

Review

Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: Protean additions to the autoimmune central neuropathies

Fatima Ali^a, Merrill Rowley^b, Bindu Jayakrishnan^b, Suzanne Teuber^a,
M. Eric Gershwin^a, Ian R. Mackay^{b,*}

^a Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, Davis, CA, USA

^b Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

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ABSTRACT

Stiff Person Syndrome (SPS) is a rare autoimmune neurological disease attributable to autoantibodies to glutamic acid decarboxylase (anti-GAD) more usually associated with the islet beta cell destruction of autoimmune type 1 diabetes (T1D). SPS is characterized by interference in neurons with the synthesis/activity of the inhibitory neurotransmitter gamma amino butyric acid (GABA) resulting in the prototypic progressive spasmodic muscular rigidity of SPS, or diverse neurological syndromes, cerebellar ataxia, intractable epilepsy, myoclonus and several others. Remarkably, a single autoantibody, anti-GAD, can be common to widely different disease expressions, i.e. T1D and SPS. One explanation for these data is the differences in epitope engagement between the anti-GAD reactivity in SPS and T1D: in both diseases, anti-GAD antibody reactivity is predominantly to a conformational epitope region in the PLP- and C-terminal domains of the 65 kDa isoform but, additionally in SPS, there is reactivity to conformational epitope(s) on GAD67, and short linear epitopes in the C-terminal region and at the N-terminus of GAD65. Another explanation for disease expressions in SPS includes ready access of anti-GAD to antigen sites due to immune responsiveness within the CNS itself according to intrathecal anti-GAD-specific B cells and autoantibody. Closer study of the mysterious stiff-person syndrome should enhance the understanding of this disease itself, and autoimmunity in general.

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1. Introduction

Stiff Person Syndrome (SPS) is an autoimmune neurological disease characterized by progressive disabling muscle rigidity, hyperreflexia and spasms, especially affecting the lumbar and/or proximal limb muscles [1], attributable to high-affinity autoantibodies to the enzyme glutamic acid decarboxylase (GAD) required for the synthesis of the inhibitory neurotransmitter gamma amino butyric acid (GABA) [2–6]. Autoantibodies to GAD can interfere *in vitro* with GABA production, and *in vivo* could be “undermining” the entire GABAergic system throughout the central nervous system (CNS) [7,8] resulting in unbalanced excitatory and inhibitory

neurotransmission. The worsening muscle stiffness and spasms are matched by electromyography that reveals constant motor unit activity. Acute exacerbations in SPS are linked to emotional upset, startlingly loud noises, trauma and intercurrent infections, but there are few clues to initiating causes. The incidence of SPS is very low, about one per million population, women outnumber men 2:1, and there seems no racial predilection [9]. There is a reported association with the human leukocyte antigen (HLA) allele DQB1*0201 [10], also a high-risk susceptibility allele for autoimmune type 1 diabetes (T1D) that is likewise associated with autoantibodies to GAD. A proportion (~30%) of cases of SPS have an associated diabetes. SPS or other CNS dysfunction is not reported among young patients that present with “juvenile-onset” T1D and positivity for anti-GAD usually at lower levels than in SPS, but more data are needed on the characteristics of cases of SPS wherein onset of diabetes precedes or is concurrent with onset of SPS.

Historically, the first set of cases, reported by Moersch and Woltman in 1956 from the Mayo Clinic, comprised a male “index case” and 13 others with such severe rigidity as to resemble tin soldiers: hence the term “stiff man syndrome” (SMS) [11]. This prompted a case report by Asher in 1958 of a middle aged woman

Abbreviations: SPS, Stiff person syndrome; GAD, glutamic acid decarboxylase; CNS, central nervous system; T1D, type 1 diabetes; GABA, gamma amino butyric acid; PLP, pyridoxyl phosphate; aa, amino acid; HLA, human leukocyte antigen; SMS, stiff man syndrome; CSF, cerebrospinal fluid; IFL, immunofluorescence; MHC, major histocompatibility complex; IFN, interferon; BBB, blood brain barrier; GABARAP, GABA receptor associated protein.

* Corresponding author. Tel.: +61 3 96820717; fax: +61 3 9902 9500.

E-mail address: ian.mackay@monash.edu (I.R. Mackay).

with SMS, recommending a name change to “stiff person syndrome” [12]. Another early report in 1960 was the occurrence in a seven year old Chinese boy [13]. Diagnostic criteria nominated in 1967 included a unique pattern of muscular rigidity and objectively beneficial effects of neuromuscular blocking agents monitored by electromyography [14]; as the disease became better understood, these were modified to include a prodrome of episodic aching stiffness of axial muscles, progression of stiffness, painful spasms elicited by triggers, increased lumbar lordosis, normal sensory and cognitive function, and a therapeutic response to benzodiazepine drugs [15]. By 1991 the accumulation of female cases reported over the past three decades led to adoption of the name “stiff person syndrome” (SPS) [16]. In the classification proposed by Barker et al in 1998 [1] SPS was divided into three categories of a) stiff trunk syndrome, b) stiff limb syndrome and c) progressive encephalomyelitis with rigidity. After Solimena et al in 1988 identified autoantibodies in serum to human brain GAD (anti-GAD) in a patient with SPS, epilepsy and T1D [6], high levels of anti-GAD became a reliable laboratory marker for the diagnosis, substantiated by several observations on a strong association of autoantibodies to GAD with SPS [2–5].

Although the neurological disease traditionally associated with high levels of anti-GAD is SPS, various case series cite substantial frequencies as well of cerebellar ataxia [17], therapy-resistant epilepsy [18], myoclonus [19,20] and others, even to a mimicking in one case of Creutzfeldt–Jakob disease [21]. Representative frequencies of neurological syndromes in patients with highly raised levels of anti-GAD, derived either from an endocrine clinic [22], or from a study based on screening for paraneoplastic disease [23], are shown in Table 1. The results in fact suggest that testing for anti-GAD among the miscellany of “degenerative” neurological diseases could disclose hitherto unsuspected examples of autoimmune central neuro–myelopathies. On the other hand classical SPS can be associated with autoantibodies other than anti-GAD, including anti-amphiphysin as in paraneoplastic SPS [24–26], anti-GABA receptor associated protein [27] and anti-gephyrin [28]; each of these autoantigens is associated with GABA-mediated neurotransmission.

2. Structural and functional features of the GAD65 and GAD67 isoforms

GAD is a pyridoxal 5'-phosphate (PLP) dependent enzyme, and is the rate limiting enzyme responsible for synthesis of the inhibitory neurotransmitter GABA from glutamate. GAD exists as two isoforms, GAD65 and GAD67, according to their molecular weights, each encoded by a different gene. The enzyme has mainly a neural tissue distribution in regions containing neuro-inhibitory cells, and is present also in pancreatic islet beta cells, with GAD65 being a major autoantigen in type 1 diabetes [29]. The two isoforms differ

in their enzyme activity. GAD67 is located in the cytoplasm, is constitutively active, and provides for the steady basal production of GABA, whereas GAD65 is present mainly in synaptic vesicles, undergoes auto-inactivation during enzyme activity and occurs in the cell primarily as apoenzyme, providing for a pulse in production under circumstances that demand a rapid surge of GABA synthesis and release [30,31]. Understandably, deficient production or release of GABA accounts for the neuraxial hyper-excitability and muscle spasms in SPS.

The two isoforms GAD65 and GAD67 have been divided according to their linear sequence into three functional domains (GAD67, brackets): an amino (N)-terminal domain, amino acids (aa) 1–188 (1–197); a middle PLP-binding domain containing the active catalytic site of the enzyme, aa 189–464 (198–473); and a carboxy (C-terminal) domain, aa 465–585 (474–594) [29] (Fig. 1A). The isoforms show a general overall sequence similarity, with the middle and C-terminal domains having 74% identity, but differing (25% identity) for the N-terminal domain, mainly in the first 100 aa. These contain the membrane-binding sequence that co-localizes GAD65 with the GABA-transporter on the membranes of synaptic vesicles [32], and ensures that GAD65 is properly anchored to synaptic vesicles, and that GABA biosynthesis and packaging into the synaptic vesicles are efficiently coupled [33].

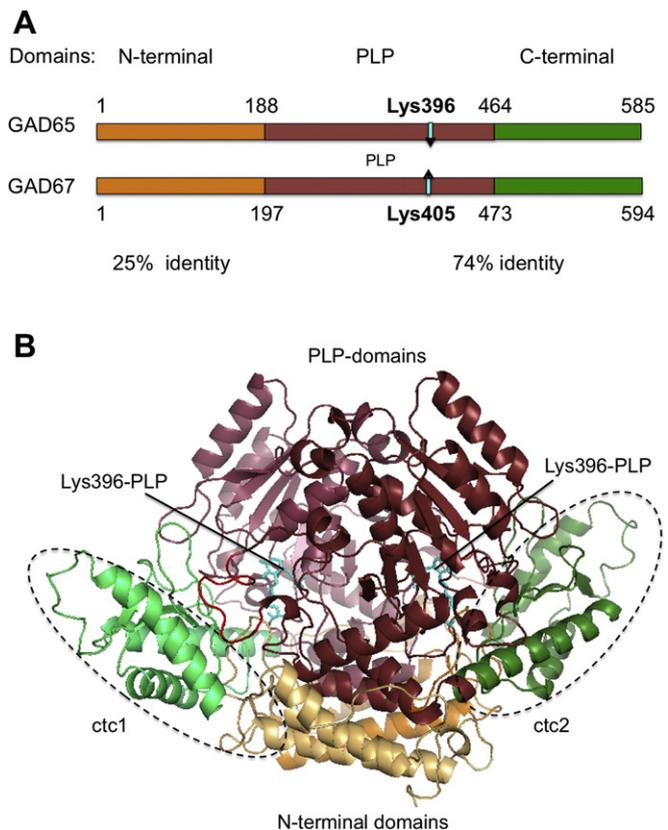


Fig. 1. Structural features of glutamic acid decarboxylase (GAD) A) Depiction of “traditional” three domains of GAD monomers, GAD65 and GAD67, showing N-terminal domains (orange), PLP-domains (ruby red), C-terminal domains (green), and binding site in the PLP domain of the co-factor L- Pyridoxal 5' phosphate (PLP, cyan with black arrow), at Lys 396 for GAD65) and Lys 405 for GAD67. (B) Dimeric structure of GAD65 (amino acids 84–585) showing the structural locations of the three domains, colored as in Fig. 1(A) with the other monomer in lighter colors, the catalytic loop (red), and the PLP co-factor that forms a Schiff base with Lys 396 (cyan) and the two C-terminal epitope clusters ctc1 and ctc2 are indicated by dotted ovals. Note that ctc1 includes some residues from the N-terminal domain and ctc2 includes some residues from the PLP-domain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Neurological diseases accompanying levels of high anti-GAD.

Disease	Percent frequencies	
	Series i, n = 50	Series ii, n = 62
Stiff-person syndrome	44%	26%
Cerebellar ataxia	34%	63%
Epilepsy, intractable	8%	31%
Others (see below)	14%	72%

Series i [22] from 61 patients referred with high levels of anti-GAD of whom 50 had a neurological disorder; series ii [23] from 62 patients derived from screening for paraneoplastic diseases. The percentages cited (totals > 100%) reflect cases with overlapping diagnoses. “Others” include a wide variety of CNS disorders, brain-stem syndrome, extrapyramidal syndromes, corticospinal spasticity, limbic encephalitis, peripheral neuropathies, ocular features (nystagmus, myoclonus). An accompanying neoplasm was cited in 4 cases in Series i, and there was clinical and/or serological evidence of accompanying thyrogastric autoimmune diseases in both series.

In the Monash Autoimmunity and Structural Biology Laboratories, crystal structures have been derived for both isomers GAD65 and GAD67, with each N-terminally truncated by 83 and 89 amino acids respectively [30]; thus there is no structural information available for the N-terminal regulatory sequence (Fig. 1B). However N-terminally-truncated GAD molecules contain the catalytic domain, retain full enzymatic activity and are immunoreactive [34,35]. Both the GAD65 and GAD67 enzymes form obligate dimers and for both isoforms the catalytic sequence of each subunit is juxtaposed in the cleft between the two monomers [30]. A notable structural difference in the catalytic and C-terminal sequences of GAD65 versus GAD67 is the increased flexibility of C-terminal sequences of GAD65 consequent on a disordered structure of the tethered loop (catalytic loop) that in GAD67 covers the active site so as to allow for sustained production of GABA. The disordered catalytic loop of GAD65 confers increased mobility which provides for easy access for binding of the PLP-cofactor and subsequent enzyme activation and then auto-inactivation [30]. The increase in flexibility in the catalytic region, and in a short C-terminal sequence of GAD65, could explain the antigenicity of GAD65 for T1D sera (see below).

The structural studies, together with data on ablative effects of mutated residues on the binding of human monoclonal antibodies (mAbs) to GAD65, and competition studies using human recombinant Fab (rFab) disclosed that antibody epitopes were tightly clustered in the region surrounding the C-terminal flexible region [34]. These epitopes segregated into two distinct C-terminal clusters (ctc1 and ctc2) located to opposite faces of the C-terminal domain, according to their preferential reactivity with one or another of two sets from a panel of human mAbs [34] (Fig. 1B). mAbs that engaged ctc1 (prototypically mAb b78), and equivalent GAD-reactive T1D sera, tended to be enzyme inhibitory and associated with a slowly progressive course of T1D as seen in latent autoimmune diabetes of adults (LADA) and autoimmune polyendocrine syndrome type 2 (APS-2) whereas those that engaged ctc2 (prototypically mAb 96.11) tended to be non-enzyme inhibitory and associated with rapidly progressive T1D [34]. Anti-GAD65 in SPS sera have been shown to engage preferentially the b78-associated epitopes i.e., those that react with ctc1 (see below).

3. B-Cell studies, anti-GAD and SPS

Some 70% of patients with SPS, and 70–80% with T1D, have antibodies to GAD [7], and in SPS a proportion of such antibodies are synthesized intrathecally [7,36a]. Thus anti-GAD are typically found in peripheral blood, and as well at lower levels in cerebro-

spinal fluid (CSF) in the CNS [7]. Anti-GAD in CSF is revealed electrophoretically as monoclonal bands, suggesting that only a fraction of the entire peripheral polyclonal response to GAD occurs in the CNS, yet the presence intrathecally of anti-GAD could facilitate ready access of anti-GAD to relevant neuronal autoantigens. Notably the intrathecal monoclonal immunoglobulins (Igs) are demonstrably reactive with GAD65 [36b].

The interesting relationships, and contrasts, between these two autoimmune diseases, SPS and T1D, and the antibody they share, include the following. First, serum levels of anti-GAD are usually substantially higher in SPS, although ranges of levels in SPS and T1D overlap; thus, given that some 30% of patients with SPS develop an associated autoimmune diabetes at some point in their illness, extremely elevated levels of anti-GAD levels could cause an eventual impairment of the function of neuroendocrine beta islet cells, as well as that of CNS neurons. The more moderate levels of anti-GAD, or the B cells that produce these, that pertain in classical T1D could be insufficient for transit across the blood–brain barrier, and so fail to elicit neurological symptoms. However, given that the intrathecal anti-GAD in SPS is indeed oligoclonal, then the breach of the blood–brain barrier must be by B cells and not their synthesized antibodies, since these would not be subject to any selection.

The next consideration is that whereas autoantibodies to GAD occur in 70%–80% of patients with SPS, as in autoimmune T1D, the properties of the anti-GAD response in the two diseases differ (Table 2). In SPS levels of anti-GAD in serum are usually highly raised, are reactive with GAD65 but almost equally so with GAD67 [7,37], can be detected by immunoblotting using purified GAD from rat brain [38], are inhibitory for the enzymatic activity of GAD [7], and react by immunofluorescence (IFL) with cells in pancreatic islets, whether or not there is coexisting diabetes, but also on sections from different regions of mammalian brain [39,40] (Fig. 2). The cerebellar granular layer is favored for diagnostic purposes. In a provocative study, Viannelo et al. [41] used IFL on cultured hippocampal neurons, on which GABAergic synapses are well represented, and staining patterns differed according to different neurological disorders, SPS, ataxia or epilepsy; the tentative suggestion was that differences in GAD epitope recognition differentiated clinical phenotypes. In T1D on the other hand anti-GAD are usually at low to moderate levels and mostly specific for GAD65, react predominantly with highly conformational epitopes and so are rarely detected by immunoblotting [39,40,42], seldom inhibitory for the enzymatic activity of GAD [43] and, although reactive with pancreatic islets, do not react by IFL with brain [38]. However since this latter statement is based on early (1990s) reports and on

Table 2
Comparison of immune responses to GAD in SPS and diabetes.

	SPS	Type 1 diabetes
Autoantibody levels	Moderate to very high [9,43]	Low to moderate [43]
Frequency		
anti-GAD65	70%+ [7,9]	60% [40]
anti-GAD67	50–60% [7,9]	12% [40], cross-reactive
Immunofluorescence +ve		
Pancreas	GAD65 only [40]	GAD65 & other reactants [40]
Brain	GAD65, 67, others [40]	Minimal if any reactivity [38]
Enzyme inhibition +ve	60% [7,43]	Rare, 2% [7]
Western blotting +ve	GAD65 only [9,38]	Rare, <10% [49]
B Cell epitopes		
GAD67, conformational	(see text)	(see text)
GAD65, linear	C-terminal [2,38,46]	C-terminal (rare) [45a,46]
GAD65, linear	N-termini, aa 4–22 [43,46–49]	None ascertained
GAD67, conformational	GAD67 specific	GAD67 or 65
IgG isotypes, subtypes	IgG1 (IgG4, IgE)	IgG1
T cell epitopes	aa 81–171, 313–403	aa 161–243, 473–555
Serum transfer to animals	Yes [44]	No [44]
Associated autoimmune diseases	Thyrogastric cluster [22,23]	Thyrogastric cluster [22,23]

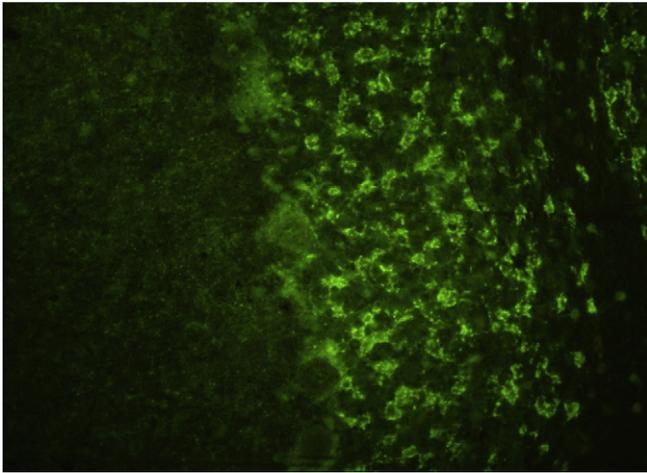


Fig. 2. Anti-GAD reactivity of SPS serum by immunofluorescence on mouse cerebellum. An image showing strong reactivity by IFL with the cytoplasm of cells in the granular layer of the cerebellum ($\times 400$), kindly provided by Bob Wilson, Supervising Scientist, Division of Immunology, Pathology Queensland. Presently the serum level by anti-GAD ELISA of antibody to GAD65 is >2000 units, the test by IFL for islet cell antibody is positive, and the test for anti-IA-2 is negative. The patient is a female aged mid-thirties with clinical features of SPS and occasional seizures whose onset of SMS was at ~ 12 yr of age, coincidentally with diabetes mellitus, with later development of hypothyroidism with anti-thyroid antibodies, and celiac disease. She has declined offered immunotherapies. Case details were kindly provided by Dr Stephen Reddel, with the patient's consent.

an unpublished communication to us from a neuroimmunology diagnostic laboratory, a systematic study is needed of the reactivity with brain of anti-GAD in classical T1D. Finally, after experimental injection of human anti-GAD into the cerebellum of rats, IgG autoantibodies from patients with SPS but not patients with T1D induced features of SPS [44]. We speculate that the above-described differing properties, to some degree at least, reflect recognition of epitopes of GAD additional to those recognized by sera in classical T1D.

3.1. Antibody epitopes in SPS and T1D

In T1D the GAD65-reactive conformational epitope sites have been well-defined. These are virtually exclusively located in the PLP- and C-terminal domains of GAD65, as judged by the good reactivity of T1D sera with GAD65 preparations that lack the first 100 amino acids [34,35] and the lack of reactivity with epitope(s) in the N-terminal regulatory domain [45a]. Also, whilst some sera from patients with T1D do have anti-GAD67 reactivity, this is almost always due simply to cross-reactivity with GAD65 [45b]. By contrast, autoantibodies to GAD65 in SPS recognize not only the conformational epitopes on GAD65 defined for sera in T1D, but also epitopes of GAD67 [38]. Moreover, several studies on SPS sera describe a major linear epitope in the first 100 amino acids that constitute the regulatory sequence in the N-terminal domain of GAD65 that is not reactive with T1D sera [43,46–49]. As well, for SPS sera, there are linear epitope regions in the extreme C-terminal sequence of GAD65 [43,46]. Interestingly, using IFL and blocking procedures, SPS sera that are immunoreactive for anti-GAD react by IFL with human islets that contain GAD65 but lack GAD67, whereas on rat brain react with GAD65 and GAD67 [40]. Thus, SPS sera in contrast to T1D sera appears to exhibit a different pattern of antibody reactivity with GAD65 and GAD67 and a different epitope selection; notably in SPS there is immunoreactivity to GAD67 to conformational epitopes, demonstrable in some 60% of cases, and at least some of these anti-GAD67 antibodies could be those

detectable by IFL on brain. The lack of cross-reactivity of anti-GAD65 and anti-GAD67 became evident using IFL on brain tissue, since some of the IFL reactivity on brain was not inhibited with GAD65 [40,46].

The properties that confer potent autoantigenic capacity on GAD65 are uncertain, so that the development is timely of crystal structures of GAD65 and GAD67 which now allow for closer study of the various biochemical and electrostatic characteristics of the two structurally similar but antigenically different isoforms [50]. Comparison of GAD65 and GAD67 reveals that GAD65 possesses a greater total accessible surface area for binding as well as more hydrophobicity, and differences exist in solvation energy data [50]. Moreover, the binding site of GAD65 for antibodies displays greater polarity and more negatively charged amino acid residues on its surface whereas for GAD67 the C-terminal exhibits a subdued, neutral electrostatic field and hence possesses less polarity [50]. Finally, the C-terminal and catalytic loop residues of GAD65 display more flexibility and mobility than their GAD67 counterparts [30], recalling here that Paus and Winter [51] suggested that increases in protein flexibility and charge were associated with augmented protein antigenicity. Thus differing structural properties of the two GAD isoforms would explain at least to some degree why, for T1D sera, only the GAD65 isoform is independently autoantigenic. The apo form of GAD65 particularly satisfies the conditions, in that once the enzyme loses its PLP cofactor and becomes inactive, it undergoes structural rearrangement that diminishes the stability of GAD65 and creates greater mobility in and around the active site, so possibly enhancing propensities to immunogenicity [50].

The above principles, although reasonably applicable to the anti-GAD65 response associated with T1D, are less applicable to the anti-GAD67 response associated with SPS. Raju et al. [43], using mAbs and polyclonal antibodies specific to GAD65 peptides, observed that C-terminus specific mAbs, particularly the human-derived mAb b78 that recognizes an epitope localized to aa 451–585, and the rabbit-derived polyclonal antiserum 7996 raised to C-terminal aa 570–585, had the greatest capacity for enzyme inhibition. Also Raju et al. [43] contrasted the differences in binding between the sera of patients with SPS and T1D by blocking reactivity with recombinant Fab (rFab) derived from the mouse mAb N-GAD65 which recognizes the linear epitope at the position aa 4–22 of the N-terminal domain of GAD65. The rFab N-GAD65 inhibited the binding to GAD65 of SPS sera, but not of T1D sera, although mAb N-GAD65 did not block the enzymatic activity of GAD. These studies are consistent with previous epitope mapping studies in the 1990s based on polypeptides derived from GAD65 (see above). For example, Kim et al. [47] in 1994 found that SPS-associated anti-GAD recognized a linear NH₂-terminal epitope which distinguished it from T1D-associated anti-GAD, and Piquer et al. [48] in 2005 studied 18 SPS patients and found that 17 (95%) contained anti-GAD65 reactive with aa 1–95 at the amino (N) terminus.

3.2. The immunogenic stimulus in T1D and SPS

Notwithstanding close structural similarities between GAD65 and GAD67, only GAD65 is primarily autoantigenic in T1D, and presumably serves as the immunogen for induction of disease. Studies in NOD mice have established the paradigm [52] that in T1D the autoimmunogenic stimulus, whether to insulin, GAD or other provocative antigens, occurs as the initial event in pancreatic islets from whence activated antigen-loaded dendritic cells migrate to the regional (pancreatic) lymph node and therein induce a response comprising antigen-reactive immunocytes, T cells particularly, and B cells as well; these T cells exit the node and “home” to pancreatic islets where they engage the cognate auto-antigen and effect beta cell destruction [53]. In the later stages the

process can be self-perpetuated by development of tertiary lymphoid structures in the target organ itself [54]. Equivalent data for human T1D are necessarily limited, but in one study the pancreatic lymph node contained T cells reactive to the insulin autoantigen [55]. Such a scheme is not applicable to the anti-GAD response in SPS since data are completely lacking on the site and nature of the initiating immunogenic stimulus to autoantigenic GAD, and particularly whether this is centered on islets as in T1D, or at some other peripheral site, or in the CNS itself.

4. Autoantibodies other than anti-GAD in SPS

As noted, anti-GAD are lacking in ~30% of patients with SPS, partly due to a different autoantigen being implicated but, so far, only three have been identified. Each is involved in some way with pre-synaptic GABA synthesis, or the post-synaptic GABA receptor signaling pathway. Amphiphysin is a proline-rich, hydrophilic protein present throughout the CNS, especially concentrated in presynaptic terminals [56a], and has a regulatory role in synaptic vesicle exocytosis. De Camilli and colleagues [24,25] in 1993 identified anti-amphiphysin antibodies in SPS after analysis of data from anti-GAD negative patients; this small subpopulation comprised only women, all with breast cancer. The association was confirmed of this paraneoplastic variant of SPS with breast cancer, and symptoms abated once the cancer was treated [24]. Murinson et al. [26] characterized this subtype of SPS in more detail by comparing 112 females with SPS and antibodies to GAD with 11 with SPS and antibodies to amphiphysin; all of the latter were female, the mean age at symptom onset was 58 years, none had T1D and stiffness affected the neck and arms more frequently than of the legs and spine as seen in anti-GAD-associated SPS. Treatment of the associated malignancy ameliorated SPS symptoms, and abolished these in some. Like anti-GAD sera, SPS is reproducible by passive transfer to rats of human IgG antibodies to amphiphysin [56b].

Other autoantigens in SPS include the GABA_A receptor-associated protein (GABARAP) to which sera of 70% of SPS patients were responsive in one study [27]. GABARAP is a post-synaptic polypeptide which, combined with gephyrin, facilitates synthesis of GABA_A receptor and its surface expression. It is present both intracellularly and on the axonal processes and facilitates signal transduction pathways of GABAergic neurons. The binding of autoantibodies to GABARAP would sabotage the normal processing of GABA through the GABAergic system, so causing symptoms. Gephyrin is another SPS autoantigen, but autoantibodies are poorly defined since there is just one case report of a patient with an undifferentiated mediastinal tumor with dysarthria and marked stiffness of the neck and upper body [28].

5. T Cell studies in SPS

T cell activation is prerequisite for adaptive immune responses, and for most autoimmune responses. T-cell responses to GAD65 have been well investigated in autoimmune diabetes in NOD mice, and as well in human T1D. In normal protective immune responses to foreign antigens, the process is initiated in the periphery, and the same likely pertains in autoimmune diabetes i.e., in the affected islet beta cells. The response is facilitated by a permissive genetic background, especially the presence of susceptibility haplotypes of the MHC that favor presentation of potentially autoantigenic peptides. Induction of autoimmune immunocytes then occurs in regional lymph nodes (see above) and destruction is executed by entry into the target tissue of specifically activated CD4 and CD8 T cells that engage peptide antigens exposed together with MHC molecules on islet beta cells. There have been identified in NOD mice and likewise in humans several islet cell autoantigens and

their peptide epitopes for T cells, varying according the MHC/HLA susceptibility alleles of the host. Fenalti et al. [34] selected for study five particular immunodominant T-cell epitope sequences on the crystal structure of human GAD65, each restricted by the diabetes susceptibility allele HLA DRB1*0401. Four of these epitopes formed a contiguous surface-exposed patch comprising parts of the two major B-cell epitope clusters, ctc1 and ctc2 (see above), illustrating the close relationship of T- and B-cell epitope sites on GAD65 that pertains for other autoantigens as well. Of the five epitope sites, three were contained within the C-terminal domain, one within the PLP domain and one within the N-terminal domain. However, for the anti-GAD response in SPS, information is scanty on immunogenic T-cell epitopes and, although the same HLA susceptibility alleles are prominent in both T1D and SPS [10], T-cell peptide epitopes may well differ.

In SPS the initiation of autoimmune responses to GAD65 and GAD67 could either be in the periphery with subsequent relocation to the CNS, or develop *ab initio* in the CNS itself, and be perpetuated by development of ectopic lymphoid neogenesis in the CNS, as occurs in pancreatic islets in NOD mice with insulinitis [54]. Although the latter scenario is consistent with evidence for immune responsiveness intrathecally, within the blood–brain barrier, it seems less likely than reactive lymphocytes in some way penetrating the blood–brain barrier and being sustained therein by antigenic contact. Postulated events are shown in Fig. 3.

Effector functions of T cells in anti-GAD-associated SPS have attracted attention. In an autopsy study of a single SPS patient, with comparison of findings using four controls, qualitative and quantitative analysis of lymphocytes in the ventral horn region of the spinal cord revealed mild “lymphocyte cuffing” but only slight inflammation, hardly supportive of the occurrence of T cell–B cell interaction in production of disease [57]. Other single case studies on interaction between GAD65 and presumed specifically reactive T cells point to production of a particular cytokine profile that is stimulatory for autoantibody synthesis. In one study T cells were collected from an SPS patient with T1D and, after stimulation with GAD65, caused a weak increase in interferon (IFN) gamma production; after treatment with prednisolone, there was clinical improvement and decreased production of IFN gamma [58]. In a similar study on a non-T1D SPS patient, GAD65 failed to elicit any meaningful T cell stimulatory responses although, when a GAD65-specific T-cell line was developed by in vitro stimulation, the cloned T cells did specifically respond to the GAD65 peptide sequence 341–360 [59]; the elicited cytokine profile tended to be non-inflammatory, with raised levels of IFN gamma and IL-10 and a low level of IL-4. In another study [60] the in vitro cytokine profile of an SPS patient was followed over 46 months and, as in the above case [59], the profile became more Th₂ prominent over this time, with GAD65 stimulation leading to marked IFN gamma and IL-13 production and low levels of IL-4; consistent with the previous report [59], the best response was to the GAD65 peptide sequence, aa 339–352. The T cell epitope sequence aa 341–360 was not among the peptides mapped by Fenalti et al. [34], but those sequences were all DR4-restricted whereas the 341–360 sequence of GAD65 was DR3-restricted [59]. There were no other relevant aa 341–360 peptide sequences in a published archive of known T-cell responsive sequences in T1D [61]. All-in-all, the reports do give some indication in SPS of helper T-cell activity but are not indicative of any inimical effects of T cells *per se* as pertains in autoimmune insulinitis in NOD mice and probably in human T1D as well. Other points to be made are first, that CNS neurons lack cell surface MHC molecules and so would be resistant to T-cell attack, and second, that “the temporary reversal of symptoms with pharmaceutical therapies implies a functional impairment of transmission rather than the destruction of the GABAergic neurons” [62]. Attention therefore shifts to B cells and antibody as effectors.

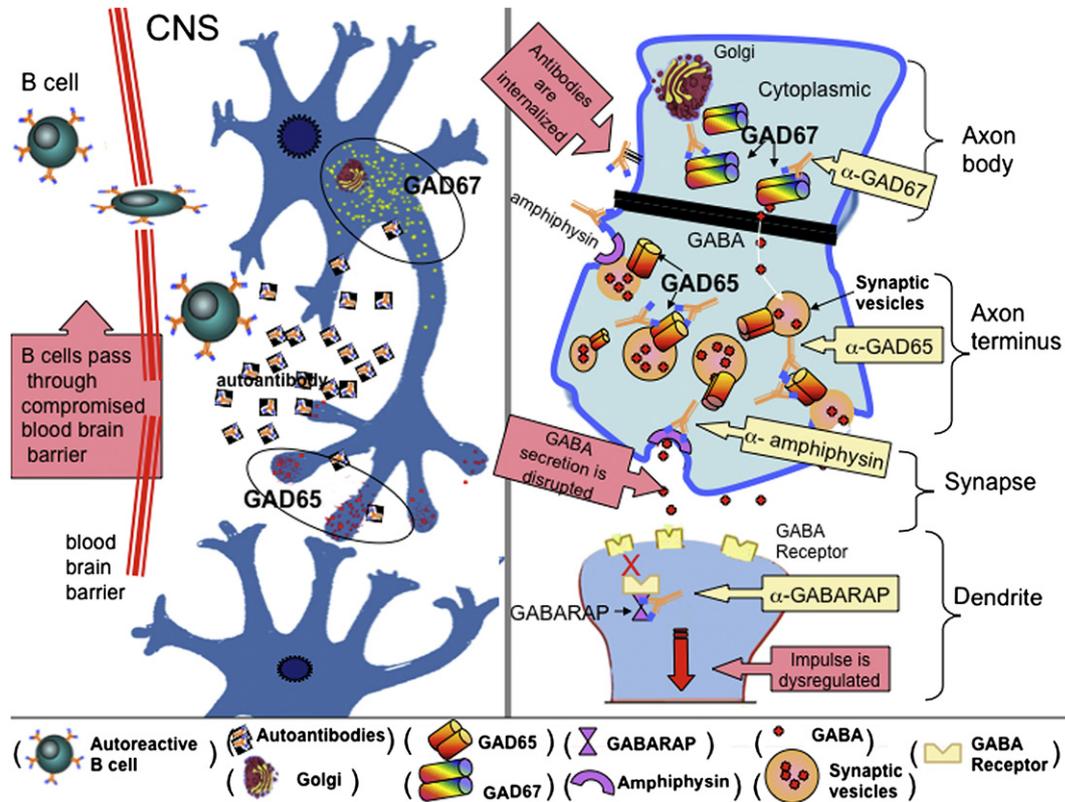


Fig. 3. Cartoon depicting proposed intrathecal immunopathology in SPS. Left panel: B (and T) cells reactive with epitopes of GAD gain access to the CNS via a compromised blood–brain barrier (BBB) that has become permissive for their transit. Shown are the distributions of GAD isoforms, in the cytoplasm (GAD67) and the synaptic vesicles (GAD65), of GABAergic neurons, and intrathecal synthesis by B cells of anti-GAD that encounters GABAergic neurones in the CNS. Right panel: Cell body and axon terminus of a GABAergic neuron, synaptic cleft and connecting dendrite. Anti-GAD antibody is internalized and reacts with GAD67 in neuronal cytosol, and with GAD65 in synaptic vesicles, resulting in disruption of synthesis/secretion of GABA, non-activation of GABA receptors and features of SPS. Also shown are other SPS-relevant autoantibodies that can disrupt GABAergic transmission (see text), to amphiphysin so interfering with the assembly of GAD65 in presynaptic secretory vesicles at the axon terminus, and to the post-synaptic GABA receptor associated protein (GABARAP), each likewise interfering with inhibitory neuronal signaling (For the interpretation of reference to color in this figure, please refer to the web version of this article.).

6. Lessons from treatment of SPS

6.1. B Cells and antibodies

SPS has the hallmarks of an antibody-mediated B-cell dependent autoimmune disease, noting that B cells in the CNS itself produce anti-GAD65 antibody [36a] and thereby could perpetuate the pathology. This concept is the basis of current therapies including prednisolone, regular plasmapheresis as used in extreme situations or when all other treatments have failed, and intravenous immunoglobulin G (IVIg) which is becoming the standard therapy for anti-GAD positive SPS. The rationale is anecdotal accounts of benefit, followed by a randomized cross-over trial involving sixteen patients [63,64] wherein high dose IVIg (2 g/kg) compared to placebo resulted in great relief of stiffness of the trunk, abdomen and face, a decrease in time to walk thirty feet, an average 33% decrease in anti-GAD levels in serum, and in many patients an overall improvement in quality of life. Thus the benefits of IVIg outweigh the increased risk of complications, cardiovascular such as deep vein thrombosis, and anaphylaxis especially in patients with concomitant IgA deficiency.

On the same reasoning it seems logical that therapeutic use of an anti-CD20 agent such as rituximab, by depleting CD20 + B cells, would diminish anti-GAD65 production and mitigate disease. There is, however, little information on the extent to which rituximab penetrates the blood–brain barrier or whether rituximab would be more efficacious given intrathecally. In fact, the literature

reveals cases wherein rituximab did prove beneficial [65,66] but balanced by others showing no improvement despite B cell reduction in blood [67]. In a case of SPS, thyroid ophthalmopathy and diabetes treatment with rituximab induced “obvious improvement” in SPS and thyroid ophthalmopathy, and a decrease in levels of anti-GAD but with no amelioration of features of diabetes [66]; the authors speculated that different pathogenic mechanisms may operate in SPS and diabetes. Contrariwise, there is the report [67] on female monozygotic twins of whom each, remarkably, was afflicted with SPS, but rituximab had little effect either on symptoms, anti-GAD65 levels, enzyme inhibitory activity or epitope recognition by autoantibodies; in fact, both patients had an increase in numbers in blood of anti-GAD65 specific memory B cells during follow up. These and other observations [68] suggest that anti-CD20 resistant memory B cells are the precursors of anti-GAD autoantibody. Accepting that there is more to B-cell pathogenesis of autoimmune disease than mere production of antibody (anti-GAD in SPS), more information should be sought on how various cell types in SPS and other B-cell-dependent autoimmune diseases respond to antigenic stimulation, interact to produce autoantibodies, acquire cytokine-producing potential, and propagate disease.

An enduring question for SPS (and indeed for autoantibody effector mechanisms in general) is how autoantibodies, anti-GAD in the case of SPS, reach and damage an intracellular target [69]. Although GAD65 is an intracytoplasmic enzyme and thus unlikely to be directly exposed to autoantibodies, whether in blood or CSF,

some studies do suggest that IgG antibodies can be transported into neurons by particular endocytosis mechanisms [70], possibly via ganglioside binding or Fc-receptor mediated binding. Alternatively the vesicular GABA transporter (VGAT) may participate, since the C-terminal of this transmembrane transporter protein projects into synaptic vesicles, which in turn could be recognized within the cell by extracellularly bound antibodies [71].

6.2. Drugs affecting GABAergic neurotransmission

Additional to pathogenetic inferences to be drawn from outcomes of anti-B cell therapies for SPS [63–67], there are inferences from the symptomatic relief obtained from pharmacological therapies. Benzodiazepine drugs were first used empirically in the 1960s for symptomatic relief of SPS although requisite doses are typically high with risks of side effects or oversedation. The efficacy of benzodiazepines is telling in view of their recognized pharmacological rationale, since certain types of GABA receptor (GABA-R) contain a specific benzodiazepine binding site. GABA-Rs contain an ion channel that conducts chloride ions across neuronal cell membranes with inhibitory effects. GABA-Rs have two binding sites for GABA, and it is the subset of GABA_A receptors that contain a separate binding-activating site for benzodiazepines; receptor engagement enhances the efficiency of GABA by decreasing the excitability of neurons [73]. Since differing GABA-Rs have varying distributions according to region of the CNS, the distributions of GAD/GABA could differ correspondingly, partly explaining why there is variable reactivity by region when SPS antisera are tested by IFL on CNS tissue. Baclofen is a synthetic derivative of GABA and an agonist for GABA receptors that particularly modulates the GABA_B receptor. Its use orally or intrathecally reduces muscle spasms and rigidity with minimal sedation. It is used long-term via an intrathecal pump [72] despite small risks of infection, leakage or pump failure.

7. Conclusions

Why and how an autoimmune response to GAD can become differentially directed, either to GABA-dependent inhibitory central neurotransmission as in SMS, or to the GAD65 isoform in pancreatic islets as in T1D, has puzzled investigators. Particular features of the autoimmune response in SPS and T1D include first, antibody-driven effector processes versus T-cell driven effector processes, and second, epitope targeting in SMS uniquely involving an N-terminal aa 4-22 sequence and an independent responsiveness to the GAD67 isoform. We note a proposal [23] prompted by the example of myasthenia gravis wherein there is a “marker” antibody (to cytoplasmic muscle striations) that accompanies a “true” pathogenic autoantibody to acetylcholine receptors on the plasma membrane, so that in SPS and anti-GAD associated neuropathologies there could be, as well as anti-GAD, an as yet unidentified autoantibody to a critical component on the plasma membrane of neurones that form inhibitory GABAergic synapses.

Tribute

This issue of the Journal of Autoimmunity honors the distinguished career of Chella David, a prolific contributor to the analysis of HLA-based genetic predisposition to human autoimmune diseases, and a generous donor of unique transgenically-derived strains of HLA-modified mice. We are privileged to participate, and one of us (IRM) is personally appreciative of Chella David's thoughtful overview (Chapter 20, *Genetics and Autoimmunity: HLA and MHC Genes*) for “THE AUTOIMMUNE DISEASES Edition IV”. The editors and authors also note that this paper is part of a special

series in the Journal of Autoimmunity and its companion journal, Autoimmunity Reviews, which recognizes important contributions and key figures that have played a major role in our understanding of autoimmunity. This has included Susumu Ikehara, Ian R. Mackay, Noel Rose and Harry Moutsopoulos, as well as a special symposium on therapeutics and geoepidemiology [74–81]. This present paper closes in, albeit from different directions, on our central question in common: Why and how does autoimmunity happen?

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