
This supplementary material has been provided by the authors to give readers additional information about their work.
eTable. Clinical features used in classifying patients with SPS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Classic SPS</th>
<th>SLS</th>
<th>Jerking SPS</th>
<th>PERM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of rigidity</td>
<td>Axial</td>
<td>Distal limb</td>
<td>Axial</td>
<td>Axial and limb</td>
</tr>
<tr>
<td>Brainstem signs</td>
<td>–</td>
<td>±¹</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Brainstem myoclonus</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>±</td>
</tr>
<tr>
<td>Long tracts involvement</td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>Clinical course</td>
<td>Slow progression</td>
<td>Relapsing and remitting</td>
<td>Slow progression</td>
<td>Rapid progression</td>
</tr>
<tr>
<td>CMUA</td>
<td>Axial muscles</td>
<td>Limb muscles</td>
<td>Axial muscles</td>
<td>Axial and limb muscles</td>
</tr>
</tbody>
</table>

¹ Transient.

Abbreviations: ++, dominant sign; +, present; ±, reported; −, absent; CMUA, continuous motor unit activity on electromyogram; PERM, progressive encephalomyelitis with rigidity and myoclonus; SLS, stiff limb syndrome; SPS, stiff person syndrome.
eAppendix. Clinical data and methods

2 patients whose plasmapheresis-derived purified IgG was injected into mice

Patient 25 (age 71 years, female) had the stiff person syndrome (SPS) variant, progressive encephalomyelitis with rigidity and myoclonus (PERM), of 19 months duration with rigidity, reflex myoclonic spasms, and type 1 diabetes mellitus but no other neurological or autoimmune disease. She had been treated with intravenous corticosteroids and plasmapheresis, but with no apparent clinical improvement. Her plasma glutamic acid decarboxylase (GAD) autoantibody titer was 5451 U/mL, whereas the purified and concentrated IgG fraction used for passive transfer had a titer of 9237 U/mL. The GAD autoantibody affinity was 0.17 nmol and the predominant GAD autoantibody IgG subclass in serum was IgG1 (68%). On frozen rat brain sections, the serum demonstrated immunohistochemical staining typical of GAD autoantibodies but no evidence of other autoantibodies. On primary neuronal cultures, the serum demonstrated autoantibodies that bound to surface determinants of a small percentage of neurons transmitting \(\gamma\)-aminobutyric acid (GABAergic).

Patient 12 (age 38 years, female) had classic SPS of 21 months duration with rigidity, superimposed spasms, and anxiety. She had been treated with mycophenolate mofetil and plasmapheresis. The clinical response to plasmapheresis is not known. Her plasma GAD autoantibody titer was 836 U/mL, whereas the purified and concentrated IgG fraction used for passive transfer had a titer of 1283 U/mL. The GAD autoantibody affinity was 0.14 nmol and the predominant GAD autoantibody IgG subclass in serum was IgG1 (71%). Perhaps related to her low GAD autoantibody levels, her serum did not demonstrate immunohistochemical staining typical of GAD autoantibodies and did not show any binding to surface determinants on neuronal cells.

Methods.

Subclass and affinity analyses for GAD autoantibodies. For subclass analyses, a nonsaturating volume of each serum sample was incubated with 50 \(\mu\)L of iodine 125–labeled GAD65, and the complexes were immunoprecipitated using 10 \(\mu\)L of each subclass-specific sheep antihuman IgG (IgG1-IgG4) (Binding Site Ltd, Birmingham) and 30 \(\mu\)L donkey antisheep IgG (Binding Site Ltd) previously adsorbed with 30% normal human serum. Parallel controls and blanks without subclass-specific immunoglobulins were performed to determine nonspecific immunoprecipitation, as described elsewhere (Vincent and Newsom-Davis. Exp Immunol. 1982;49[2]:257-265).

For affinity analysis, the \(^{125}\)I-GAD65 was serially diluted (1:2) 10 times to provide a range of different concentrations and incubated with nonsaturating volumes of serum and immunoprecipitated with antihuman IgG as above. Bound and free \(^{125}\)I-GAD65 were converted to nanomoles and the dissociation constants (KD) for GAD autoantibodies derived from Scatchard analysis (GraphPad Prism).
Processing central nervous system tissue from mice. Mice deeply anesthetised by halothane inhalation were transcardially perfused with chilled phosphate-buffed saline solution (PBS), 25 mL/mouse, followed by a freshly made filtered solution of 4% paraformaldehyde (PFA; Sigma) in PBS, 150 mL/mouse. Brains and spinal cords were removed and post-fixed in the same fixative for 30 minutes. All tissues were cryoprotected overnight at 4°C in a solution of 30% sucrose in Tris-buffered saline (TBS; 50mM; Tris pH, 7.6). Brains were sectioned into regions and mounted in tissue-Tek within cryomoulds on dry ice and stored at −80°C. Cryostat sections (12 μm thick) were cut on to chrome gelatin-coated slides, dried overnight in a vacuumed chamber, and stored at −20°C until stained.

Immunostaining used Shandon Sequenza slide racks with the cover plate system (Thermo Fisher Scientific). Sections were washed with PBS and nonspecific binding was blocked with 10% NGS in 0.3% PBS and Triton X-100 (Sigma-Aldrich) (PBST) at room temperature for 1 hour. Primary antibodies were applied in 10% NGS in 0.3% PBST and incubated at room temperature for 1 hour. After 3 washes in PBS, the secondary antibodies were applied in the same solution and incubated for 1 hour. Slides were washed and coverslips applied using fluorescent mounting medium (Dako). Slides were allowed to dry at 4°C overnight before examination under confocal microscopy. The primary and secondary antibodies used are described in below. An in situ cell death detection kit (Roche) was used to detect apoptosis.

Commercial antibodies. Primary antibodies used were hamster anti-CD3 (1:50; Caltag Laboratories, Burlingame [CA94010]), rat anti-CD45 (1:50; Caltag Laboratories [CA94010]), rat anti-F4/80 (1:100; Serotec), rabbit anti-GFAP (1:1000; Dako), mouse anti-NeuN (1:1000; Chemicon), rabbit anticalbindin (1:1000; Swant), rabbit anti-AC3 (1:200; Cell Signalling Technology), rabbit antisynaptophysin (1:100; NeoMarkers), and antimouse CD16/CD32 (Fc©III/II receptor) (1:50; BD Pharmingen). Secondary antibodies (Alexa Fluor, Molecular Probes, Invitrogen) were specific for the species of the primary antibodies and conjugated with fluorophores with excitation maxima compatible with the confocal microscope lasers.
eFigure 1. Successful immunoadsorption of serum from patient sample 1 against recombinant GAD

Immunoblotting against recombinant human glutamic acid decarboxylase 65 (rhGAD65) after adsorption of serum from a patient with stiff person syndrome (SPS) (patient 1 [Table 2]) with rhGAD65. Lanes 1 and 6 represent the SPS serum before adsorption. Lanes 2 to 5 show the same volume of serum (10 µL) adsorbed with increasing amounts of rhGAD65 (2, 3, 4, and 5 µg). Lanes 7 to 10 show increasing dilutions of the serum (1:10, 1:100, 1:1000, and 1:5000) adsorbed with a fixed amount of rhGAD65 (2.5 µg).
eFigure 2. Binding to recombinant GAD

A, Representative Western blot of recombinant human glutamic acid decarboxylase 65 (rhGAD65) with patient serum (1:100). Arrow head marks the band representing GAD65. Samples showing positive reactivity are indicated by an asterisk. Number in parenthesis refers to patient number in Table 2. Abbreviations: C-SPS, classic stiff person syndrome; GADmAb: anti–GAD monoclonal antibody (1:1000); MG, ; NHC, normal healthy control; SLS, stiff limb syndrome. B, Integrated optical density (IOD) results for immunoblotting rhGAD65 with patient serum were plotted against the GAD autoantibody (Ab) titers. IOD values are normalized to the positive control (anti-GAD monoclonal antibody, 100%). There was a positive correlation by Spearman rank correlation test.
eFigure 3. Protocol for passive transfer

Study profile and time line for passive transfer of human IgG from a patient with high glutamic acid decarboxylase autoantibody (GADAb) titer (A) and low GADAb titer (B) into GAD65-enhanced green fluorescent protein (EGFP) transgenic mice. BT indicates behavioral tests; C-SPS, classic stiff person syndrome; IP, intraperitoneal; LPS, lipopolysaccharide; PERM, progressive encephalomyelitis with rigidity and myoclonus.
eFigure 4. IgG levels in mouse brains after passive transfer

Immunoblot showing human IgG heavy (50 kDa, arrow head) and light chains (25 kDa, arrow head) in brains of mice passively transferred with patient IgG (A and B) or control IgG. Human serum IgG as a control was used at 1:4 serial dilutions. There was significant correlation between the human IgG detected in immunoblotted mouse brain extracts (integrated optical densities [IOD]/prot in milligrams) and the concentration of human IgG (in milligrams per milliliter) injected into mice. The levels in the 2 glutamic acid decarboxylase autoantibody (GAD-Ab)–treated mice are demonstrated by the patient numbers. The control IgG was at a higher concentration.

Comment [mc2]: Please spell out abbreviation.
eFigure 5. Double immunofluorescence on GAD antibodies.

Indirect immunofluorescence micrographs of anti–glutamic acid decarboxylase monoclonal antibodies (GADmAb, fluorescein isothiocyanated conjugated) and stiff person syndrome serum (SPS, patient sample 1, phycoerythrin) on hippocampus (Hp, original magnification ×20) and cortex (Ctx, original magnification ×100). The merged result in the third column shows colocalization of anti-GADmAb with SPS serum immunoreactivity.