenicity of the GAD isoforms merits scrutiny, particularly in view of the potential of GAD-65 to be used in immunotherapies. Both isoforms have similar activities in catalyzing GABA synthesis but exhibit clear differences in parts of their amino acid sequences and in their deduced tertiary conformation. GAD-65 is assumed to be more flexible in the *C*-terminal region (24), which is a potentially consequential feature, since these unique mobility regions in the GAD-65 molecule coincide with the region that exhibits key antigenic epitopes (24). However, this does not mean that the *N*-terminus of the molecule is not relevant. The GAD-65 *N*-terminal region is the site of palmitoylation, which is a feature not found in other T1D islet antigens and which, in turn, appears to determine the subcellular localization of GAD-65. This was highlighted by the report of Solimena et al. (25), showing that substitution of the first 29 amino acids of GAD-67 with the first 27 amino acid residues of GAD-65 redirects the normally cytosolic 67-kDa isoform to the Golgi complex, which is the usual localization of GAD-65, thus indicating that the first 27 amino acids of GAD-65 contain an intracellular localization signal. Other studies have also shown that post-translational modifications of the molecule in this region are highly relevant to its function. GAD-65 is palmitoylated on two cysteine residues in the *N*-terminal region and that feature determines its subcellular orientation (26). While GAD-67 remains persistently cytosolic, palmitoylated GAD-65 is incorporated into SNLV membranes, which results in the outward orientation of SNLV-associated GAD-65 facing the cytosol. It is in this position that GAD-65 forms a small protein complex containing the vesicular inhibitory amino acid transporter (VGAT). Meaningfully, this allows the local production of GABA by GAD-65 and the rapid transport of GABA by VGAT into the SNLV storage compartment in the beta cell (2).

Other relevant research has postulated that the *N*-terminal domain of GAD-65 (mainly expressed in pancreatic islets) involves a two-step modification, which leads to membrane anchoring involving post-translational hydroxylamine-sensitive palmitoylation (26). The anchored GAD-65 can be released from the membrane through an apparent enzyme activity in islets, indicating that the membrane anchoring and release steps are subject to regulation. The hydrophobic modifications and subsequent membrane anchoring and orientation of GAD-65 to microvesicles may be significant for its role as an islet autoantigen.

It is noteworthy that human vascular endothelial cells can process and present immune-relevant GAD-65 epitopes to autoreactive T cells (27). The in vitro transmigration by autoreactive T cells across an endothelial cell monolayer is stimulated by presentation of cognate peptide/HLA complexes on the endothelial cell surface and is also leukocyte function-associated molecule 1 (LFA-1)-dependent. In consequence, it is possible that the presentation of GAD-65 by islet endothelium in vivo could promote transmigration of circulating islet autoantigen-specific T cells that have been primed in regional lymph nodes against the pertinent GAD-65 epitopes.

There is also a well-documented body of research showing that the early humoral autoimmunity in T1D is directed against epitopes pri-

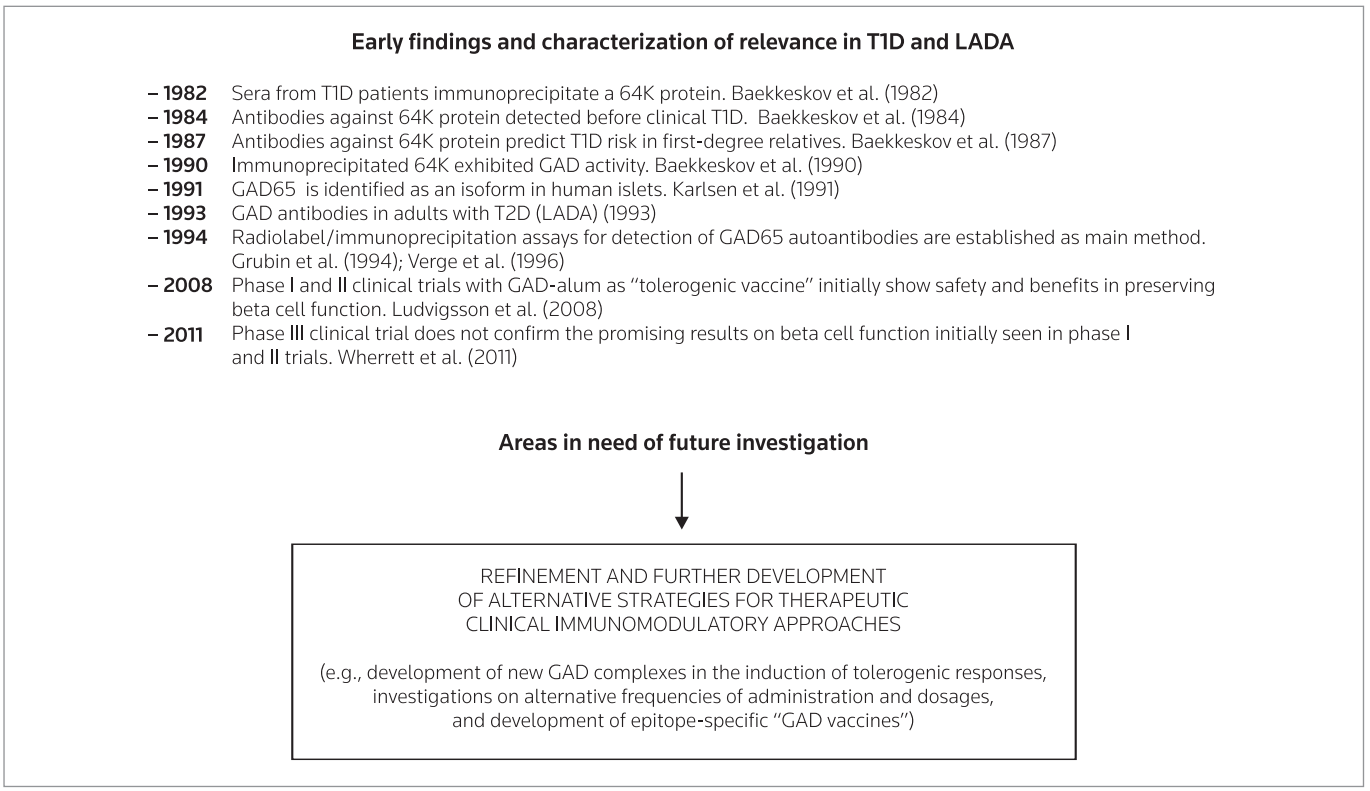


Figure 1. Highlights of GAD-65 in diabetes: past, present and an exciting future; T1D, type 1 diabetes; LADA, latent autoimmune diabetes in adults; GAD, glutamic acid decarboxylase.

marily in the middle region of the molecule, but also the *C*-terminus (28, 29). Additionally, recent thought-provoking structural crystallography studies aided by monoclonal antibody testing of antigenic determinants (30) have indicated that the more flexible *C*-terminal region of GAD-65 exhibits a grouping of autoantibody- and T-cell antigenic determinants.

Moreover, these studies indicated that there is close proximity of the B- and T-cell-related epitopes in the GAD-65 molecule, with the important implication that the antigen–antibody complexes could contribute to GAD-65-induced T-cell reactivity. While the importance of the middle region and the *C*-terminus as the major sites of immunoreactive epitopes have become well established, immunoreactive epitope localization is frequently not stable over time and can spread later to epitopes in the *N*-terminal region of GAD-65 (31, 32). Nevertheless, not all studies have substantiated time-related intramolecular epitope spreading (33, 34). The dynamic nature of GAD-65 humoral autoimmunity in T1D is likely to continue to grow as an area of research in order to develop more precise and effective diagnostic assays and immunotherapeutic approaches.

CLINICAL RELEVANCE FOR TYPE 1 DIABETES DIAGNOSIS

Estimation of risk

Autoantibodies against GAD are a valuable predictor of risk and progression to overt autoimmune diabetes. The combined positivity to GAD-65 and other islet autoantigens in the determination of T1D autoantibodies has a reliable and accurate predictive application (35-37). Interestingly, the intramolecular increase in antigenic determinants described for GAD in the previous section also has an intermolecular counterpart in the spreading of islet autoantigenic proteins during development of autoimmune diabetes, whereby the number of autoantibodies against different islet autoantigens generally increases sequentially (38). While insulin-directed autoantibodies have frequently been reported to be among the first to appear in animal models of T1D and in patients, and appear to be the more relevant single autoantibody type in the prediction of risk and progression to clinical T1D (39), it is also true that GAD autoantibodies are often found at early preclinical stages (40). This early presence and the relative ease with which they are assayed have made GAD autoantibodies the most commonly used autoantibody type for a first screen to assess risk of progression to the insulin-requiring stage of the disease. Additionally, an increase in intermolecular islet antigen spreading has enormous predictive value, as the number of autoantibodies against diverse islet autoantigens has been shown to correlate with risk and rate of progression to overt diabetes (41-43).

Therefore, in the body of knowledge that has developed from the original seminal studies on single and multiple humoral autoimmunity and prediction of T1D risk, a consensus has emerged to the effect that the detection of a single autoantibody type against islet pro-

teins has a moderate predictive power. However, screening for a single autoantibody type such as GAD-65 autoantibodies provides a valuable warning sign should the screen show positivity, and this single positivity is an indication that additional islet autoantibody assays are indicated in order to accurately assess risk and predict onset of T1D. The pertinent literature is plentiful and a variety of prediction models have been developed. Some models, for instance, show that triple autoantibody positivity against different islet proteins implies a risk of developing diabetes of 48-86% over 5 years and 64-86% over 10 years (35, 43), while other models have estimated that the risk of T1D onset with triple autoantibody positivity approaches 100% after 5 years (41).

Specific strategies to assay for islet autoantibodies have become part of clinical practice and depend on both the predictive value and relative methodological accessibility of the assays. However, aside from a trilogy of assays as determinants of risk, the need to use easier-to-implement and easier-to-monitor assays has led to the standardization of T1D autoantibody assays in current clinical practice. This has resulted in an alternative approach in which the most used strategy to screen at-risk populations for T1D is to assay for autoantibodies against the islet antigens insulin, GAD-65 and receptor-type tyrosine-protein phosphatase-like N (IA-2) (35). More recently, antibody assays against the zinc transporter 8 (ZnT-8) have been considered as an added screen (44). In either case, it is evident that GAD testing is a common denominator assay in either strategy for multiple autoantibody testing in order to estimate risk and progression rate to the insulin-requiring stage in T1D. In fact, GAD autoantibody determinations constitute the first assay in the examination of autoantibody reactivity in T1D in usual clinical healthcare practice.

The need to coordinate methodological protocols has resulted in standardization strategies, such as the adoption of a serum reference standard for GAD and IA-2 antibodies by the World Health Organization (WHO). Importantly, in 2000, it also led to the creation of the Diabetes Antibody Standardization Program (DASP) by a combined effort of the Immunology of Diabetes Society (IDS) and the Centers for Disease Control and Prevention (CDC). DASP involves an international network or consortium of 47 key laboratories in 18 countries, which provide technical support and training, proficiency testing and support development of methodologies, reference methods and materials. Its activities have also led to the current recognition of four major islet autoantigens in the clinical laboratory assessment of humoral T1D-associated autoimmunity –GAD, insulin, IA-2 and ZnT-8–, again underscoring the continuing relevance of GAD assays as a tool in the estimation of diabetes risk. The need to provide a basis for comparison of results has also continued to evolve into the harmonization efforts to produce unified protocols that can take into account differences in the use of standards such as the WHO standard relative to the use of common standards used by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) consortia laboratories (45).

While GAD autoantibodies are a first-line tool in the assessment of T1D risk and the combined positivity of GAD and other islet autoantibodies yields a highly accurate predictor of risk, the determination of GAD autoantibodies also has unique features in clinical application due to its age-related incidence in susceptible populations. It is well established that other islet autoantibodies such as insulin autoantibodies and receptor-type tyrosine-protein phosphatase N2 (phogrin) are highly accurate T1D risk predictors in childhood (46), and the age of detection of insulin autoantibodies in particular exhibits close associations with age of T1D diagnosis in children (39). In contrast, the presence of GAD-65 autoantibodies appears to be related to adult ages, and high titers have been shown to exhibit a correlation with longer-term diabetic complications such as retinopathy 15 years after T1D onset (47).

Therapeutic applications

The maintenance of production of self-antigens by the thymus and similarly specialized immune cells is a major factor in the maintenance of immune tolerance to these self-antigens (48, 49). More specifically and in regard to the topic of this review, it has been shown that the administration of GAD-65 to non-obese diabetic (NOD) mice can prevent the autoimmune loss of pancreatic beta cells, possibly by maintenance of exposure to the immune system, thereby promoting the recognition of "self" and immune tolerance. As a result of this body of investigations, a great deal of interest has motivated the examination of the therapeutic value of administering GAD in humans. Clinical studies have been conducted to assess if the administration of GAD-65 as a tolerogenic vaccine in patients recently diagnosed with T1D can prevent or ameliorate the loss of functional beta cells. This interest in turn led to the implementation of phase I and II trials in which patients with recent-onset T1D received subcutaneous GAD-65 formulated with aluminum hydroxide (GAD-alum vaccine). The results of the phase I and phase II studies showed promising effects for the GAD-alum vaccine in the prevention of the decline of insulin secretion, without adverse effects. Additionally, the GAD specificity of the response was substantiated by endpoints indicating that there was GAD-induced humoral and cellular autoimmunity (23). Subsequently, phase III trials were conducted to further assess the efficacy and dose-responsiveness of the GAD-alum treatment protocol in preserving beta cell function, results of which were recently made public. Unfortunately, the analysis of the endpoints related to insulin secretion and preservation of beta cell function, as shown in Figure 2, did not substantiate the earlier encouraging findings and led to the conclusion that translating the approach used successfully in animal models presents challenges to obtain similar effectiveness in patients for applications in clinical practice (50).

ROLE IN LADA

The incidence of both T1D and T2D has continued to expand worldwide and the understanding of the role of autoimmunity in diabetes has evolved along with the detection of new populations and subgroups. While the presence of autoantibodies against islet proteins continues to be the hallmark of classic T1D, a number of studies have shown that diabetes-related autoantibodies can be detected in 2-12% of patients with diabetes types that have not been associated with autoimmunity in their pathoetiology, such as T2D (51). The presence of T1D autoantibodies has led to the identification of unique subpopulations of diabetes that exhibit overlap or similarities with both T1D and T2D. As the detection and biochemical characterization of these novel subgroups presents conceptual, epidemiological and clinical challenges, there is a need to assess the value of

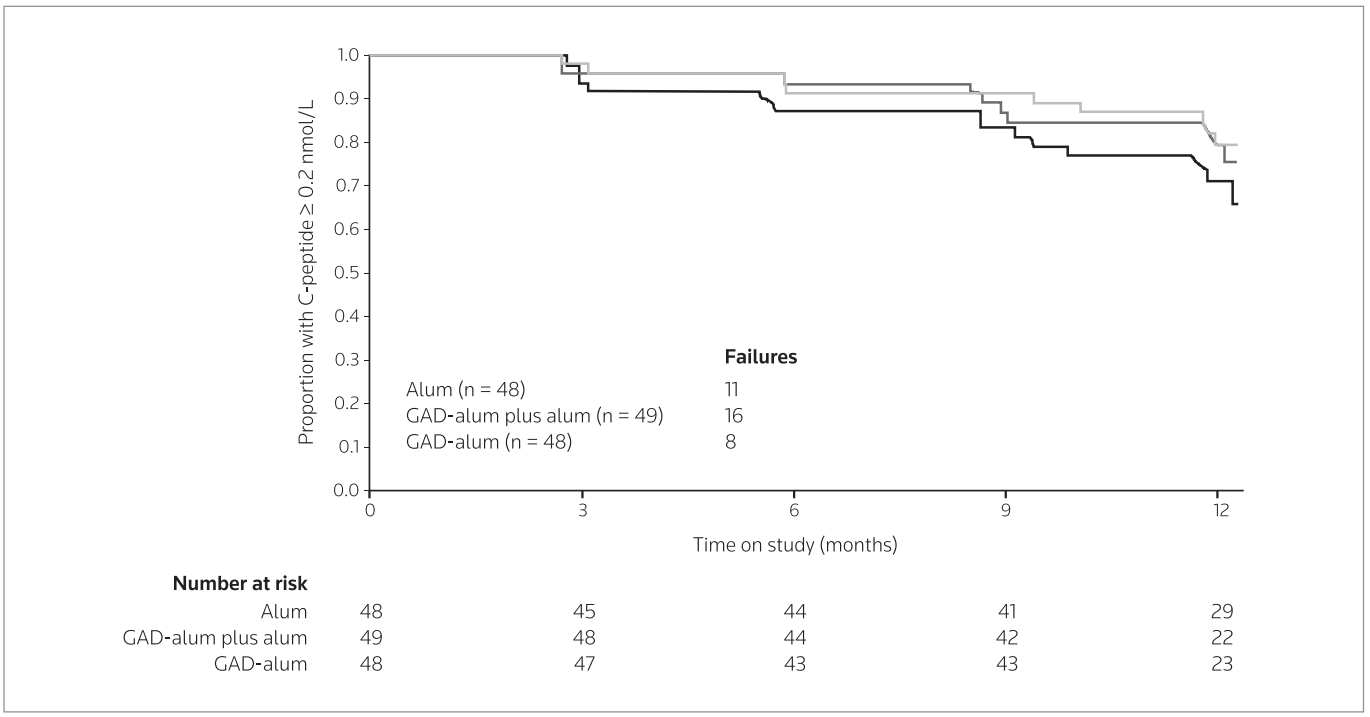
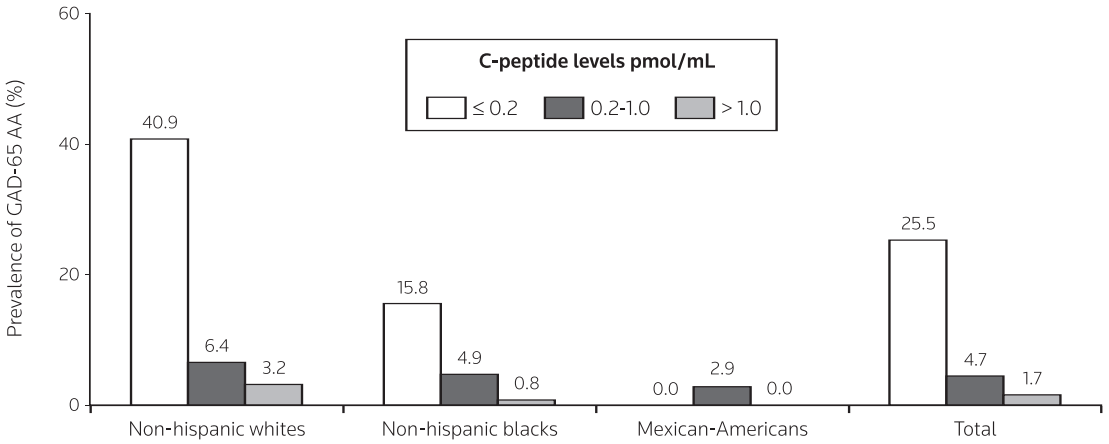


Figure 2. Proportion of patients with 2-hour peak C-peptide levels ≥ 0.2 nmol/L. Data are adjusted for age, sex and baseline value of C-peptide. GAD, glutamic acid decarboxylase. Reproduced with permission from Wherrett D.K. et al. and the Type 1 Diabetes TrialNet GAD Study Group *Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: A randomized double-blind trial*, Lancet 2001, 378(9788): 319-27.

specific markers that permit the accurate detection and treatment of these sui generis cohorts. A growing body of research has documented the case of patients who are generally non-obese adults, who present a T2D phenotype and puzzlingly also have circulating islet autoantibodies. These characteristics have led to the term LADA, sometimes also termed type 1.5 diabetes (51). Currently, the diagnosis of LADA relies primarily on the detection of GAD-65 autoantibodies in the serum of patients with clinically diagnosed T2D. In this regard, GAD-65 testing provides the critical first identifier to detect this unique diabetic disease phenotype, which in addition to the presence of GAD-65 autoantibodies, includes impaired insulin secretion and, in a significant number of cases, treatment with insulin as part of the therapy (52, 53). Additionally, it has also been demonstrated that, as is the case for patients with canonical T1D, individuals with LADA also possess T cells reactive to islet proteins, which is not the case in classic T2D without humoral islet autoimmunity. While the specific characteristics of LADA are the subject of ongoing examination and the possibility that both classic T2D and autoimmune diabetes may be present in the same patient can currently not be conclusively discarded, LADA remains the consensus term to describe older populations exhibiting GAD humoral autoimmunity.

The detection of GAD-65 and IA-2 autoantibodies in patients with diagnosed T2D by conventional criteria (American Diabetes Association [ADA] or WHO) commonly occurs in 5-10% of these individuals and may be higher if insulin is used as a therapy. The epidemiological consequences of this demographic assessment are many. First, there may be similar numbers of GAD-65-positive older patients with diabetes with GAD-65 autoantibodies as there are children with canonical T1D. Second, there is a need to investigate if more thoroughly characterized assays for relevant reactive epitopes of antigens involved in islet autoimmunity might detect a larger proportion of patients with T2D and islet autoimmunity. Therefore, testing for GAD-65 autoantibodies and the use of biochemically defined antigens in the assays might permit the identification of immunodominant LADA-associated epitopes. Hopefully, the relative ease of LADA assays might make the use of these tests part of the clinical characterization of patients diagnosed with T2D, as it might aid in predicting the rate of progression to insulin requirement in these generally adult populations. GAD-65 antibody-positive patients may be suitable for early insulin therapy or tighter and more closely monitored identifiers of glycemic control. In this regard, GAD-65 testing continues to be a unique primary screen in this group to clearly distinguish and suitably treat GAD-65 antibody-positive older patients with diabetes.

The application of GAD autoantibody testing in LADA and LADA-related groups has continued to grow in interest and scope. For instance, studies have shown that in adult populations, the prevalence of GAD-65 humoral autoimmunity and its correlation with beta cell function exhibits specific associations with racial and ethnic characteristics of the study population (Fig. 3), and is also a relevant biomarker in elderly patients in the assessment of cardiovascular status and T1D autoimmunity (18). The relationship between epitope immunoreactivity and LADA features is an important area of investi-



GAD-65 AA +	9 8 9	3 7 1	0 4 0	12 19 10
N	22 125 279	19 144 132	6 139 193	47 408 604
P* value	< 0.001	0.006	0.07	< 0.0001

Figure 3. Prevalence of GAD-65 amino acids (AA) by C-peptide levels (pmol/mL) and race/ethnicity among all participants with diabetes, NHANES III (N = 1,059). *Overall race/ethnicity-specific comparisons. Reproduced from Barinas-Mitchell E. et al. *Islet cell autoimmunity in a triethnic adult population of the National Health and Nutrition Examination Survey (NHANES) III*, Diabetes 2004, 53(5): 1293-302.

gation that has been shown in studies covering LADA-associated subpopulations. There appears to be a correlation between severity of development to insulin requirement and the presence of autoantibodies against both the middle region and the *C*-terminus in patients with new-onset T2D (54). Additionally, more recent observations suggest that some of the more widely known concepts about development of GAD epitope autoimmunity may need to be further elucidated and perhaps reassessed. While epitope spreading is a well-accepted and -documented phenomenon overall in autoimmune diabetes, the specific role of reactive epitopes in autoimmunity in LADA is likely to become an even more active area of investigation in view of a diversity of findings. A recent LADA-related comprehensive study in the U.K., termed United Kingdom Prospective Diabetes Study (UKPDS) (55), included the assessment of GAD-65 epitopes in 242 patients who had tested positive at the time of diagnosis, whose epitope immunoreactivity was assessed over a 6-year period, and appears to be in contrast to the concept of epitope spreading over time. The results from the study indicated that GAD-65 autoantibodies were directed mostly (in > 70% of the subjects) against middle-region or *C*-terminal epitopes but the immunoreactivity of the epitopes did not exhibit changes over the 6-year period. Other recent LADA studies have also shown novel different GAD-65 epitope associations that relate to ethnicity. In a report by Jin et al. (56) in Chinese populations, the major epitopes of humoral autoimmune reactivity were located in the middle region and *C*-terminus of GAD-65, in line with the current consensus. However, another LADA-related study in adult (mean age of 48 years) Japanese patients exhibiting slowly progressive insulin-dependent diabetes (57) described a unique epitope in the *N*-terminus of GAD-65 that was found to be prevalent in this subgroup and also shown to be inversely related to length of time before insulin is required therapeutically, suggesting the relevance of the GAD-65 *N*-terminus in LADA in specific cohorts. GAD-65 epitope specificity in LADA populations may also show associations with comorbidities such as thyroid autoimmune disease (AITD). In the study by Jin and coworkers (58), Chinese patients with LADA and AITD also exhibited a higher frequency of antibodies against both the middle and *C*-terminal regions of GAD-65 than those without thyroid autoimmunity, suggesting that epitope-specific detection of autoantibodies may aid in the prediction of thyroid autoimmune comorbidity in LADA. In addition to the higher likelihood of thyroid autoimmunity, it was also found that patients with LADA who had antibodies with both middle and *C*-terminus reactivity had lower levels of C-peptide and exhibited a requirement for insulin treatment. Although prima facie, the main difference in the clinical presentation between the studies is the presence of autoimmune thyroid disease in the Chinese report, the data must be interpreted with caution, as they cannot firmly be construed to represent overall specific epitope reactivities in the corresponding cohorts. Additionally, the described genetic associations, such as HLA haplotypes related to diabetes risk, provided interesting albeit limited insight in these studies. The Japanese study, describing GAD *N*-terminus reactivity, indicates that in their LADA-related group, the HLA DQA1*0303DQB1*0401 haplotype was more common than in control subjects, while in the related Chinese study (56), the differences between the immunoreactive GAD-65 *C*- and *N*-terminus epitopes among DQA1*-DQB1* haplotypes in patients with

LADA were largely inconclusive, possibly because of insufficient population sample sizes. In consequence, although these reports provide interesting insights, it is important not to overinterpret the role of HLA genotypes in assessing their effect on humoral autoimmunity in limited studies. These considerations await much larger studies and the necessary confirmations in additional publications. Nonetheless, they also highlight the need to further the depth and breadth of research on two key areas, namely GAD epitope immune recognition as it relates to comorbidities that show associations with T2D such as thyroid autoimmunity, and also the specific roles of genetic and immune interactions between ethnic and racial subgroups in regions that are increasingly the focus of diabetes research.

CONCLUDING REMARKS

Although the expression of GAD-65 is not exclusive to the beta cell of the pancreas, the relevance of GAD-65 as a specific biomarker in diabetes risk prediction, as well as a potential avenue for immunoregulatory therapies, has continued to expand as an area of investigation. The application of assays to detect autoantibodies against GAD-65 continues to be a primary screen for the detection of autoimmune diabetes. The knowledge gained from our understanding of the structural features of the GAD-65 molecule and their implications in the development of immunoreactive autoantigenic epitopes has provided useful tools to better identify risk and prediction to insulin-requiring stages in susceptible populations, not only in classic T1D, but also in more recently characterized forms of the disease, as is the case of LADA. These areas of research are likely to remain the focus of a continuing large body of investigations for the foreseeable future.

The use of GAD-65 as an immunomodulatory therapy also merits discussion. The current evidence describes promising effects on the preservation of beta cell function with the use of GAD-65 as a tolerogenic vaccine, at least in phase I and II studies. While a large and recent phase III trial did not substantiate the earlier and promising findings, questions and potential alternative avenues of therapy with GAD-65 still remain. These include different presentations of the GAD-65 molecule complexes, as well as alternative frequencies, dosages and routes of administration of the GAD-65 vaccine. These considerations need to be closely scrutinized and deserve further research. Even if the larger phase III trial provided disappointing results, the overall body of data are by no means discouraging and deserve further efforts. Future diabetes-related research on GAD-65 is likely to continue to broaden and deepen the knowledge and potential clinical applications of this unique versatile molecule.

DISCLOSURES

The authors state no conflicts of interest.

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