Neurol Sci (2002) 23:145–151

REVIEW

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Glutamic acid decarboxylase autoantibodies and neurological disorders

Received: 16 May 2002 / Accepted in revised form: 10 July 2002

Abstract Glutamic acid decarboxylase (GAD) is the enzyme that catalyses the production of GABA, a major neurotransmitter of the central nervous system. Antibodies to GAD (GAD-Ab) were first recognised in a patient affected by stiff-person syndrome; subsequently they were reported in a large number of cases with type 1 diabetes. Recently GAD-Ab have been described in a number of patients affected by chronic cerebellar ataxia, drug-resistant epilepsy and myoclonus. These cases usually harbour other autoantibodies or are affected by organ-specific autoimmune diseases. The role of GAD-Ab is still unclear; the lack of experimental models makes it difficult to investigate their potential pathogenetic role. However two mechanisms have been suggested: the reduction by GAD-Ab of GABA synthesis in nerve terminals or the interference with exocytosis of GABA.

Key words GAD autoantibodies • Stiff-person syndrome • Chronic cerebellar ataxia • Epilepsy • Polyglandular autoimmunity

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Introduction

Autoantibodies (Ab) to neuronal antigens are usually detected in the serum and cerebrospinal fluid (CSF) of patients with paraneoplastic neurological disorders. However, anti-neuronal Ab can also be found in a number of other disorders of the central nervous system (CNS) of autoimmune origin, such as stiff-person syndrome (SPS) [1]. In this case, Ab are directed against the enzyme glutamic acid decarboxylase (GAD); this group of anti-neuronal Ab is known as GAD-Ab.

GAD is the enzyme that catalyses the conversion of glutamic acid in the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and is present in GABAergic neuronal cytoplasm and secretory vesicles. It is also expressed in pancreatic β-cells [2], testis, fallopian tube, liver, kidney and adrenal glands [3]. GAD is produced in two isoforms of 65 and 67 kD, encoded by two different genes localised on chromosomes 10 and 2; they probably derive from a common ancestral gene [4, 5]. Both GAD proteins are synthesised as soluble molecules, but only GAD-65 is modified in the NH_2-terminal domain and bound to the GABA vesicle membrane [6]. GAD-65 and -67 are similar in the median and carboxylic part of the protein, but are quite different in the aminic terminal, so they recognise different intracellular targets [7]. Both isoforms are detectable in the CNS while only GAD-65 is present in pancreatic cells.

GAD autoantibodies

GAD-Ab were first reported in 1988 by Solimena et al. [8] in a patient affected by SPS and epilepsy.

After the discovery of GAD-Ab in patients with SPS, Baekkeskov et al. [9] found these Ab in the serum of patients affected by type 1 diabetes without neurological disorders [9, 10].

At present, GAD-Ab are considered a marker of autoimmune diabetes since they can be found in the serum of the majority of patients, even before clinical onset of the disease [11, 12]. Indeed, GAD-65 is currently regarded as an important β-cell antigen able to induce type 1 diabetes and is probably involved in the development of the disease in non-obese diabetic mice [13, 14].

Although GAD-Ab can be detected in patients with SPS and type 1 diabetes, the autoantibodies present different characteristics in the two conditions. In SPS, Ab recognise GAD epitopes in linear form as evidenced by denatured protein staining in immunoblotting, which is usually negative in diabetic patients [9]. This is consistent with the different epitope specificity in the two groups of diseases [15–17]. Indeed, further studies have shown that Ab from diabetic patients recognise epitopes in the central and C-terminal sites of the protein, while in SPS they react with the C- and N-terminal sites of GAD-65 [16, 18]. Lastly, in SPS, Ab link the active enzyme site since co-incubation of purified GAD and SPS sera inhibits GAD enzymatic activity, as different from diabetic sera [15].

Several methods have been developed to detect GAD-Ab. To date, immunohistochemistry and radioimmunoassay represent the most reliable procedures for their identification in clinical practice.

Immunohistochemistry

Frozen sections of rat cerebellum are incubated with serial dilutions of patients' sera (and, when available, with CSF). Subsequently the sections are incubated with peroxidase- or FITC-conjugated rabbit anti-human IgG, IgA and IgM and the reaction is developed with diaminobenzidine tetrahydrochloride and hydrogen peroxidase when the peroxidase system is used. Positive sera stain the axon hillocks of Purkinje cells and diffuse nerve terminals in the molecular and granular layers of cerebellum [8, 19, 20].

Western Blot

Total homogenate of human cerebellum is separated by sodium dodecylsulphate gel electrophoresis and transferred to nitrocellulose membrane. Strips are incubated with patients' sera and polyclonal anti-GAD antibody (used as positive control) and then exposed to peroxidase-conjugated rabbit anti-human IgG Fab'. Blots can also be developed with chemiluminescent reagents. Immunoblot can also be performed using recombinant human GAD-65 (rGAD-65) transferred to nitrocellulose and developed with chemiluminescence. Positive sera recognise a band of 64 kD [21, 22].

Radioimmunoassay

The assay is performed with a commercially available kit following the manufacturer's instructions. Serum samples are incubated with ^{125}I-labelled human rGAD-65 and protein A-Sepharose is added. After centrifugation the precipitates are counted for ^{125}I with a gamma scintillation counter. The results are interpolated in the standard curve constructed using dilution of a positive control serum [23]. This method is widely applied to detect GAD-Ab in diabetic patients and it has the advantage to provide a useful quantification of these Ab.

Enzyme-linked immunosorbent assay

Ezyme-linked immunosorbent assay (ELISA) is performed using recombinant human GAD-65 (rGAD-65) reacting with patients' serum at different dilutions, developed with a colorimetric method and using an ELISA plate reader to calculate the optical density [24].

Other methods

GAD-Ab may be detected with other methods such as immunoprecipitation assay [25] and radiobinding assay, which are highly sensitive for type 1 diabetes diagnosis but rarely used owing to their difficult protocols [26, 27].

GAD autoantibodies and neurological disorders

A recent study has shown that the spectrum of neurological disorders associated with GAD-Ab is wider than previously believed [28]. In addition to SPS, these Ab have been found in a series of cerebellar ataxia cases [29–32], in drug-resistant epileptic patients [33, 34] and finally in a patient with branchial muscle myoclonus [35]. We briefly reviewed the clinical and immunological characteristics of these patients.

Stiff-person syndrome

Clinical features

SPS is usually a sporadic disease (excluding rare familiar cases); the autoimmune variant more commonly affects women [36]. The syndrome is of insidious onset, usually more frequent in middle age (30–60 years), and is characterised by painful spasms in axial muscles that progress to involve proximal leg muscles, usually asymmetrically in

GAD-Ab-positive cases [36]. Patients sometimes show symmetric spine deformity, typically lordosis or loss of normal spinal curvature.

Symptoms are initially intermittent and muscle contractions are exaggerated in response to sound, electrical stimulation and touch. Stiffness and spasms later become continuous, precipitated by voluntary movements and preventing normal gait [37].

The disease is clinically elusive and should be considered in patients with unexplained stiffness and spasms [38].

Several variants have recently been characterised and divided according to the relative distribution of clinical signs (Table 1) [39]. Progressive encephalomyelitis with rigidity is characterised by a subacute course leading to death within three years. Jerking stiff person syndrome presents a predominant brainstem involvement. Stiff limb syndrome is a focal form with predominant involvement of the spinal cord [40]. Within the autoimmune group a paraneoplastic variant has been demonstrated and is associated with anti-amphiphysin [41] and anti-gephyrin [42] Ab.

Pathophysiology

Electromyography (EMG) shows involuntary motor unit firing at rest; contraction of antagonist muscles fails to induce motor unit relaxation in leg muscles. In some patients, continuous motor unit activity in paraspinal muscles at lumbar levels presents a full interference pattern [43].

In 1963, Howard showed marked clinical improvement after benzodiazepine administration, suggesting involvement of the inhibitory system using GABA as neurotransmitter [44]. Continuous motor unit activity is also suppressed by epidural anaesthesia as well as by drugs with central action, such as baclofen and tizanidine [45].

Floeter et al. proved that the clinical features of SPS were compatible with dysfunction of the central inhibitory GABAergic mechanism [46]. Not all spinal GABAergic neurons are uniformly affected in SPS patients, whose differences suggest a heterogeneous pathophysiologic mechanism. Clinical variants of SPS (such as stiff-limb syndrome) may represent different examples of this heterogeneity [46].

Immunology

The association of SPS with a number of autoimmune diseases such as pernicious anaemia, autoimmune thyroid diseases, vitiligo, myasthenia gravis and type 1 diabetes has been already reported. Demonstration of GAD-Ab in the serum of 60%–70% of patients (Table 1) [1] suggests that an autoimmune mechanism of damage could be implicated in this subgroup of SPS cases. The oligoclonal bands and intrathecal synthesis of IgG usually detected in their CSF support this concept as recently stressed by Dalakas et al. [47].

Favourable responses to plasmapheresis [48], intravenous administration of immunoglobulin [49, 50] and immunosuppressive therapy [51, 52] have also been reported.

Cerebellar ataxia

Clinical features

In the series recently reported by Honnorat and coworkers [30], fourteen patients (13 women) with chronic cerebellar ataxia collected from different European centres showed high titres of GAD-Ab in the serum. The patients had a median age of 55 years at onset of disease. The cerebellar symptoms progressed slowly in almost all these patients and were clinically similar to familial (SCA2) or toxic cerebellar degeneration. All these patients presented severe gait ataxia while only a few exhibited limb ataxia; nystagmus and dysarthria were observed in most cases. In ten patients, the neurological symptoms prevented enjoyment of a completely independent way of life. The chronic evolution of symptoms and the follow-up excluded a paraneoplastic origin.

Brain magnetic resonance imaging (MRI) in some patients showed cerebellar atrophy with no evidence of brainstem involvement.

Immunology

Similarly to SPS, almost all these patients were also affected by a polyglandular autoimmune disorder characterised by type 1 diabetes (ten cases), thyroiditis, pernicious anaemia,

Table 1 Stiff-person syndrome (SPS) and its clinical variants

	Clinical features	Autoantibodies associated	Therapy
Classic SPS	Stiffness and rigidity in axial muscles and superimposed spasms	GAD-Ab (in 60%–70%) Anti-amphiphysin (5%) Idiopathic (35%)	Improvement after benzodiazepine, plasmapheresis or immunosuppressive therapies
Progressive encephalomielitis with rigidity	Widespread rigidity, myoclonus, brainstem signs, cognitive changes	–	Poorly responsive to therapy (death within 3 years despite therapy)
Jerking SPS	Brainstem myoclonus	GAD-Ab in few cases	Improvement after benzodiazepine, plasmapheresis or immunosuppressive therapies
Stiff-leg syndrome	Stiffness of one or both legs sparing the trunk	GAD-Ab in few cases	Improvement after benzodiazepine, plasmapheresis or immunosuppressive therapies

myasthenia gravis and psoriasis in addition to several other organ-specific autoantibodies in their serum.

In ten patients, CSF analysis showed oligoclonal IgG bands with an high IgG index. In the few patients in whom both serum and CSF were analysed, the titre of GAD-Ab was higher in the CSF, exhibiting intrathecal GAD-Ab synthesis.

Successful treatment of these cases with plasmapheresis or high dose of immunoglobulins has been reported [53] (and personal observation).

Epilepsy

Clinical features

GAD-Ab have recently been reported in patients with drug-refractory epilepsy [33, 34]. A man affected by complex partial seizures with temporal-lobe abnormalities on MRI, harbouring high levels of GAD-Ab in the serum and CSF, was described in 1998 [33]. CSF examination showed an inflammatory pattern with a high IgG index and oligoclonal bands [33]. The patient was also positive for islet-cell Ab (ICA) and improved after immunosuppressive therapy.

Recently GAD-Ab have been found in sera from 8 of 51 patients with drug-refractory epilepsy. Interestingly, patients with the highest GAD-Ab titre (measured by radiobinding assay) had a history of temporal-lobe epilepsy. In some patients, titres were as high as found in SPS cases [34]. MRI showed left hippocampal sclerosis in one patient. Finally, it is noteworthy that SPS cases sometimes present epileptic seizures [1].

Immunology

Few of these patients showed other autoimmune disorders, but serological positivity for anti-cardiolipin Ab (ACA), anti-thyroid peroxidase Ab (TPO-Ab) and anti-gliadin Ab was reported. One patient, with the second highest titre of GAD-Ab, had a history of hypothyroidism and oligomenorrohea. CSF was negative for oligoclonal bands and the IgG index was normal in most cases.

Myoclonus

A woman affected by palatal myoclonus and seizures, with high titres of GAD-Ab in the serum (in addition to ICA, anti-nuclear Ab and anti-parietal-cell Ab) has been reported [35]. She recovered after administration of benzodiazepine and phenytoin.

In our series of neurological patients harbouring GAD-Ab, a patient presented with myoclonus of floor of the mouth muscles. He was affected by type 1 diabetes and recovered after benzodiazepine administration (unpublished observations).

Palatal myoclonus is characterised by rhythmic, unintentional jerks of palatal muscles. Imbalance in the GABAergic connection between the cerebellum and inferior olivary nucleus is thought to underlie the disease.

Neurological diseases and polyglandular autoimmunity

Neurological disorders with GAD-Ab are usually associated with organ-specific autoimmune diseases or other autoantibodies. The association of two or more organ-specific autoimmune disorders (clinically evident or latent) is usually known as polyglandular autoimmune disease (PGAD), which is divided into four groups (Table 2) [54–56]. So far,

Table 2 Classification of polyglandular autoimmune diseases (PGAD)

Type 1 PGAD
Presence of at least two of:
Chronic mucocutaneous candidiasis
Idiopathic hypoparathyroidism
Addison's disease
Type 2 PGAD
Presence of both
Addison's disease
Thyroid autoimmune disease and/or type 1 diabetes
Type 3 PGAD
Thyroid autoimmune disease and/or other autoimmune diseases (excluding Addison's disease and/or hypoparathyroidism):
Type 1 diabetes
Chronic atrophic gastritis (with or without pernicious anaemia)
Vitiligo, alopecia, or myastenia gravis
Hypergonadotropic hypogonadism
Non-organ specific (or intermediate) autoimmune diseases (e.g. SLE, Sjogren's syndrome, rheumatoid arthritis)
Type 4 PGAD
Association not falling into any of the previous categories (e.g. alopecia and/or vitiligo and type 1 diabetes, myastenia gravis and type 1 diabetes)

SLE, systemic lupus erythematosus

SPS and myasthenia gravis have been the only neurological disorders reported in PGAD. On the basis of data presented in this review, we assume that ataxia, epilepsy and myoclonus have to be included as possible neurological manifestations of PGAD.

We stress the importance of identifying GAD-Ab in neurological patients affected by other autoimmune organ-specific disorders; nevertheless, it is important to check for other autoimmune diseases in GAD-Ab-positive neurological patients. This may help recognise a subgroup of neurological patients in whom procedures capable of modifying immunological derangement could be of some benefit.

Role of GAD autoantibodies

Specificity of GAD-Ab detection

Many arguments exclude the hypothesis that anti-GAD Ab merely reflect the presence of type 1 diabetes. Indeed, several reported cases were not affected by diabetes. In addition, GAD-Ab titres were significantly higher in neurological patients than in diabetic cases and in two of our cases, CSF titres were higher than in the serum, indicating intrathecal synthesis [33, 57]. This finding, coupled with the high IgG index and oligoclonal bands, supports the hypothesis of an active autoimmune process in the CNS. The wide range of neurological disorders associated with GAD-Ab is noteworthy; interestingly, in all these clinical conditions the GABAergic system appears to be involved. At present, we do not know what underlies the different clinical manifestations; however, the finding that different neurological diseases may be associated confirms a common pathogenetic mechanism. Indeed, in the major series of SPS, some patients were also affected by epilepsy [8], while the case with palatal myoclonus presented generalised seizures [35]. Finally, a woman with SPS later developed ataxia [29].

Possible role of GAD-Ab

The role of GAD-Ab in neurological diseases is still unclear and the lack of experimental models makes it difficult to investigate their pathogenetic role. It has been postulated that a humoral immune response to GAD could lead to functional impairment of GABAergic synaptic transmission in SPS and subacute cerebellar ataxia (supported by the improvement after benzodiazepine administration). Recently, two mechanisms have been suggested for the Ab-induced suppression of GABA release: reduction of GABA synthesis in the nerve terminal or interference with exocytosis of GABA.

Using neurophysiological methods, some authors have shown a dose-dependent modulation of basket-cell-inhibitory potentials by GAD-Ab. A down-regulation of GABA synthesis in basket-cell terminals, with a reduction of GABA release on postsynaptic Purkinje cells, has been demonstrated [58–60].

Other authors have shown that serum and CSF from SPS patients proved to reduce GABA synthesis in rat cerebellar extracts in vitro. This effect was dose-dependent and strongly correlated with the presence of GAD-65 Ab. Few GAD-65-positive diabetic sera altered GABA production, with no apparent correlation with the GAD-Ab titre [17, 61].

The GAD molecule is localised in the cytoplasm of neurons and pancreatic beta cells and is never exposed to the outer side of the cell membrane during GABA exocytosis. GAD-Ab from serum and CSF are therefore unlikely to have access to their target molecule in intact cells. This finding interferes with the hypothesis of in vivo inhibition of GABA synthesis by GAD-Ab. However, Ab may penetrate living cells, including neurons [62, 63].

We can also assume that GAD-Ab directed to the catalytic site of molecule may not need to access GAD to display a function. The Ab could mimic the structure of GABA and cross-react with GABA binding sites accessible on the neuronal surface, as anti-DNA Ab cross-react with DNA binding sites in DNAse I [64].

Finally, it must be considered that type 1 diabetes associated with GAD-Ab is a T-cell mediated disease; this cellular mechanism may even be implicated in the pathogenesis of neurological disorders associated with GAD-Ab as suggested by the recognition of GAD-65 epitopes by peripheral blood T cell of SPS patients [65].

Sommario *La decarbossilasi dell'acido glutammico (GAD) è l'enzima che catalizza la sintesi dell'acido gamma-aminobutirrico (GABA). Anticorpi diretti contro tale enzima (GAD-Ab) sono stati per la prima volta riscontrati in una paziente affetta da stiff-person syndrome (SPS) e successivamente riportati in un elevato numero di pazienti affetti da diabete tipo 1. Recentemente sono stati riscontrati GAD-Ab in pazienti affetti da atassia cerebellare ad evoluzione cronica, epilessia farmaco-resistente e mioclono del velo palatino. In tutti i casi i pazienti erano inoltre affetti da altre malattie autoimmunitarie organo e non-organo specifiche e, a livello sierologico, erano presenti altri autoanticorpi. Il ruolo dei GAD-Ab rimane ancora dubbio; la mancanza di modelli sperimentali rende difficoltoso stabilire l'eventuale ruolo patogenetico. Due meccanismi sono recentemente stati ipotizzati: la riduzione della sintesi di GABA da parte dei GAD-Ab a livello delle terminazioni sinaptiche o l'interferenza con l'esocitosi di GABA.*

Acknowledgments We thank Prof. C. Betterle for informative discussion about polyglandular autoimmunity and Mrs J.M. Flaming for revision of the manuscript.

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