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REVIEW

A critical update on the immunopathogenesis of Stiff Person Syndrome

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ABSTRACT

Background Stiff Person Syndrome (SPS) is a relatively rare but often overlooked autoimmune neurological disorder that targets antigens within the brain's inhibitory pathways resulting in incapacitating stiffness and spasms that impact on the patients' quality of life. Although a number of immunomodulating therapies significantly improve the patients' symptoms, the exact pathogenic mechanisms remain unclear.

Materials and methods The current literature on SPS was reviewed and combined with the authors' experience with many patients and various laboratory studies. The majority of the patients have high-titre anti-GAD (Glutamic Acid Decarboxylase) antibodies in the sera and CSF suggesting dysfunction of the GABAergic neurotransmission. These antibodies are excellent disease markers but their pathogenic role remains uncertain.

Conclusions This review provides a critical assessment on the immunobiology of SPS, describes the identification of anti-GABARAP antibodies as a new antigenic target in the GABAergic synapse and identifies the areas for future research.

Keywords Autoimmunity, GABAergic synapse, GABARAP, glutamic acid decarboxylase, nervous system.

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Introduction

Stiff Person Syndrome (SPS) is a sporadic, rare neurological disorder affecting mostly women, which can be manifested as paraneoplastic or idiopathic [1]. It is a disorder associated with a number of different autoantibodies (GAD, GABARAP, amphiphysin and gephyrin), which target antigens predominantly expressed in the inhibitory synapses of the central nervous system. In the non-paraneoplastic and most common variant, a number of immune-modulating therapies such as intravenous immunoglobulin (IVIg) and plasmapheresis significantly improve symptoms [2,3], which suggests that the disorder is very likely antibody-mediated. A very strong association has been documented with several DQβ1 and DRβ1 MHC class-II alleles [1], although no single predominant allele(s) has been identified. Despite all the recent progress, it is not yet fully understood which is the primary auto-antigen in idiopathic SPS and how the autoantibodies might cause disease. In this review, we provide a critical analysis of the current status of autoimmunity, discuss the role of the various autoantibodies in the disease pathogenesis and identify areas for future research.

Main clinical signs

Stiff person Syndrome is typically characterized by muscle rigidity of the lumbar, trunk and proximal limp muscles, changes in posture, episodic painful muscular spasms precipitated by noise, touch and emotional upset and an altered startle response. Muscle rigidity is caused by sustained muscular contractions concurrently occurring in agonist and antagonist muscles, affecting the body's posture and often resulting in lumbar hyperlordosis, because of contraction of paraspinal and abdominal muscles. Rigidity is typically absent during sleep. Painful spasms and accompanying falls can be triggered by unexpected sounds such as ringing of the phone or a siren. These falls have been described as log-like or statue-like, as the patients have no control over their falling bodies [4]. Fear, anxiety and phobias are very common among SPS patients often giving the impression of a psychogenic disorder that delays diagnosis. These symptoms can either be explained as a result of the falls or there might be a dysregulation in the areas of the brain such as the amygdala and hippocampus [5,6]. The average age of symptom onset is 35 years with a lag of 6·2 years till diagnosis [1]. SPS remains a clinical diagnosis and requires a great deal of suspicion and exclusion of other disease mimics

such as primary psychogenic disease, myelopathies, multiple sclerosis or Parkinson's disease [1]. The diagnosis is aided by electrophysiology, which shows continuous activity of voluntary contractions from agonist and antagonist muscles, despite the patients' attempt to relax [7], because of continuous firing of depolarized motor neurons. A good number, at least 35%, in our early series of SPS patients have Type 1 diabetes (T1DM), which either precedes the onset of SPS or develops during the course of the disease [1,8]. In a recent study, 46% of SPS patients developed T1DM after the onset of SPS, while the rest have been diabetics for a few months to 15 years [9]. A few other organ-specific autoimmune diseases can be associated with SPS patients, including Hashimoto's thyroiditis, Grave's disease, pernicious anaemia, coeliac disease and vitiligo [1,10]. Antibodies against proteins of the GABAergic synapse, namely GAD, GABARAP, amphiphysin and gephyrin, are characteristic of SPS (Fig. 1). Various other autoantibodies, not necessarily related to SPS but rather representative of the abnormal immune repertoire, have been observed in our series of patients, including anti-nuclear, anti-thyroid, anti RNP, anti-gliadin and anti-intrinsic factor antibodies [1].

Anti-GAD antibodies: a good disease marker

The most common autoantibody, found in approximately 80% of SPS patients, both in the serum and CSF is against the enzyme GAD [1,11]. Glutamic Acid Decarboxylase (GAD) catalyses the decarboxylation of L-glutamate to γ-aminobutyric acid (GABA), the most common inhibitory neurotransmitter in the brain and spinal cord. GAD is mainly synthesized in GABAergic neurons in the Central Nervous System (CNS) and in the β-cells of the pancreas. The enzyme has two isoforms, derived by different genes, GAD65 (a membrane associated form) and GAD67 (a soluble form). These isoforms are 65% identical and they mostly differ in the amino-terminal region (only 23% identity). This explains their different sub-cellular localization [12]. Both are synthesized in the cytoplasm where GAD65 is then modified and attached to the inner surface of synaptic vesicles in GABAergic neurons or microvesicles in the pancreatic β-cells [13]. The anti-GAD65 antibodies are found in only 1% of the normal population and in 5% of patients with other neurological disorders (OND). In clinical practice, these antibodies

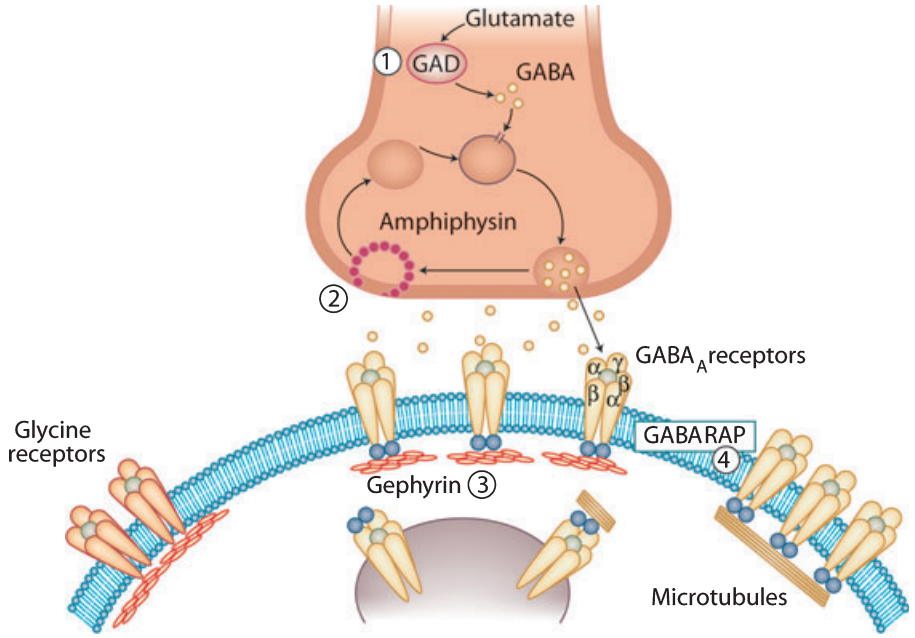

Figure 1 Autoantigens associated with GABAergic synaptic transmission in Stiff Person Syndrome (SPS) patients include both pre-synaptic and post-synaptic proteins. The pre-synaptic antigens are glutamic acid decarboxylase (GAD) (1), the enzyme that synthesizes GABA, and amphiphysin (2), a synaptic vesicle protein responsible for endocytosis of vesicle plasma membranes following GABA release. In the post-synaptic membrane, the target antigens are gephyrin (3) and GABA-A receptor-associated protein (GABARAP) (4). Gephyrin is a tubulin-binding protein needed for clustering the receptors of both inhibitory neurotransmitters, the glycine receptors in the spinal cord and the GABA-A receptors in the brain. GABARAP is a linker protein between gephyrin and GABA-A receptors and promotes the recruitment of gephyrin and organization of the GABA receptors. The most common autoantigen in SPS is GAD, which is seen in 85% of patients, followed by GABARAP, which is seen in 65%. Amphiphysin is seen in 5% of patients, and gephyrin has been seen in only one case (Adapted from reference [66]).

are routinely measured in the serum using methods such as RIA or ELISA.

The autoimmune hypothesis in SPS was proposed by Solimena and co-workers who were the first to describe the GAD65 auto-antibodies by showing binding of a patient serum to GABAergic neurons in rat and human brain sections [14]. The serum also recognized a unique band in a Western blot and stained rat pancreatic β-cells. Auto-antibodies to GAD65 are detected in up to 80% of SPS patients, while auto-antibodies to GAD67 are only detected in < 50% of the patients and at much lower titres [11,15,16]. In the CSF, autoantibodies against GAD65 are detected in 75% of the patients [15]. It is suggested that this is due to intrathecal synthesis of anti-GAD65 IgG [17]. The titres of the antibodies in the CSF are 50-fold lower than in the serum but the rate of synthesis is 10-fold higher [5] suggesting a role for a mature population of clonal B-cells within the confines of the blood–brain barrier (BBB). This was further confirmed in a study, which demonstrated that the binding avidity of the CSF IgG was 10 times higher compared with that of serum IgG [18]. Finally, the different epitope specificity noted between paired serum and CSF specimens further suggests local stimulation of B cells within the CSF compartment [19].

Anti-GAD65 antibodies are also found in T1DM (up to 80% of patients) where they serve as a marker for the disease and as a risk predictor [20]. They are found only in the serum of (most) patients, occasionally before the onset of diabetes, and most significantly in much lower titres (50- to 100-fold less in typical cases) in comparison with the SPS patients [1,21]. There is a significant difference in epitope specificity between the GAD65 antibodies found in diabetes or SPS despite the fact that GAD is identical in sequence and antigenicity whether expressed in the pancreas or in the brain [22]. This difference was shown by experiments where sera from T1DM failed to recognize GAD65 on a Western blot or GABAergic neurons in brain sections, while sera from SPS showed strong reactivity at the same IgG concentration [8,17,22]. In general, in T1DM the antibodies are thought to recognize conformational epitopes, while the antibodies in SPS mostly recognize linear and denatured epitopes especially in the $–NH_2$ terminal region of the GAD65 molecule [12,22,23]. A recent study also showed that the decarboxylase catalytic site is particularly antigenic [24]. It remains to be determined whether this epitope differentiation is related to the difference in titre levels between SPS and T1DM.

The mechanism which induces GAD autoimmunity remains unclear. Potential loss of tolerance was addressed by looking for expression of GAD in the thymus. It was shown that both GAD65 and GAD67 are expressed in the thymus [25] and more specifically GAD65 was localized in antigen-presenting cells in human tissue [26]. Molecular mimicry has also been implicated. A patient infected with West Nile virus, later developed GAD65-positive SPS; a stretch of 12 amino acids highly homologous between the virus and GAD65 suggested that loss of tolerance after the infection could have led to anti-GAD65 autoimmunity [27].

Anti-GAD antibodies: are they pathogenic?

It is still a lingering question whether GAD65 antibodies are pathogenic in SPS. In a number of studies, such a role has been postulated employing all the standard approaches for brain autoimmunity, i.e. demonstrating a functional effect, producing an animal model either by passive transfer or active immunization, looking for evidence of maternal transfer and correlating antibody parameters with disease severity. Several hypotheses have been formulated with regard to how the antibodies might produce symptoms.

The dominant hypothesis still is that the pathogenic role of the anti-GAD65 antibodies is exerted through the dysfunction of GABAergic inhibitory neuronal circuits, which allows for the continuous firing of α-motor neurons. Experiments using transcranial magnetic stimulation suggested the possibility that focal disinhibition in the motor cortex leading to motor cortex hyperexcitability could affect brain control over spinal circuits, [28], [29]. Immunotherapy can improve such manifestations [30]. An alternative hypothesis proposes that loss of inhibition locally in the inhibitory circuits of the spine could enhance their activity and lead to muscle stiffness. Two such GABAergic inhibitory spinal circuits have been tested (H-reflexes testing using vibration-induced inhibition and reciprocal inhibition), but only one of them was found to be abnormal, thus making this hypothesis questionable [31]. Loss of inhibition in spinal interneurons could, however, lead to excessive responses to impulses from the muscle and skin thereby explaining the stimulus-sensitive induction of spasms. An interesting report is that of mice mutant for the Trak-1 protein, which exhibit increased muscle tone similar to that seen in SPS. Trak-1 interacts with GABA-A receptors and its loss of function leads to GABA-A receptor down-regulation especially in the lower motor neurons [32]. All the above support the notion that in SPS, the clinical symptoms can be attributed to dysfunction of the GABAergic neuronal circuits. The therapeutic effect of GABA-enhancing drugs, such as diazepam, tiagabin, or balofen, provides additional support.

In a biochemical study, SPS (n = 10) GAD65-positive sera reduced GABA synthesis in crude rat cerebellar extracts. This was not the case with high-titre sera from T1DM, again demonstrating the difference in specificity between the GAD65 antibodies in SPS and T1DM [15]. We have confirmed the inhibition of GAD65 activity *in vitro* and also showed that the capacity of SPS sera to inhibit the enzymatic activity of GAD65 correlated with their binding to a conformational C-terminal epitope. These studies suggest that the presumed loss of function in inhibitory circuits may be due to interference with

GABA synthesis. This concept was further supported by our *in vivo* MRS spectroscopy studies, which showed a decrease in GABA levels in the sensorimotor cortex [33]. The mechanism by which the antibodies inhibit GAD remains unknown [19].

Loss of function of inhibitory circuits, either central or spinal, may be also due to interneuron loss. However, only a small number of GAD65-positive SPS patients were reported to have GABAergic cell loss in the cerebellar cortex or loss of spinal motor neurons in the intermediate and medial ventral horn together with loss of interneurons (n = 5). In a few cases, perivascular inflammation has also been observed (n = 3) [34].

Intracerebellar injection of GAD65-positive sera from SPS patients in rats blocked the potentiation of the corticomotor response, which is modulated by the cerebellum. In the meanwhile, an injection in the lumbar paraspinal region induced continuous motor activity and increased excitability in anterior horn neurons. Control sera from GAD65-positive patients but without any neurological symptoms had no effect on the animals [21]. These experiments taken together, suggest that the spinal cord neurons are affected with the specific target being the inhibitory synapse.

In a case report, a mother who had SPS and high titres of GAD65 antibodies failed to pass the disease to her offspring (after 6-year follow-up and 8-year follow-up) despite the fact that the infants also acquired high GAD65 titres for up to 24 months after birth [35]. This could either mean that the autoantibodies *per se* are not pathogenic or that the BBB of the offspring remained intact, unlike the mother's, and thus the antibodies did not penetrate into the CNS. It is hard to draw conclusions from this study as even in the case of myasthenia gravis (MG) where the antibodies are well documented to be pathogenic, only 20% of infants develop maternally transferred MG. This is due to the fine specificity of the MG antibodies; this might also be the case in the maternally transferred anti-GAD65 antibodies [36].

Very few active immunization experiments have attempted to induce SPS. Chang *et al.*, have reported GABAergic neuronal loss in the brainstem of mice following active immunization [37]. In contrast, several groups have immunized animals against GAD65 to investigate the effect of GAD on T1DM. None has reported any neurological or neuromuscular problems [38,39], although it is not clear whether the generated antibodies could pass through the BBB. In NOD (non-obese diabetic, a spontaneous animal model for diabetes) mice in particular, the immunization against GAD65 seems to be able to induce tolerance and protect from T1DM. Again, no neurological symptoms were reported in these animals [20].

Whether or not the anti-GAD65 antibodies may exert a direct role in the pathogenesis of SPS was also indirectly examined in several studies where the clinical parameters of the disease were correlated with anti-GAD65 antibody titres [2,5,40]. No correlation was found between GAD65 titres in the serum or CSF and disease severity, disease duration or response to therapy. In a number of other autoimmune disorders, e.g. MG and Lambert–Eaton myasthenic syndrome, there is a positive correlation between antibody titres and disease severity [5,41]. Such a lack of correlation suggests that these antibodies may not be pathogenic. It is broadly accepted that intrathecally produced IgG has access to the brain parenchyma. What is needed further is to examine whether these antibodies can interfere with intracellular antigenic targets such as GAD65.

The clinical spectrum of anti-GAD autoimmunity

Apart from SPS, anti-GAD65 antibodies have been found in a few other conditions such as cerebellar ataxia, temporal lobe epilepsy, myoclonus and non-paraneoplastic limbic encephalitis [42,43]. According to one report, GAD-associated cerebellar ataxia seems to affect mostly women (> 80%) with an underlying T1DM or polyendocrine autoimmunity. The symptoms include gait ataxia, limb ataxia, dysarthria and nystagmus [9,42]. Coexistence of SPS and cerebellar ataxia is not uncommon [44]. We have encountered five such patients who also had a 2.5-fold higher intrathecal anti-GAD65 antibody synthesis compared with SPS patients without cerebellar disease. Of interest, immunotherapy improved only the SPS symptomatology but not the cerebellar [45]. The differences between patients with cerebellar ataxia and SPS regarding GAD65 antibody titres, intrathecal synthesis of GAD65 antibodies and the presence of oligoclonal CSF bands are not significant [9,42]. In a functional study, rat cerebellar slices were incubated with GAD65-positive CSF from a patient with cerebellar ataxia. Using whole cell patch clamp, it was shown that GABA-mediated transmission from basket cells to Purkinje cells was suppressed. In the Purkinje cells, the amplitude of inhibitory post-synaptic currents was greatly reduced, while GABA release in the pre-synaptic terminals of the basket cells was also reduced [46,47]. It is not clear whether these data support the notion that there is a common pathogenic mechanism between SPS and cerebellar ataxia. We have proposed that in SPS-cerebellar patients, the anti-GAD65 antibodies may recognize additional antigenic targets on the inhibitory interneurons causing ataxia because of the lack of necessary feedback inhibition from the Purkinje cells [44]. As no cerebellar atrophy is noted in these patients, the presumed inhibitory effect is probably caused by a functional blockade, rather than a destructive process.

Antibodies against GAD65 have also been found in a series of eight out of 51 patients with drug-refractory epilepsy [48]. Few of them showed other immunological disorders and their CSF was negative for oligoclonal bands in contrast to SPS and cerebellar ataxia patients. The sera of two patients were tested

electrophysiologically and it was shown that the GAD65 autoantibodies bound on cultured hippocampal GABAergic neurons and caused a significant increase in the frequency of IPSPs, suggesting an increased inhibition [49,50]. Overall, remains unclear whether GAD65-positive sera from patients without SPS exert a more significant effect in neuronal preparations.

Is SPS caused by a different antibody?

The unconvincing data about the direct pathogenicity of anti-GAD65 antibodies paved the way for exploring other auto-antibodies in SPS. There are paraneoplastic variants (5% of total SPS patients, mostly suffering from breast cancer), which have anti-amphiphysin and anti-gephyrin antibodies ($n = 1$) [51–53]. Amphiphysin is a cytosolic pre-synaptic vesicle-associated protein with a role in endocytosis [54]. Gephyrin is also cytosolic, but post-synaptic. It is an integral part of the inhibitory synapse, associated with both GABA and glycine receptors. Although these antibodies have no clear pathogenic role, the antigens which are directed against are integral components of the inhibitory synapses located either pre-synaptically where GAD65 is present, or post-synaptically (Fig. 1).

There are little data from passive transfer and *in vivo* animal experiments. In a single report, the disease was successfully transferred to rats using serum from a patient suffering from paraneoplastic SPS with high-titre antibodies against amphiphysin. The animals developed a condition with dose-dependent stiffness and spasms [55]. In a follow-up study, it was shown that the IgG fraction of the same sera could reduce GABA-induced calcium influx in cultured rat embryonic motor neurons. The effect was abolished by selectively depleting the amphiphysin antibodies [56].

Another target protein within the inhibitory synapse is GABARAP (GABA receptor-associated protein). GABARAP is expressed in the cytosol and in axonal processes, and interacts with gephyrin to assemble the GABA-A receptor. Its absence can prevent the assembly and surface positioning of the GABA-A receptor [57]. Antibodies against GABARAP have been recently found in 70% of GAD65-positive SPS sera. It was shown that the serum down-regulated the density of GABA-A receptors in neuronal processes of cultured hippocampal neurons. This has obvious functional implications for GABA neurotransmission and so this protein is more likely to be a target autoantigen [58]. The obvious question is whether anti-GABARAP is a pathogenic antibody causally related to SPS. As GABARAP and GAD65 antibodies coexist in 70% of cases, it remains unclear whether malfunctions attributed to anti-GAD65 could be really related to GABARAP antibodies. Of interest, 12 SPS patients with severe disease had higher anti-GABARAP antibody titres compared with eight patients with lower titres and milder symptoms [58] suggesting that GABARAP antibodies may correlate with disease severity, unlike the GAD65 antibodies.

The GABARAP discovery, using a blind search with reverse proteomics, provides an unbiased confirmation that in SPS, autoimmunity is targeting the inhibitory synapse. Along these lines followed the targeted search for molecules involved in GABA metabolism; however, no autoantibodies were found against GABA transaminase [24]. Another obvious candidate autoantigen is the GABA receptor itself. One could envisage how autoimmunity spreads and involves proteins at both sides of a synapse. An inflammatory process could be implicated, which could lead to a release of both pre- and post-synaptic antigens. The spreading of humoral autoimmunity in multiple antigens is, after all, typical of autoimmune diseases. The inhibitory synapse hypothesis bears similarities to the evolved picture in MG where apart from the acetylcholine receptor, MuSK and titin are also targeted in up to 20% of MG patients [59]. Like the GABAergic synapse, antigenic targets in the neuromuscular junction are not only limited to post-synaptic but also to pre-synaptic proteins and channels. What is also attractive is that in contrast to MG, the SPS-related autoantibodies seem to cause dysfunction rather than destruction of the synapse, as evidenced by the normal MRI and the rapid reversal of the symptoms after successful immunotherapy.

Cellular autoimmunity

In addition to the antibodies, one might also consider the possibility of SPS being a T-cell-mediated disease. In a series of eight patients, T cells from peripheral blood recognized two dominant epitopes from GAD65 (aa 81–171 and aa 313–403). In T1DM, the dominant epitopes were different (aa 161–243 and aa 473–555) [60]. In the same study, the autoantibodies were mainly IgG1 and IgG3 suggesting a Th1 helper T-cell response. In another study, *in vitro* T-cell proliferation was shown in four of five SPS patients after 5 days of incubation with recombinant GAD65 protein. T cells from patients with GAD65 antibody-positive cerebellar ataxia and T1DM were used as controls but did not respond to GAD65 any better than control cells from healthy individuals. The cytokine profile or the responding T cells could not determine whether this was a Th1 or a Th2 response [61]. In a smaller series of four patients, GAD65-specific T cells were cloned from the CSF of patients that had a high index of intrathecal production of IgG [62]. In addition, from patients with T1DM a CD4-positive T-cell line was developed, but did not cause SPS in an adoptive transfer animal model [63]. Finally, in a very recent study, it was shown that a mouse GAD65-CD4 (+) response caused encephalomyelitis. What is of particular interest is that the T cells were found in the brain tagging GAD65 expression [64]. This animal model

could provide clues on how T cells could target and disrupt the function of GABAergic neurons. An active T-cell response could be driving the disease in the initial stages as no significant T-cell infiltrations have been observed in the CNS of SPS patients post-mortem [65].

Discussion

Stiff Person Syndrome, although rare, remains a clinically fascinating disease. As the diagnosis is made entirely on clinical grounds, it is very often misdiagnosed, necessitating the need to find a diagnostic marker. In addition, there is a need to explore and critically assess if there is any pathogenic antibody with specificity for SPS.

A fundamental question is whether SPS fulfils the criteria for a neuro-autoimmune disorder. Specific autoantibodies (anti-GAD, anti-GABARAP, anti-amphiphysin) have been identified in the patients' sera and high anti-GAD65 antibody titres distinguish SPS from diabetes or other autoimmune disorders and serve as an important diagnostic tool. Furthermore, SPS patients respond to immunomodulatory therapy [66]. A controlled trial with IVIg has clearly demonstrated that this therapy is highly effective and relieves patients' symptoms significantly [2]. In a few instances, GAD65 antibodies have shown some pathogenic potential *in vitro*, as they were able to inhibit GABA synthesis in neuronal extracts and also to inhibit GABAergic transmission in brain slices. However, in all these *in vitro* experiments, it has been assumed that the anti-GAD65 antibodies were the pathogenic factor in the patients' sera. As we now know that at least 70% of GAD-positive patients contain anti-GABARAP antibodies, it is an obvious question whether some of the effects described in the *in vitro* experiments could be attributed to GABARAP or to another antibody. In some functional experiments, like those where GABA synthesis is inhibited, one could assume that is indeed the anti-GAD65 specificity that holds the key role. But, one could reasonably argue, that in synaptic transmission experiments the anti-GABARAP antibodies could be acting in synergy. An important negative factor, however, has been the lack of active immunization data. A single study using GAD65 as an autoantigen reports neuronal loss but without any symptoms. There is also no passive transfer model using GAD sera but only one example where SPS was transferred to mice but with sera containing anti-amphiphysin antibodies, the rare paraneoplastic form of SPS.

As discussed earlier, if the GAD65 or the other aforementioned antibodies are indeed pathogenic, they could affect neuronal synapses in several ways. It might be that even a very low concentration of IgG in the brain parenchyma can affect synaptic transmission at the neuronal level. If the main antigen is expressed in the brain, it could constantly stimulate resident B cells to produce pathogenic antibodies. B cells are capable of doing that [67] as they are also capable of crossing the BBB and entering the brain. Although in SPS the anti-GAD65 titre in the serum remains high, there is considerable evidence of intrathecal antibody synthesis with antibodies in the CSF recognizing different epitopes than those in the serum.

Perhaps the predominant criticism against anti-GAD65 antibodies being pathogenic is that GAD is an intracellular protein. The same, however, is true for GABARAP, amphiphysin and gephyrin. How do these antibodies after crossing the BBB or synthesized intrathecally can penetrate vesicles or neurons and eventually block either the function or synthesis of GAD? In other autoimmune disorders of the CNS, e.g. limbic encephalitis, the target of the auto-antibodies is extracellular (NMDA or AMPA receptors) [68]. It has been suggested, that during GABA exocytosis, GAD65 peptide fragments could be presented on the neuronal surface and that might act as a target for the autoantibodies. Alternatively, GAD may be recognized by the immune system because is membrane associated to the vesicles through heat shock proteins. However, these hypotheses have not been experimentally tested.

Therefore, there is a need for further research to explore what blocks GABA neurotransmission in the brain and results in clinical stiffness. Synaptic inhibition is mediated by GABAergic or glycinergic interneurons. Loss of glycinergic inhibition is the basis of the Startle Disease caused by glycine receptor mutations. Antibodies against these receptors have been described [70]. Search for GABA involvement in SPS could be centred on developing an animal model, either by active or passive immunization and *in vitro* studies of the GABAergic synapse using brain slice electrophysiology both in wild-type and GAD knockout animals.

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