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GAD antibody-spectrum disorders: progress in clinical phenotypes, immunopathogenesis and therapeutic interventions.

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GAD antibody-spectrum disorders: progress in clinical phenotypes, immunopathogenesis and therapeutic interventions

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***Abstract*:** Antibodies against glutamic acid decarboxylase (GAD), originally linked to stiff person syndrome (SPS), now denote the *"GAD antibody-spectrum disorders"* (*GAD-SD*) that also include autoimmune epilepsy, limbic encephalitis, cerebellar ataxia and nystagmus with overlapping symptomatology highlighting autoimmune neuronal excitability disorders. The reasons for the clinical heterogeneity among GAD-antibody associated syndromes remain still unsettled, implicating variable susceptibility of GABAergic neurons to anti-GAD or other still unidentified autoantibodies. Although anti-GAD antibody titers do not correlate with clinical severity, very high serum titers, often associated with intrathecal synthesis of anti-GAD-specific IgG, point to *in-situ* effects of GAD or related autoantibodies within the central nervous system. It remains, however, uncertain what drives these antibodies, why they persist and whether they are disease markers or have pathogenic potential. The review, focused on these concerns, describes the widened clinical manifestations and overlapping features of all GAD-SD; addresses the importance of GAD antibody titers and potential significance of GAD epitopes; summarizes the biologic basis of autoimmune hyperexcitability; highlights the electrophysiological basis of reciprocal inhibition in muscle stiffness; and provides practical guidelines on symptomatic therapies with gamma-aminobutyric acid-enhancing drugs or various immunotherapies.

Keywords: autoantibodies, cerebellar ataxia, encephalitis, GAD autoimmunity, hyperexcitability, stiff person syndrome

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Introduction

GAD (glutamic acid decarboxylase) is a pyridoxal 5'-phosphate-dependent enzyme, widely expressed within the central nervous system and pancreatic β-cells, that catalyzes the conversion of the excitatory neurotransmitter l-glutamate to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA).[1] Autoantibodies against GAD were first detected in 1988 in a patient with stiff person syndrome (SPS), epilepsy and type-1 diabetes mellitus (DM-1), pointing out to an immunological connection between SPS, DM-1 and epilepsy and highlighting since then that disruption of GABAergic neurotransmission results in neuronal excitability.[2] Over the ensuing years, anti-GAD antibodies have been also connected with other autoimmune neurological diseases associated with neuronal excitability that now comprise the "GAD antibody-spectrum disorders" (GAD-SDs) and include SPS, autoimmune epilepsy, cerebellar ataxia, limbic encephalitis, myoclous and nystagmus.[3]

As the clinical spectrum of GAD-SD in now widened and their overlapping symptomatology more clearly recognized, a number of puzzling clinical connections, diagnostic dilemmas and pathogenetic mechanisms have emerged. New information about GAD epitopes and the importance of GAD titers has further strengthened their specificity in defining GAD-SD. While low GAD antibody titers directed at different epitopes are seen

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in 80% of patients with DM-1,[4] and up to 30% of patients with GAD-SD also have DM-1, only patients with typical GAD-SD neurological syndromes exhibit very high titers. The paper aims to describe the evolved clinical manifestations of GAD-SD; discuss why antibody titers matter in diagnosis and immunopathogenesis; highlight how GABAergic neurotransmission results in such diverse clinical phenomena with reciprocal inhibition and muscle stiffness; and summarize the best therapeutic options to treat autoimmune neuronal excitability. Considering that SPS is not as rare as has been thought, but still misdiagnosed, based on the large number of patients seen in our clinic, the review is hoped to increase the awareness of these syndromes for practicing neurologists and facilitate early diagnosis and prompt therapy initiation.

Clinical manifestations

SPS

SPS, first described by Moersch and Woltman in 1956,[5] is the commonest and most characteristic clinical subtype of GAD-SD. Although its precise frequency is unclear, based on the large number of patients referred to us in the last 30 years and having the opportunity to screen, examine, treat and follow many such patients in-person, rather than from chart reviews and retrospective data collection, we believe it is a fascinating disorder, more common than previously thought, but still underrecognized or misdiagnosed. SPS is twice as common in women than men, frequently represented among African-American women, with an average age of onset at around 30–35 years.[6,7] Patients typically present with muscle spasms and stiffness, concurrently in the thoracolumbar paraspinal and abdominal muscles, resulting in difficulties turning and bending, and progressive muscle rigidity with hyperreflexia and spasms, mainly in the truncal and proximal leg muscles.[8] Severe truncal stiffness resembles a "statue" or a "freezing"-like appearance and patients often describe that they walk like a "tin-man". They often have an accompanying severe anxiety, often misdiagnosed as a primary anxiety disorder, and task-specific phobias[9] that include fear of walking and falling. Symptoms of muscle spasms and stiffness can be precipitated by unexpected stimuli, including sounds, like a phone ringing or a siren, sudden touches or emotional upset. In some cases these events can cause severe and continuous painful spasms, along with stiffness in the thoracic muscles with breathing difficulties, tachycardia and hyperhidrosis, a condition we have labeled "status spasticus", requiring emergency admission for intravenous diazepam.[10] Electrophysiological studies have revealed continuous activity of motor unit firing at rest, confirming that stiffness is caused by co-contractions of agonist and antagonist muscles.[11–13] Normal physiology is governed by reciprocal inhibition, which means that when one muscle (i.e. biceps) contracts, its antagonist (i.e. triceps) is automatically inhibited. Stimulated gamma neurons of the agonist muscle send information to the spindles to contract, while the antagonist's gamma neurons do not discharge due to inhibition of GABA interneurons (Figure 1). In pathologic situations of impaired GABAergic neurotransmission, as occurs in SPS due to reduced GABA from the cerebral motor pathways, the gamma motor neurons discharge continuously because inhibitory signals are inhibited, resulting in bursting overstimulation of the muscle spindles expressed as simultaneous hypercontraction of both agonist and antagonist muscles (Figure 1). This is clinically manifested with muscle rigidity and stiffness and electrophysiologically as continuous motor unit activity in agonist and antagonist muscles. Up to 80% of SPS patients have autoantibodies against GAD, the rate-limiting enzyme for GABA synthesis.[14] These antibodies may interfere *in vitro* with GABA production and *in vivo* with the entire GABAergic system,[15,16] explaining the unbalanced neurotransmission and the ensuing enhanced hyperexcitability expressed as spasms and stiffness. Since GAD is also expressed in pancreatic cells and patients with DM-1 have low-titer anti-GAD antibodies, as discussed below, up to 35% of SPS patients may also have DM-1 along with and other autoimmune diseases, such as vitiligo, pernicious anemia, celiac disease or thyroiditis.[4,17,18]

The diagnostic criteria for SPS, as revised in 2009,[17] include: (1) stiffness of the axial muscles, particularly the abdominal and thoraco-lumbar paraspinals, leading to hyperlordosis; (2) superimposed painful spasms triggered by unexpected tactile or auditory stimuli; (3) severe anxiety with task-specific phobias especially in anticipation of physically challenging tasks; (4) electromyographic evidence of continuous motor unit activity of agonist and antagonist muscles; (5) absence of other neurological findings that may suggest an alternative diagnosis; and (6) highly positive GAD-antibody titers by

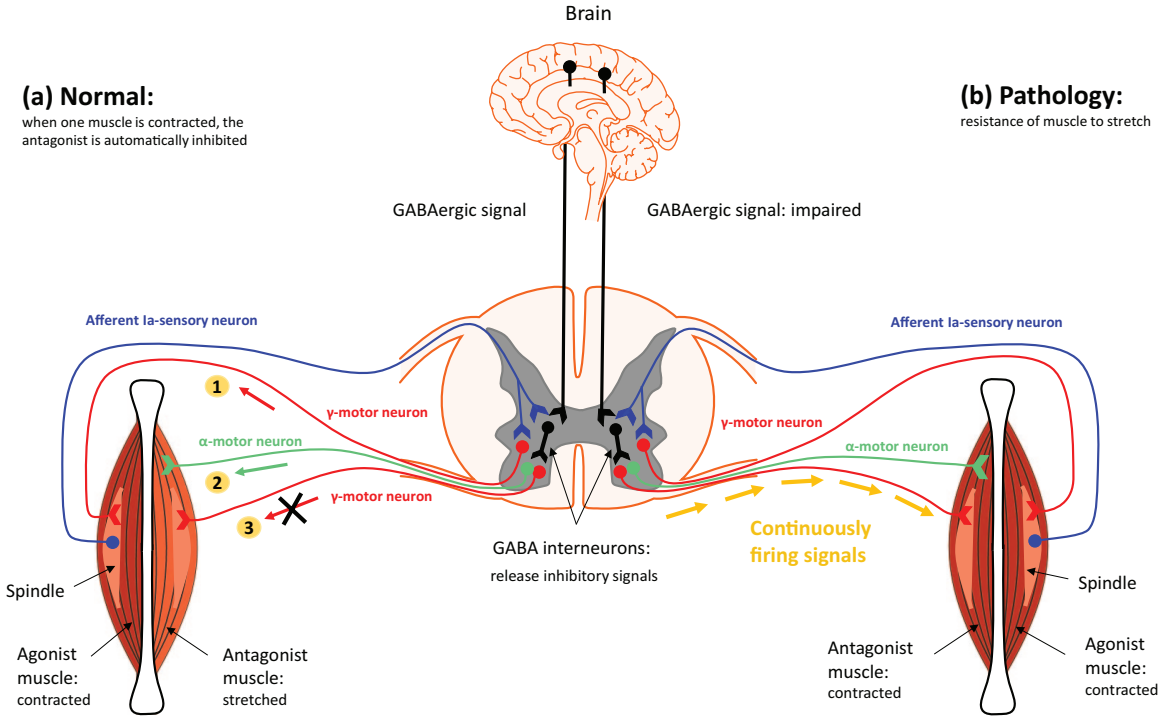

Figure 1. Reciprocal inhibition and stiffness generation in stiff person syndrome patients.
(a) Normal function: when one muscle is contracted, its antagonist is automatically inhibited. Afferent Ia sensory neuron fires, bringing information to the spinal cord, and stimulates the gamma neurons. Then, the gamma-motor neurons of the agonist muscle send signals to the spindle to contract [1], while the gamma motor neurons of the antagonist muscle do not discharge (X) due to inhibition of GABA interneuron [3]. As a result, the alpha-motor neuron of antagonist stretches (relaxes) the muscle (2) (interneuron: releases inhibitory mediators).
(b) Pathology: if the motor neuron is continuously firing signals, while there is no inhibition of the GABA interneuron to the antagonist muscle, the whole muscle will continuously be stimulated and will become hypertonic (spastic), without the ability to stretch (relax), due to concurrent contraction of the agonist and the antagonist muscles, as happens in stiff person syndrome that presents with stiffness and hyperexcitability.

immunocytochemistry, Western blot, enzyme linked immunosorbent assay (ELISA) or radioimmunoassay. Although these criteria best describe "classic or typical SPS", some patients with positive anti-GAD antibodies may not exhibit all the aforementioned symptomatology.

In our longitudinal study of 57 anti-GAD-positive SPS patients, which represents the largest clinical series of personally examined patients every 6 months for a two-year period to assess disease progression, the most common initial symptom was the insidious onset of proximal leg stiffness followed by rigidity in the lumbosacral paraspinals, thoracic and abdominal muscles. Axial muscle stiffness (truncal and proximal legs), lumbar hyperlordosis and impaired gait were first signs in 68% of these patients with 28% of them also having various degrees of facial muscle stiffness.[19] About 15% of the patients with typical SPS symptomatology also had ataxia, dysarthria and dysphagia, overlapping with the cerebellar variant, as described below, an important distinction because these patient subsets do not fully respond to immunotherapies.[20] Exaggerated reaction to various external stimuli and "startle response" were present in all patients except two. Marked anxiety related to unprotected falls or in anticipation of physically challenging situations was seen in 52 of 57 patients; 21 patients experienced chronic anxiety combined with intermittently depressed mood. Simple phobias, such as fear of walking in open and crowded places, crossing a street or taking escalators, were reported by more than 10% of patients with several also having task-related phobias, such as fear of public speaking. Most patients had been misdiagnosed with conversion or functional disorder because their falls were attributed to avoidant behavior and heightened mental anticipation. Other common misdiagnoses were myelopathies, dystonias or Parkinsonism. Many patients reported muscle

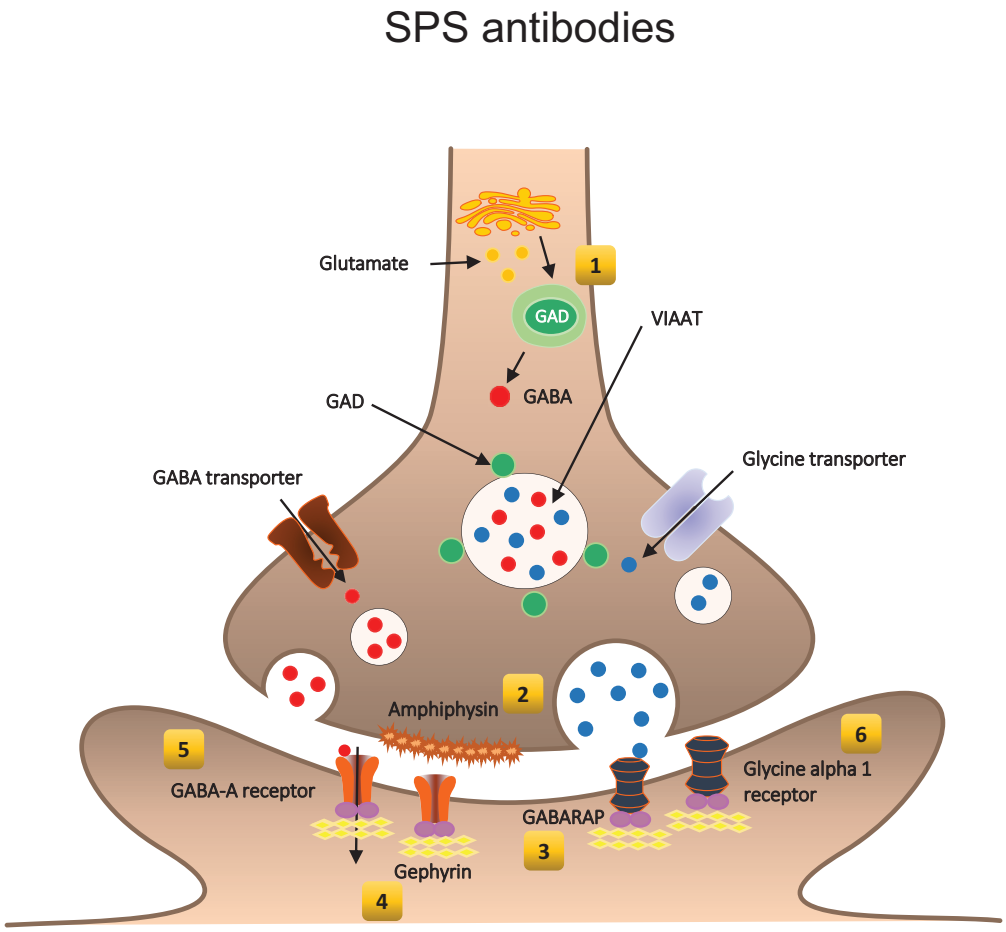

Figure 2. Autoantigenic targets associated with the Central Nervous System inhibitory synapses in patients with stiff person syndrome.
The pre-synaptic antigens are GAD (1), the enzyme that synthesizes GABA, the main inhibitory neurotransmitter, and amphiphysin (2), a synaptic vesicle protein responsible for endocytosis of plasma membranes following GABA release. Post-synaptically, the main targets are GABA-A Receptor Associated Protein (GABARAP) (3), gephyrin (4), a tubulin-binding protein needed for clustering both GABA-A (5) and glycine receptors (6). The most common antigen in stiff person syndrome is GAD followed by glycine receptor (vesicular inhibitory amino acid transporter; VIAAT). Modified from Dalakas.[118]
GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase.

pain along with painful spasms and some had been on narcotics.

Although some patients may manifest concurrent neuropsychiatric symptomatology that when prominent necessitates the need for psychiatric advice, others have been labeled as having a functional disorder. Formal neuropsychiatric testing in 10 consecutive patients seen at the (National Institutes of Health NIH) did not meet Diagnostic and Statistical Manual for Mental Disorders IV criteria for phobic disorder.[9] It was felt by our mental health colleagues at the National Institutes of Mental Health (NIMH) that the patients perceived their fears and anxiety as realistic, arising from the possibility of falls caused by SPS.[10] This still, however, remains a puzzling interpretation since we do not see such phenomena in other neurological disorders that present with weakness and falls.

Apart from the antibodies against GAD, other antibodies may also be positive in patients with SPS and hyperexcitability syndromes (Figure 2). We had found antibodies against GABAA receptor-associated protein in about 70% of the patients,[19] but these findings have not been replicated. Another autoantibody found in 10–15% of SPS patients is against glycine-a1 receptor (GlyR), a key inhibitory neurotransmitter. Anti-GlyRa1 antibodies, first described in progressive encephalomyelitis with

rigidity and myoclonus (PERM),[21,22] as discussed below, may be pathogenic as they recognize extracellular epitopes of the receptor expressed in the spinal cord, brainstem and cerebellum. Low-titer autoantibodies against GABAA receptor are also found in 10% of patients with SPS, cerebellar ataxia, epilepsy or encephalitis.[3] In about 5% of patients, SPS can be paraneoplastic, associated with antibodies against amphiphysin[23,24] and in a single case against gephyrin.[25] Apart from GlyR all targeted antigens are predominantly cytoplasmic and it remains to be determined whether they can transiently exhibit an extracellular domain during neurotransmission and exocytosis that may account for pathogenicity.[26]

Cerebellar ataxia

Anti-GAD antibody-associated cerebellar ataxia is the second most frequently encountered GAD-related neurological disorder within the GAD-SDs. It affects more women than men, often with comorbid DM1 or polyendocrine autoimmunity.[27–31] These patients exhibit gait and limb ataxia, nystagmus, severe dysarthria, dysphagia and oculomotor dysfunction, most often overlapping with the typical SPS symptomatology that worsens the overall clinical picture.[32] CSF analysis may show oligoclonal bands, without protein elevation, and intrathecal anti-GAD antibody synthesis.[20,28] Although in an old prospective study of 320 patients with sporadic cerebellar ataxia only six (2%) had GAD antibodies,[33] the frequency is probably higher today.

Whether the antibodies play a role in the pathogenesis of cerebellar ataxias is unclear.[34] Recent studies showed that a monoclonal GAD65Ab interferes with GABAergic neurotransmission in brain slice preparations and *in vivo* elicits in animals neurophysiological and behavioral effects mimicking cerebellar ataxias.[35] Intracerebellar administration of IgGs from CSF of patients with GAD-associated cerebellar ataxia impairs cerebellar modulation of motor control and contributes to lack of coordination.[36–40] The anti-GAD antibodies seem to act on nerve terminals of GABAergic interneurons depressing the release of GABA, resulting in hyperexcitability and eventually loss of Purkinje cells with diffuse proliferation of Bergmann glia.[41,42] Furthermore, a human monoclonal GAD65Ab elicits some pathogenic effects resembling those induced by cerebrospinal fluid (CSF) IgGs.[35,39] These patients may have overlapping clinical manifestations with epilepsy and SPS.[43] The magnetic resonance imaging (MRI) of cerebellar ataxia patients is normal with rare instances of mild cerebellar atrophy,[28] implying a functional blockade rather than a destructive process, hence the need to pursue immunotherapies.[17,44]

Autoimmune epilepsy

GAD antibodies were first associated with drug refractory temporal lobe epilepsy in 1998.[45] It is considered the third most common GAD65 neurological autoimmunity, and probably one of the most common causes of autoimmune epilepsy.[46] In early retrospective studies of 200 cases, GAD-antibodies were most frequent in patients with chronic pharmaco-resistant epilepsy, who often presented with temporal lobe epilepsy, epilepsia partialis continua or refractory convulsive and non-convulsive status epilepticus,[47–49] without inflammatory markers in the CSF or MRI but higher frequency of autoimmune comorbidities.[48,49]

In other retrospective series, anti-GAD antibodies were detected in 22% of patients with various epilepsies and concurrent autoimmune associations.[50] In a cohort of 233 patients with all types of epilepsy, the percentage of GAD-Abs was only 2.3%,[51] but when dissecting out the patients with focal epilepsy, GAD-Abs were present in 16% of all cases,[52] while among patients with temporal lobe epilepsy the percentage was up to 21.7%.[53] In a series of 1510 epileptic patients, three had musicogenic reflex seizures (MRSs) with two of them having GAD-associated epilepsy.[54] MRSs have also been reported in a patient with SPS comorbidity.[55] Although this clinical manifestation is extremely rare, MRS may be a distinctive type of epilepsy highlighted by anti-GAD antibodies, necessitating the need to test for GAD antibodies in all suspected MRS cases, even with normal structural MRI.[54] Among 13 children with epilepsy and mean age of 6 years (range 1–13 years), seven with suspected autoimmune epilepsy were positive for neuronal surface antibodies (N-Methyl-D-Aspartate Receptors in 3, Voltage Gated Potassium Channel-complex in one and GAD in another). Immunotherapy in nine neuronal surface antibody-positive cases was reported effective.[56]

Cytotoxic T lymphocytes have been found in histological preparations of temporomesial tissue from patients with pharmaco-resistant epilepsy associated with GAD antibodies who underwent temporal lobectomy.[57] These T cells may release perforin

and granzyme, leading to necrosis, apoptosis or electrical silencing of the respective neurons.[58] It has been suggested on these findings that epilepsy may be caused by potentially neurotoxic $CD8^+$ cells against GABAergic interneurons.[59]

The anti-GAD antibodies, by inhibiting GABAergic pathways, may result in hyperexcitability, which can explain epileptogenesis. The intrathecal synthesis of GAD antibodies has been proposed to suggest degeneration of GABAergic neurons and release of cytoplasmic proteins into the CSF, leading to an antibody-mediated immune response.[2] Such a humoral response may potentially inhibit the function of GAD, decrease the conversion of glutamate to GABA and eventually result in excessive excitatory neurotransmission that lowers the seizure threshold, contributing to manifestation of drug-resistant epilepsy.[60] At least 5% of SPS patients have seizures,[61,62] although in our experience the epilepsy in SPS is not refractory but rather easily controlled. On the other hand, only a small number of patients with GAD-associated epilepsy seem to respond to routine immunomodulating therapies, requiring more aggressive immunosuppressants.[59]

Limbic encephalitis

Autoimmune limbic encephalitis with anti-GAD antibodies clinically presents like the classic autoimmune or paraneoplastic limbic encephalitis,[63] with impaired working memory, psychiatric symptoms, seizures or altered level of consciousness.[64] In some patients there are oligoclonal bands in the CSF and intrathecal synthesis of GAD-Abs[65] but the causative role of GAD antibodies remains unclear.

PERM

PERM, described the same year as SPS by Campbell and Garland, was considered as an SPS-spectrum disorder.[66] PERM is now a distinct syndrome characterized by muscle stiffness, spasms, myoclonus and brainstem dysfunction with oculomotor abnormalities, dysphagia, gait ataxia prominent autonomic involvement and depressed level of consciousness.[67] It seems equally present in men and women although in our small series all patients were men. The hallmark of this disorder is the presence of anti-GlyR antibodies. As mentioned earlier, up to 15% of SPS patients with anti-GAD antibodies also harbor low titers of anti-GlyR-Abs.[68–70] An underlying tumor, especially thymoma or lymphoma, can be detected in about 20% of PERM patients.[71] Another autoantibody detected in four patients with PERM is anti-DPPX,[62] characterized by diverse symptomatology including prominent gastrointestinal manifestations, seizures, encephalopathy, sleep disturbance and dysautonomia. Limited histological data on PERM has demonstrated inflammatory and microglial changes and cell loss in the pons, medulla, cerebellum, spinal cord and autonomic ganglia.[8] Some PERM patients had increased T2 fluid-attenuated inversion recovery signal of spinal cord and brainstem on MRI.[67] In our small series, PERM is highly responsive to immunotherapies, especially if started early.

Nystagmus and abnormal eye movements

Isolated oculomotor dysfunction, characterized mainly by downbeat nystagmus and saccadic intrusions/oscillations but rarely opthalmoparesis, can be also associated with anti-GAD antibodies. In our experience, oculomotor dysfunction is not unusual among all GAD-positive patients with SPS, especially those with cerebellar ataxia.[72–75] The most common isolated GAD-positive oculomotor dysfunction is persistent horizontal or downbeat nystagmus, presumably related to excitability of vestibular nuclei driving the motor neurons of the ocular muscles, resulting in upward slow phase with quick compensatory downward phase or horizontal saccades.[73,76,77] Within the GAD-SDs, opsoclonus and myoclonus have been also observed.[78,79]

The importance of GAD antibody titers in the diagnosis of SPS-SD and distinction from DM-1

The importance of high GAD titers in connection with true neurological syndromes, compared with atypical or non-specific entities and DM-1, has been recently re-emphasized, pointing out that anti-GAD antibody titers do matter for diagnosis, even though their pathogenicity is still unclear. Patients with the described typical neurological diseases within the GAD-SDs normally have very high anti-GAD antibody titers compared with moderate–low titers in atypical syndromes and very low titers in DM-1.[74] Several assays are being used to detect anti-GAD antibodies, generating at times confusion in clinical practice, including quantitative radio-immunoassays (RIAs) and ELISAs.[27,61,80] These assays, initially developed

to detect the low titers of GAD antibodies in DM-1 patients, require adaptations with serial serum dilutions to ensure the accurate detection of high titers characteristic of SPS-SD patients. Other qualitative assays such as tissue immunohistochemistry, cell- based assays or line blots are mostly useful to detect structural epitopes of GAD65 antibodies.

Depending on the laboratory and the method used, reference values may be expressed in different units. A major US clinical laboratory uses RIA and defines high titers as > or =20 nmol/L.[81,82] According to their experience, these titers are found in classic SPS (93% positive) and in related autoimmune neurologic disorders, while values in patients who have DM-1 without a polyendocrine or autoimmune neurologic syndrome usually have titers < or =20 nmol/L. Other US and European laboratories use ELISAs, where the cutoff for positivity is >5 IU/mL. According to various clinical studies in SPS patients, titers are considered high when they are above 10,000 IU/mL.[74] The same applies to our own laboratory where we use ELISA. Titers within the range of 5–2000 IU/mL are seen in DM-1; only titers >10,000 IU/mL are associated with a neurologic disorder. In a recent study, the significance of serum anti-GAD65 antibody titers in connection with true neurological disease was re-confirmed by setting a cutoff value of 10,000 IU/mL in ELISA based on their specificity in concurrent testing by immunohistochemistry and cell-based assay. High (>10,000 IU/mL) titers conferred specificity for an autoimmune neurological disease in 94% of the patients, including SPS, cerebellar ataxia, chronic epilepsy, limbic encephalitis, or overlapping conditions; in contrast, lower concentration antibodies were seen in a broader spectrum of heterogeneous disorders.[83] The high titers were also associated with measurable anti-GAD antibodies in the CSF.

Collectively, the practicing neurologists need to be aware that anti-GAD antibody titers do matter: if high (>10,000 IU/mL), they are diagnostic for a true GAD-SD, necessitating immunotherapy; lower (<10,000 IU/mL) titers are associated with atypical or non-specific neurological disorders requiring further investigation, whereas very low titers (<2000 IU) are typically seen in DM-1 or are of unclear significance.[74] Importantly, GAD-Abs can also be detected within the various IVIg preparations and anti-GAD antibodies can be detected in patients receiving IVIg.[84] Most importantly, there is no association between GAD-Ab titer and disease severity or response to therapy without significant titer reduction after immunotherapies with either IVIg or rituximab based on two controlled studies we have performed.[85,86]

Intrathecal synthesis of GAD antibodies

There is strong evidence that in SPS, as well as in patients with other GAD-SDs, there is intrathecal synthesis of GAD antibodies. In a pivotal study, using the Link's formula, the ratio of GAD antibody concentration in the CSF to that in the serum was divided by the ratio of albumin concentration in the CSF to that in the serum; on this basis, values >1 are indicative of robust intrathecal synthesis.[57] In clinical practice, when the serum GAD antibody titers are above 10,000 IU/mL, GAD antibodies are also detected in the CSF[78] and, in these circumstances, a diagnostic lumbar puncture may not be necessary, especially in SPS patients where the stiffness in the lumbar paraspinals requires a radiology-guided puncture.

The demonstration, however, of intrathecal GAD antibody synthesis comprises the strongest evidence linking a neurological syndrome to autoimmunity, as suggested.[87] In clinical practice, testing the CSF for GAD antibodies is helpful in patients with serum titers below 10,000 IU/mL and in patients with seronegative GAD-spectrum disorder, especially those with encephalitis, and in patients with a seemingly functional disorder resembling SPS symptomatology.

GAD epitopes and their potential significance

GAD exists in two isoforms, GAD65 and GAD67, each encoded by a different gene, *GAD1* and *GAD2*, located in chromosomes 2q31.1 and 10p12, respectively.[88] GAD65 represents the membrane form of the enzyme. It is found in synaptic vesicles in the nerve endings and it is mostly utilized under circumstances where there is an urgent need of GABA synthesis and release.[89,90] GAD67 represents the soluble form, it is abundant in the cytoplasm and it is implicated in functions such as synaptogenesis, but not neurotransmission. The two isoforms are divided according to their linear sequence into three functional domains:

Table 1. Diagnostic work-up.

Clinical evaluation	Electrophysiology	Immunological studies	Neuropsychiatric examination
• Axial rigidity • Episodic spasms often triggered by unexpected external stimuli or emotional upset • No other neurological diseases that could account for stiffness	Continuous co-contraction of agonist and antagonist muscles with inability to relax	Sera and CSF (when applied) are tested by ELISA and CBAs for relevant autoantibodies: • Anti-GAD • Anti-GlyR • Anti-GABAA receptor • Anti-amphiphysin • Anti-gephyrin GAD seronegative patients need to be examined carefully as they may have a functional disorder	Structured interviews, whenever clinically required, for the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) Axis I (SCID-I/P) to explore the origin of anxiety and phobic symptoms

CBA, Cell-based Assay; CSF, cerebrospinal fluid; ELISA, enzyme linked immunosorbent assay; GABAA, gamma-aminobutyric acid A; GAD, glutamic acid decarboxylase; GlyR, glycine-a1 receptor.

an amino(N)-terminal domain, amino acids-aa 1-188 (1-197); a middle PLP-binding domain, where the catalytic center of the enzyme resides, aa 189-464 (198-473), and a carboxy (C)-terminal domain, aa465-585 (474-594).[91] The isoforms show an overall similarity within the middle and C-terminal domains, having 74% identity, while the N-terminals have 25%.[4] More specifically, patients with SPS and cerebellar ataxia show strong immunoreactivity to b78 epitopes, whereas those with DM-1 recognize more commonly the b96.11 epitopes.[87,92,93] Antibodies against b78 inhibit the enzymatic activity of GAD and provoke depression in the inhibitory synapsis in rat cerebellar slices, whereas these events are not observed with antibodies against the b96.11 epitope.[35] Patients with DM-1 harbor antibodies directed against conformational epitopes exclusively located in the PLP- and C-terminals domains.[94,95] In contrast, patients with SPS predominantly recognize linear epitopes in all three domains[96,97] and in the first 100aa that constitute the regulatory sequence in the N-terminal domain of GAD65 and does not react with DM-1 sera.[92,98–101] The GAD-Abs therefore in SPS exhibit a different epitope pattern of antibody reactivity with distinct biological effects, compared with DM-1.

Whether different epitope patterns exist among GAD-related syndromes is unclear. In one study, GAD-Abs from patients with limbic encephalitis were more likely to recognize epitopes in the N-terminal domain, compared with those with SPS, cerebellar ataxia or epilepsy, with the latter showing more reactivity to the C-terminal domain of the enzyme.[35,102] In our study, however, of 27 patients with diverse GAD-related syndromes, no differences in epitope specificities were found except in three patients with epilepsy.[103] Accordingly, the current data cannot explain the diverse clinical presentation based on different epitope binding patterns.

Diagnostic work-up and concerns in diagnosing GAD-negative SPS

The diagnostic work-up for SPS includes the clinical criteria mentioned earlier in conjunction with electrophysiological data, and relevant autoantibody seropositivity mainly highlighted by anti-GAD with cutoff titers >10.000 IU/mL by ELISA, as mentioned earlier (Table 1).[15,104] The main difficulty remains the seronegative SPS that represents close to 20% of patients with seemingly clinical SPS. Considering that functional components can co-exist in some patients with high-GAD titers, the main concern in practice is to ensure that patients with seronegative SPS do not have a functional disorder or another neurological disease mimicking SPS. Adherence to strict clinical criteria, neurophysiologic testing and neuropsychiatric assessment, if needed, are essential. Although an empirical trial with diazepam is often used, it does not ensure diagnostic accuracy because it cannot differentiate an organic from a functional disorder.

Pathogenesis of GAD-SD and biologic basis of autoimmune hyperexcitability

The pathogenesis of GAD-associated syndromes is still uncertain. Despite the key role of

autoantibodies in defining a rather heterogeneous group of overlapping disorders, it is not yet clear whether GAD antibodies are pathogenic or markers of aberrantly activated innate and acquired immunity.

Rats treated intracerebroventricularly with SPS-IgG showed a stiffness-like behavior, a decline of motor function as measured by time on the Rotarod test and a decrease in forelimb grip strength as compared with control IgG-infused rats. Additional studies of passive transfer of GAD-Abs from patients into rats or mice have shown continuous motor activity with repetitive muscle discharges and abnormally enhanced reflexes with increased excitability of anterior horn cells.[38,39,105] Whether these effects are related to anti-GAD or other antibodies directed at different synaptic antigens is unclear. On the other hand, diffusion of IgG and quantification of enhanced green fluorescent protein-labeled neurons after SPS IgG injection into mice did not generate any symptoms.[106] Furthermore, stereotactic injection of GAD-Abs into the hippocampus of rats *in vivo* did not alter spontaneous and evoked GABAergic synaptic transmission.[107,108] In contrast to anti-GAD antibodies, animals treated intraperitoneally[109] or intrathecally[110] with IgG anti-amphiphysin Abs have exhibited stiffness-like behavior.

Equally controversial are the data from experiments conducted in cultured neurons. Hippocampal cultured neurons treated with sera from epileptic GAD-positive patients showed an increase of post-synaptic inhibitory potentials compared with negative controls.[111] Further, when rat cerebellar slices were exposed to serum or CSF from patients with SPS or cerebellar ataxia, a decrease of post-synaptic inhibitory currents of Purkinje cells was observed, compared with GAD-negative sera form ataxic patients.[72,112,113] Some studies have also shown epitope-dependent pathogenic actions of GAD-Abs in histological brain sections and *in vivo* preparations,[35,106,114] whereas others showed lack of internalization into hippocampal cultured rat neurons[3] (Table 2). It remains, however, unclear how GAD-Abs can cause the GABAergic dysfunction in SPS if not internalizing into neurons. The possibility that antigens during synaptic transmission transiently expose extracellular epitopes, recognized by the immune system, remains hypothetical.

Circulating GAD-reactive B cells that can differentiate into antibody producing cells have been also detected in the peripheral blood and bone marrow of patients with GAD-Ab associated neurological syndromes. Interestingly, the presence of GAD was not required for induction of GAD-antibody producing cells and GAD-Ab production by stimulated peripheral blood cells did not correlate with GAD-Ab serum levels, suggesting an additional source of GAD-Abs. This study implied that targeting both memory B cells (i.e. with rituximab) and plasma cells (i.e. with bortezomib) might be a potential treatment option.[115]

Anti-GAD antibodies are typically found in the peripheral blood and in lower levels in the CSF.[15] GAD-Abs in CSF on occasion can be detected as monoclonal bands, suggesting that only a fraction of the whole anti-GAD response occurs in the CNS and that the intrathecal production of GAD-Abs could facilitate their access to relevant neuronal autoantigens. Interestingly, the intrathecal monoclonal IgGs are reactive to GAD65.[3,4] The role of B cells remains, however, unclear. The statistically negative controlled study with rituximab (see below) cannot be interpreted to suggest that B cell suppression and antibody production may not be critical factors, because the study had an impressive effect in some patients but it was statistically negative due to a strong placebo effect. The same may also apply to the lack of correlation of antibodies titers to disease severity. Interestingly, it has been observed that mice possessing a monoclonal GAD65 specific $CD4^+$ T cell population develop a lethal encephalomyelitis-like disease in the absence of any other T cells or B cells.[116]

Therapeutic approaches

SPS patients experience severe anxiety and depression due to phobias of sustaining falls or inability in completing even simple physical tasks. Patients with significant symptoms that do not improve concurrently with the physical symptomatology need clinical and psychological support both at home and at work. Their phobias often lead to depression, or at times addiction to drugs such as benzodiazepines or narcotics, highlighting the need for multifactorial care.

For SPS and GAD-SD, two strategies of treatment are implemented: symptomatic (anti-spasmodic) or immunologic[117] interventions either independently

Table 2. Experimental animal models of glutamic acid decarboxylase antibody-spectrum disorder.

Experimental approach	Sample	Clinical manifestation	Result	Reference
(a) Stereotactical injection into rats' hippocampi	CSF	Focal epilepsy due to LE	NO changes on GABAergic transmission	Hackert *et al.*[107]
(b) Intraperitoneal injection into transgenic mice	IgG	PERM and SPS	NO differences in behavioral tests NO loss of GAD-EGFP neurons	Chang *et al.*[106]
(c) Epitope specificity: monoclonal GAD Ab in cerebellar slices and mice	Sera	CA, SPS, LE, T1D	b78 inhibits GAD enzyme activity	Manto *et al.*[35]
(d) Whole-cell patch-clamp technique on cerebellar slices	IgG from CSF	CA	Effects presynaptically with suppression of GABAergic transmission	Mitoma *et al.*,[113] Takenoshita *et al.*,[72] Ishida *et al.*[112]
(e) Whole-cell patch-clamp technique on hippocampal slices	Sera	Epilepsy, T1D	Increase of frequency of IPSPs	Vianello *et al.*[111]
(f) GAD-Abs in rat cerebellar slices	Sera, CSF	SPS, T1D, autoimmune PS	SPS: reduction of GABA production T1D/PS: NO differences in enzyme's activity	Dinkel *et al.*[15]
(g) Intracerebellar injection in rats	IgG from sera	SPS, CA, paraneoplastic CA (GAD^-), T1D	GAD^+: altered cerebellar activity, impaired learning, muscle discharges, abnormal reflexes GAD^-: NO effect	Manto *et al.*[38]
(h) Passive transfer of Abs in mice	GAD-Abs	–	Loss of GAD-EGFP neurons NO behavioral changes	Chang *et al.*[107]
(i) Intraventricular (i.c.v.) and intrathecal (i.th) injection	IgG	SPS	i.c.v.: stiffness-like behavior, impaired walking ability, sensorimotor dysfunction, normal anxiety test i.th: NO motor symptoms; normal spinal transmission	Hansen *et al.*[105]

CA, cerebellar ataxia; CSF, cerebrospinal fluid; EGFP, enhanced green fluorescent protein; GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase; IPSP, Inhibitory Postsynaptic Potentials; LE, Limbic encephalitis; PERM, progressive encephalomyelitis with rigidity and myoclonus; PS, polyendocrine syndrome; SPS, stiff person syndrome; T1D, DM-1.

or in combination, depending on symptom severity (Table 3). Symptomatic relief is often achieved with agents that enhance GABAergic transmission, such as benzodiazepines, which diminish spasms and stiffness, especially those triggered by emotional factors. The first therapeutic option is diazepam, a GABAA agonist. This drug can help most patients, although the high doses sometimes required cannot be tolerated and may lead to addiction. Other similar compounds include clonazepam, alprazolam, lorazepam and temazepam. The second category of drugs used as anti-spasticity agents are GABAB agonists. Because of better tolerance and no addiction potential, we have now started using these agents as first line therapy in lieu of benzodiazepines. The most effective among them is baclofen, to the point of now using it as first in order. Sometimes high doses (up to 60 mg) are required to induce meaningful improvement, resulting in some cognitive issues. Antiepileptic drugs that enhance the brain's GABAergic transmission also improve symptomatology, either alone

Table 3. Therapies of stiff person syndrome.

Symptomatic			Immunologic	
Drug category	**Agents**	**Effect**	**Immunomodulating**	**Effect**
GABAA agonists	Diazepam Chlonazepam Alprazepam Lorazepam Temazepam	Enhance GABAergic transmission Can help most patients High doses: not tolerated	IVIg	The only proven immunomodulatory therapy: beneficial in SPS
GABAB agonists	Baclofen	Antispasticity drug High doses my cause cognitive effects	Plasmapheresis	Not routinely used: Inconsistent and transient benefits
α2 adrenergic receptor	Tizanidine	Muscle relaxant	Corticosteroids	Mostly ineffective
Ca^{+2} inhibitor	Dantrolene	Muscle relaxant	Immunosuppressants	
Anti-epileptic drugs	Gabapentin Vigabartin Tiagabine Leveritacetam	Inhibition of GABA transmission	Azathioprine Methotrexate Cyclophosphamide Cellcept Rituximab HSMT	Mostly ineffective Rituximab, although statistically insignificant against placebo, may have impressive benefits in some patients

GABA, gamma-aminobutyric acid; HSMT, Hematopoietic Stem Cell Transplantation; IVIg, intravenous immunoglobulin; SPS, stiff person syndrome.

or in conjunction with baclofen and benzodiazepines. In our experience, the most helpful agents in this family are gabapentin and vigabatrin, which act by inhibiting GABA-transaminase. Others include tiagabine, an inhibitor of GABA re-uptake, and levetiracetam, which facilitates inhibition of GABAergic transmission. Tizanidine, a centrally acting α2 adrenergic receptor, and dantrolene, a muscle relaxant, can also help.

If the above agents do not offer a satisfactory benefit, we proceed to immunotherapy. The most widely used agent in this category is intravenous immunoglobulin (IVIg) after its proven efficacy. In a randomized, double-blinded, placebo-controlled crossover trial we conducted in GAD-positive SPS patients, IVIg resulted in significant improvements in objective stiffness parameters and activities of daily living.[61] The duration of efficacy after each monthly IVIg infusion ranges from 4 to 12 weeks in most patients. IVIg remains the only immunomodulatory therapy with proven benefit in SPS. Subcutaneous immunoglobulin may be also an option in patients with poor venous access, or when there is a demonstrable early wearing-off effect, ensuring a more sustained benefit. Plasmapheresis has been of limited and transient benefit and we do not routinely use it in spite of some anecdotal case reports.[117] Corticosteroids are surprisingly ineffective in our experience with a large number of patients, although one anecdotal report had shown limited benefit.[118] Furthermore, triggering or exacerbating diabetes is a serious consideration that further limits its use. Of paramount importance is the control of diabetes, which requires insulin most times and, if uncontrolled, seems to worsen the neurologic symptomatology.

Immunosuppressive agents such as azathioprine, methotrexate, cyclophosphamide and mycophenolate mofetil are equally disappointing, in spite of rare anecdotal reports.[119,120] The most useful drug in this category is rituximab. A randomized controlled trial[86] we conducted in patients with SPS demonstrated lack of efficacy of rituximab compared with placebo owing to a strong placebo effect. In this series, however, seven patients improved and four of them with severe disease demonstrated meaningful to impressive improvements. On this basis, we believe rituximab is a useful drug for a subset of patients who have failed therapies with GABA-enhancing drugs and IVIg. It should be stressed that anti-GAD

antibody titers may drop but not to a statistically significant level and the titers do not correlate or predict improvement.

Some SPS patients who failed conventional immunosuppressive therapy have experienced benefit after autologous hematopoietic stem cell transplantation (auto-HSCT). In one small study, three patients with SPS and one with PERM were initially treated with cyclophosphamide (Cy) 2 g/m^2 + Granulocyte-colony stimulating factors (G-CSF) and then conditioned with Cy 200 mg/kg + Anti-thymocyte Globulin (ATG) followed by auto-HSCT. All patients tolerated the procedure well and showed improved physical performance. One patient's walking distance improved from 300 meters to 5 miles and one other's ambulation improved from being confined to a wheelchair to being able to walk with a frame. Two patients became seronegative for anti-GAD antibodies and normalized their neurophysiological abnormalities. Additional studies have also shown some benefit. Although auto-HSCT was thought promising, a large study aiming at 40 patients was terminated early after recruiting 23 patients because of lack of efficacy or transient benefits, taking into account potential serious complications.[121,122] One of the many limitations of this study, as pointed out,[123] was the enrollment of patients with advanced disease. A new Hematopoietic Stem Cell Transplantation (HSMT) trial in SPS patients unresponsive to the therapies mentioned above might be, however, considered for patients with still early disease, in a controlled trial design taking into account a strong placebo effect, and the use of objective validated scales.[123]

Therapy for the other, non-SPS, GAD-SDs

Therapeutic agents in autoimmune epilepsy, nystagmus and cerebellar disease are the same in the other GAD-SDs as those discussed in SPS, except if their manifestation is acute or subacute, as seen in autoimmune encephalitis. In these circumstances, we start with intravenous steroids 1000 mg daily for 5 days, followed by IVIg and rituximab, as needed. Anti-epileptics are added in patients with epilepsy, although GAD-associated epilepsy is reported refractory to the above immunomodulatory therapies, often requiring more aggressive immunosuppressive approaches, with mycophenolate motefil or rituximab.[59] Interestingly, exogenous testosterone replacement therapy has been shown to reduce seizure frequency and intensity.[124] In some cases, epilepsy surgery should be considered, although few beneficial surgical outcomes in GAD-associated epilepsy patients have been reported.[59] Patients with longer-standing disease and those with cerebellar ataxia, dysarthria and dysphagia have overall less impressive response to applied therapies.

Conclusion

The review highlights that high-titer anti-GAD antibodies are associated with an array of distinct neurological syndromes including SPS, cerebellar ataxia, epilepsy, limbic encephalitis, abnormal eye movements. Although high anti-GAD antibodies in serum or their presence in CSF are important for diagnosis, the titers do not correlate with disease severity and do not generally predict response to immunotherapy. Despite considerable efforts, using both *in vitro* and *in vivo* preparations, the pathophysiological role of anti-GAD antibodies has not yet been clarified, suggesting that other autoantibodies affecting inhibitory neurotransmission might be of importance, because autoimmunity targeting inhibitory synaptic antigens point to a unifying theme of hyperexcitability as the underlying pathomechanism.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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