Anti-GAD antibodies: indications in clinical practice

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Abstract

The pathogenesis of type I diabetes (characterized by insulin deficiency) is caused by an auto-immune process against the pancreatic islet ß cells. It occurs when a genetic susceptibility interacts with multiple environmental agents. The evidence for an autoimmune basis comes from the recognition of insulitis and the presence of multiple auto-antibodies to ß cells constituents. In the early preclinical phase of type I diabetes, antibodies to islet cells antigens are detected, including: ICA (islet cell antibodies), IAA (insulin auto-antibodies) and anti-GAD (glutamic acid decarboxylase antibodies). The anti – GAD antibody is the main auto-immune marker. When detected in the preclinical phase of the disease, it allows us to predict future insulin dependency. Its contribution to the classification of type I diabetes in the young non-obese adults and in gestational diabetes is discussed. In the future, the use of anti-GAD could be useful in the primary prevention with antigen immunotherapy.

Keywords: antibodies anti-GAD, type I diabetes, autoimmunity.

Introduction

During the past decade, there have been profound changes in our knowledge of the etiopathology of type I diabetes.

Type I diabetes is a chronic disease with abnormal metabolism of carbohydrates, lipids, and proteins, due to an absolute or relative lack of insulin, which occurs progressively over a period of months or years. This autoimmune process is triggered by environmental factors in genetically predisposed individuals.[1] Regarding genetic factors, there is a known association with HLA DR3 and HLA DR4 haplotypes. As for the extrinsic factors, these include viruses, toxins, and dietary factors. Under the effect of these factors, and as a result of the expression of class II antigens of the HLA system, an immunological reaction against pancreatic b cells is triggered, with the activation of antibody-producing b lymphocytes and cytotoxic T lymphocytes, culminating in b cell lysis.

The autoimmune aggression phase is recognized by the appearance of several antibodies against constituents of the islet cells, including ICAs (anti-islet cell antibodies), AIAs (anti-insulin antibodies), anti-GADs (anti-glutamic acid decarboxylase antibodies), and others. In this phase, which is of variable duration and precedes the appearance of clinical symptoms or laboratory alterations, the detection of antibodies is the serological marker of underlying insulitis. Anti-GAD antibodies, with their heightened sensitivity and greater specificity, are easily detectable and become particularly important in the prediction and diagnosis of future insulin dependence.

Historical Data

In 1960, the first suspicions that type I diabetes is an autoimmune disease arose, but it was not until 1974 that this theory confirmed by the discovery of ICAs (anti-islet cell antibodies), which were the first auto-antibodies to be detected.[2] Discovered by Lernmark, ICAs were first isolated in the serum of children with recently-diagnosed type I diabetes. Later, in 1982, it was discovered that eighty to ninety percent of children with recently-diagnosed diabetes presented ICAs directed against a protein with a molecular weight of 64 kilodaltons, and this antigen was named 64 k protein.[3] Atkinson was the first to suspect that anti-64k protein antibodies are the best and earliest markers of diabetes.[4]

In 1990, Bakeskov developed specific techniques, including immunoprecipitation, for the detection of this protein targeted by the immune system, which led to the identification of this antigen as glutamic acid

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decarboxylase (GAD), an enzyme also recognized as the antigenic target of the autoimmune neurological disease known as stiff-man syndrome.[5] From 1991, through the work of many molecular biologists, the genetic sequence of GAD began to be mapped, enabling the identification of two distinct forms of GAD (GAD 65 and GAD 67), codified by two distinct genes.[6,7] Their amino acid sequences are seventy percent identical, and both forms of GAD are present in the islet cells and in the brain. In the islet cells, GAD 65 is the most abundant isoform, and seems to be the more important auto-antigen.[8] In 1993, Tobin confirmed that GAD is the initial target of the autoimmune response, coinciding with the appearance of insulitis, and proposed the idea of the possibility of preventing the disease through immunological interference at this level.

Animal studies are currently being conducted aimed at preventing diabetes through the inactivation of GAD-reactive T cells.

Anti-GAD antibodies

Anti-GAD auto-antibodies were first recognized in stiff man syndrome, a neurological disease that is often associated with type I diabetes.[9]

In this disease, the existence of antibodies against the enzyme glutamic acid decarboxylase inhibits the formation of the neurotransmitter GABA, interfering in neurotransmission. The anti-GAD antibodies react not only with the neurons, but also with the pancreatic b cells, which may explain the association of diabetes with this disease.[9,10] It has long been accepted that the autoimmune mechanism of type I diabetes may be triggered by extrinsic factors, the most widely recognized of these being viruses.[11] The recognition by Erlander of the resemblance that exists between the amino acid sequences of GAD 65 and the P2-C protein of the Coxsackie B4 virus supports the theory of molecular mimicry.[12] According to this theory, in a genetically predisposed individual, viral infection by Coxsackie results in the presentation of the viral peptide of P2-C to the immune system and, in particular, to the B and T lymphocytes, triggering and antiviral immune reaction and, subsequently, due to the antigenic resemblance, an anti-b cell reaction. The immune response could be perpetuated in the absence of the virus that initiated the process. The b cells would be destroyed, first by the viral infection and afterwards by the immune response, which would promote the release of the GAD protein, inducing an anti-GAD lymphocyte response.

It should also be noted that the immune response depends on genetic susceptibility. Anti-GAD positivity is correlated with the HLA DQ and HLA DR phenotypes. Thus, eighty-four percent of type I diabetics who are heterozygous for DR3/DR4 present anti-GAD antibodies, compared with forty-eight percent in type 1 diabetics who are heterozygous for an allele other than those previously mentioned.[13] The recognition that the destruction of b cells is mediated by T lymphocytes has enabled an understanding of the importance of anti-GAD antibodies as important immunological markers of insulitis.

The significance of anti-GAD antibodies

Adult

The classification of diabetes in non-obese adults aged between thirty-five and fifty years presents frequent diagnostic difficulties. Young diabetics include a heterogeneous group of patients in whom insulin deficiency, insulin resistance, and hyperinsulinemia often coexist. IDD (insulin-dependent, or type I diabetes) is classically characterized by sudden onset, severe symptoms, absolute dependence on exogenous insulin, and a tendency towards ketosis.[14] As it is an autoimmune disease, it can occur at any age.[15] While the onset is abrupt in children, requiring immediate insulin therapy, it is slow in adults, and can be mistaken for NIDD (non-insulin-dependent, or type II diabetes). In these cases, patients may initially be asymptomatic and not in immediate need of insulin, and are through diet and oral antidiabetic drugs over periods ranging from months to years. This slow onset with a period of insulin-independence reflects the progressive loss of b cells.[14,16,17]

NIDD is a disease of heterogeneous etiopathology which involves multifactorial genetic susceptibility, associated with environmental and lifestyle factors. It includes a wide spectrum of variations, from hyperinsulinemia to hypoinsulinemia with varying degrees of insulin sensitivity and insulin resistance. If the developed countries are witnessing an increase in the incidence of this disease with epidemic characteristics, this seems to be the result of changes from a traditional to a modern lifestyle. These cases are characterized by hyperinsulinemia and insulin resistance, with abdominal obesity and associated

dyslipidemia and high blood pressure, form part of the condition currently called plurometabolic syndrome (or syndrome X).[18,19]

However, in recent years, other forms of NIDD have been recognized, in which a lack of insulin secretion is prevalent. Some of these forms include cases of NIDD with hyperinsulinemia in the initial phase, followed by the depletion of pancreatic b cells and subsequent insulin deficiency. Other forms are associated with genetic mutations such is the case with various forms of MODY (Maturity-Onset Diabetes of the Young). This is a form of NIDD that appears before the age of twenty-five, apparently transmitted as a dominant autosomal trait, and not requiring insulin therapy for at least two years after clinical onset. There are three recognized subtypes of MODY, differentiated by underlying genetic alterations: MODY2 by mutations of the glucokinase gene, and MODY1 and MODY3 by alterations of the genes located in chromosomes 20q and 12q, respectively, that are not yet clearly understood.[20] Other forms include slow onset IDD. Hagopian and Zimmet showed that around twenty percent of adult diabetes progresses to insulin dependence, a percentage which can reach as high as fifty percent in the case of non-obese young adults.[18,19]

Progression to insulin dependence in adults may be slow, both at the onset and during its evolution, and it is a form of adult-onset, insulin-dependent diabetes with an autoimmune basis.[19,21] It usually occurs in young adults with low BMI, with lower levels of C-peptide, and with a high frequency of autoimmunity to specific organs (thyroid gland, gastrointestinal tract).

As in type I diabetes, immunological markers (ICA and anti-GAD) coexist with genetic markers (most often HLA DR3 and HLA DR4). The frequency of positivity for anti-GAD antibodies is as high as seventy-four percent in these cases.[22,23] Zimmet labeled this type of diabetes LADA (Iatent autoimmune diabetes in adults).[21] This refers to patients who are initially treated with diet and oral antidiabetic drugs over a period of time that can last from months to years, but who inexorably progress to insulin dependence. The identification of genetic and immunological markers capable of identifying these patients is essential for appropriate diagnosis and treatment.

Anti-GAD testing may be a routine procedure to detect latent insulin dependence, allowing more appropriate classification and treatment (*Table I*).[23]

TABLE I

Classification proposed by Zimmet

1 – IDDM
Known genetic contribution - HLA DR and HLA DQ
acute onset
LADA
not autoimmune
2 – NIDDM
Known genetic contribution (rare)
glucokinase
mitochondrial DNA
insulin receptors
insulin gene
Unknown genetic basis (majority of cases)
insulin resistance
defective insulin secretion
3 – GESTATIONAL DIABETES
temporary
IDD
NIDD
4 – OTHER TYPES
pancreatic
endocrine
related to poor nutrition
induced by medications
rare genetic syndromes

Anti-GAD antibodies, targeted against a well-characterized antigen, are currently considered useful in the prediction and diagnosis of future insulin dependence.[24] Anti-GADs have the twofold advantage of being as sensitive as ICAs and, more specifically, they have eighty percent sensitivity and more than ninety percent specificity in predicting insulin dependence.[25] These antibodies have a heightened predictive value, because, unlike ICAs, they may precede the onset of symptoms by ten years and persist for up to forty years after a diabetes diagnosis. Another advantage is that their levels are easy to measure, using techniques that can be easily standardized, such as immunoprecipitation. Disadvantages are that these antibodies are neither species-specific nor organ-specific, and that factors such as age and race (for example, lower frequency in Asians) influence their prevalence.

In conclusion, non-obese young adults pose frequent diagnostic difficulties. In these cases, anti-GAD testing may be very useful in predicting insulin dependence. Early insulin therapy might be an important form of immunotherapeutic intervention by preserving a greater number of functioning islet cells and reducing the risk of late microvascular complications of diabetes.

Children

Type I diabetes is rare before nine months of age and its prevalence peaks between the ages of five and fifteen years. Its evolution is generally rapid in patients under five years of age and slower in those above fifteen years of age. In children, other antibodies besides anti-GADs are important, particularly ICAs and AIAs. As regards anti-GAD antibodies, they are detected in seventy to eighty percent of children with recently diagnosed diabetes.[25,26,27] However, unlike ICAs and AIAs, anti-GAD antibodies have proven to be less sensitive (especially in boys under ten years of age).[27] Also, there seems to be no correlation between the level of anti-GADs and the degree of destruction of b cells in children, unlike that shown in adults by Petersen et al.[26,28]

Recently conducted population studies have shown that isolated screening of anti-GAD antibodies in children is not very useful in predicting type I diabetes. In children, maximum sensitivity and specificity are achieved by using various ICA markers associated with AIA and anti-GAD, in conjunction with genetic susceptibility markers (HLA) and data on tissue lesions.[26,27] Consequently, population surveys have shown sensitivity for ICAs associated with anti-GADs of ninety-nine percent in Sardinia and seventy-five percent in England and Italy. As for the type I forms of diabetes, anti-GAD antibodies appear to increase the sensitivity and the predictive value of ICAs and AIAs.

Pregnant women

Gestational diabetes includes different forms of the disease, from temporary diabetes, to NIDD, and a group of women with slow evolution to IDD. IDD that develops during pregnancy is characterized by a prolonged period of post partum remission (from months to years), which is the result of an increased need for insulin during the gestational period. This remission period is similar to the prediabetic phase and its recognition is fundamental.[28]

The value of anti-GAD antibodies as predictive markers of insulin dependence in pregnancy was suggested by Tuomilheto in a retrospective screening for anti-GADs in diabetic pregnant women. The sensitivity of this study in predicting insulin dependence was eighty-two percent and the specificity was one hundred percent, and it was observed that positivity for these antibodies may precede insulin dependence by ten years.[29]

Petersen et al. recently demonstrated that the presence of anti-GAD 65 antibodies during pregnancy is associated with a high risk of developing IDD. According to the same authors, positivity for these antibodies alone, regardless of their level, correlates with a rapid loss of b cell function, there being no relationship between their level and the time interval corresponding to the remission period.[28]

In conclusion, the routine use of anti-GADs, in association with other autoimmunity markers (particularly ICAs), may also be useful in identifying patients as they evolve towards insulin dependence.

Other implications of anti-GADs

A precise understanding of the etiopathology of diabetes is important for the possibility of its prevention. The recognition that b cell damage is progressive and may predate the onset of diabetes by years, in combination with the research around marker antibodies and the detection of genetic susceptibility markers and functional reserve markers (early loss of peak insulin secretion), have prompted multiple approaches to the prevention of this disease. Many centers have been developing immunotherapy protocols aimed at inhibiting the immune mechanism. Initially, generalized immunosuppressors, such as cyclosporine and azathioprine, were used, but they were not effective for long term prevention and were abandoned because of their side effects. Recently, monoclonal antibodies targeted against LT and nicotinamide have also been used without great success.

The most recent prevention strategies include the early use of insulin therapy. Early use of insulin therapy, by preserving b cells, seems to delay progression to insulitis by reducing antigen expression.[30] Now that GAD has been identified, cloned, and proven to be important in the development of diabetes, attempts are being made to use it in prevention. The administration of GAD is currently being studied in animal models with the objective of achieving tolerance to

this molecule and thus, inhibiting the triggering of the autoimmune response. Using the GAD antigen, the system of autoreactive T cells becomes permissive, offering a possible effective form of prevention in the near future (vaccine?).

Conclusion

Anti-GAD antibodies are currently the most important markers of autoimmunity in type I diabetes. They have the dual advantage of heightened sensitivity and specificity and they may precede clinical diabetes by several years, and persist for years after diagnosis. They are especially useful in the differential diagnosis of diabetes in non-obese adults and of gestational diabetes. Anti-GAD testing in non-obese adults may be a routine procedure for detecting insulin dependence, enabling more appropriate classification and treatment. Its application to high-risk groups will also allow the use of new forms of intervention in the prevention of this disease. ■

References

1. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 1994; 331: 1428-1436.

2. Bottazzo GF, Forin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; ii: 1279-1283.

3. Baekkeskov S, LandinM, Kristensen JK et al. Antibodies to a 64,000 Mr human islet cell antigen precede the clinical onset of insulin-dependent diabetes. J Clin Invest 1987; 79: 926-934.

4. Atkinson MA, Maclaren NK, Sharp DW et al. 64,000 Mr autoantibodies as predictors of insulin-dependent diabetes. Lancet 1990; 335: 1357-1360.

5. Baekkeskov S, Aanstoot HJ, Christgau S et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA- synthesizing enzyme glutamic acid decarboxylase. Nature 1990; 347: 151-156.

6. Erlander MG, Tillakaratne NJ, Feldblum S et al. Two genes encode distinct glutamate decarboxylases. Neuron 1991; 7: 91-100.

7. Bu DF, Erlander MG, Tillakaratne NJ et al. Two glutamate decarboxylases, 65 kDa GAD and 67 kDa GAD are each encoded by a single gene. Proc Natl Acad Sci USA 1992; 89: 2115-2119.

8. Hagopian WA, Michelsen B, Karlsen AE et al. Autoantibodies in IDDM primarily recognize the 65,000 Mr rather than the 67,000 Mr isoform of glutamic acid decarboxylase. Diabetes 1993; 42: 631-636.

9. Solimena M, Folli F, Denis-Donini S et al. Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and Type 1 diabetes mellitus. N Engl J Med 1988; 318: 1012-1020.

10. Solimena M, Folli F, Aparisi R et al. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. N Engl J Med 1990; 322: 1555-1560.

11. Szopa TM, Titchener PA, Partwood ND et al. Diabetes mellitus due to viruses - some recent developments. Diabetologia 1993; 36: 687-695.

12. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ. Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 1992; 89: 283-292.

13. Serjeantson SW, Kohonen-Corish MR, Rowley MJ et al. Antibodies to glutamic acid decarboxylase are associated with HLADR genotypes in both Australians and Asians with Type 1 diabetes mellitus. Diabetologia 1992; 35: 996-1001.

14. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28: 1039-1057.

15. Molbac AC, Christau B, Marner B et al. Incidence of insulin-dependent diabetes mellitus in age groups over 30 years in Denmark Diabetic Med 1994; 11: 650-655.

16. Groop LC, Bottazzo GF, Doniach D. Islet cell antibodies identify latent type 1 diabetes in patients aged 35-75 years at diagnosis. Diabetes 1986; 35: 237-241.

17. Tuomi T, Groop LC, Zimmet PZ et al. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993; 42: 359-362.

18. Zimmet P, MD, PHD. The pathogenesis and prevention of diabetes in adults. Diabetes Care 1995; 18; (7): 1050-1064.

19. Zimmet P: Type 2 (non-insulin-dependent) diabetes- an epidemiological overview. Diabetologia 1982; 22: 399-411.

20. Dussoix P, Vaxillaire M, Patrick B et al. Diagnostic heterogeneity of diabetes in lean young adults-classification based on immunological and genetic parameters. Diabetes 1997; 46: 622-631.

21. Zimmet PZ, Tuomi T, Mackay IR et al. Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. DiabeticMed 1994; 11: 299-303.

22. Thivolet CH, Tappaz M, Durand A et al. Glutamic acid decarboxylase autoantibodies are additional predictive markers of Type 1 diabetes mellitus in high risk individuals. Diabetologia 1992; 35: 570-576.

23. Zimmet PZ, Elliot RB, Mackay IR et al. Autoantibodies to glutamic acid decarboxylase and insulin in islet cell antibody positive presymptomatic Type 1 diabetes mellitus: frequency and segregation by age and gender. DiabeticMed 1994; 11: 866-871.

24. Palmer JP. What is the best way to predict IDDM. Lancet 1994; 343, 8910: 1377-1378.

25. Rowley MJ, Mackay IR, Chen QY et al. Antibodies to glutamic acid decarboxylase discriminate major types of diabetes mellitus. Diabetes 1992; 41: 548-551.

26. Batstra M, Pina M, Quan J et al. Fluctuations in GAD 65 antibodies after clinical diagnosis of IDDM in young children. Diabetes Care 1997; 20 (4): 642-644.

27. Chen QY, Rowley MJ, Byrne GC et al. Antibodies to glutamic acid decarboxylase in Australian children with insulin-dependent diabetes mellitus and their first degree relatives. Pediatr Res 1993; 34: 785-790.

28. Petersen J, Dyrberg T, Damm P et al. GAD65 autoantibodies in woman with gestational or insulindependent diabetes mellitus diagnosed during pregnancy. Diabetologia 1996; 39: 1329-1333.

29. Tuomilehto J, Zimmet P, MacKay IR et al. Antibodies to glutamic acid decarboxylase as predictors of insulindependent diabetes mellitus before clinical onset of disease. Lancet 1994; 343: 1383-1385.

30. Keller RJ, Eisenbarth GS. Jackson RA. Insulin prophylaxis in individuals at risk of type 1 diabetes. Lancet 1993; 341: 927-928.