ORIGINAL CONTRIBUTION

Anti–Glutamic Acid Decarboxylase Antibodies in the Serum and Cerebrospinal Fluid of Patients With Stiff-Person Syndrome

Correlation With Clinical Severity

Goran Rakocevic, MD; Raghavanpillai Raju, PhD; Marinos C. Dalakas, MD

Background: Stiff-person syndrome (SPS) is an immune-mediated central nervous system disorder characterized by fluctuating muscle stiffness, disabling spasms, and heightened sensitivity to external stimuli. Up to 80% of patients with SPS have anti–glutamic acid decarboxylase (GAD) antibodies in the serum or cerebral spinal fluid (CSF). Whether these antibodies are clinically relevant and correlate with disease severity is unknown.

Objective: To correlate anti-GAD antibody titers in the serum and CSF of patients with SPS with the degree of clinical severity.

Design: Patients studied the last 6 years.

Setting: The Clinical Center of the National Institutes of Health, Bethesda, Md.

Patients: Sixteen patients with typical SPS and elevated serum anti-GAD antibody titers.

Interventions: Antibody titers in serum and CSF were measured by radioimmunoassay, and the intrathecal anti–GAD-specific IgG production was calculated.

Main Outcome Measures: Comparison of antibody titers with stiffness index and heightened sensitivity scores based on scales that reliably measure disease severity.

Results: The mean disease duration was 11 years (range, 5-30 years). The mean anti-GAD antibody titer in the serum was 51 500 U/mL (range, 24 000-200 000 U/mL); and in the CSF, 181 U/mL (range, 30-400 U/mL). A 10-fold increased intrathecal production of GAD-specific IgG antibodies was noted. No correlation was found between antibody titers in serum or CSF with disease severity. In 4 patients, the anti-GAD antibody titers measured serially during a 2-year period did not correlate with clinical fluctuations.

Conclusions: In patients with SPS, the anti-GAD antibody titers in serum and CSF do not correlate with disease severity or duration. Anti-GAD antibodies are an excellent marker for SPS, but monitoring their titers during the course of the disease may not be of practical value.

Arch Neurol. 2004;61:902-904

From the Neuromuscular Diseases Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md.

STIFF-PERSON SYNDROME (SPS) is an immune-mediated central nervous system disorder characterized by rigidity of the axial and proximal limb muscles, intermittent superimposed spasms, and heightened sensitivity to external stimuli.[1-3] Continuous cocontraction of agonist and antagonist muscles caused by involuntary firing of motor units at rest are the clinical and electrophysiologic hallmarks of the disease.[1-4]

Stiff-person syndrome is an autoimmune disorder that responds to immunotherapies[5,6] and is frequently associated with other autoimmune diseases, autoantibodies, and certain HLA haplotypes.[1-3] A characteristic autoimmune marker for SPS seen in more than 80% of the patients is the presence of antibodies against glutamic acid decarboxylase (GAD65), the rate-limiting enzyme for the synthesis of γ-aminobutyric acid (GABA), the brain's main inhibitory neurotransmitter.[1-3] These antibodies are produced intrathecally and may impair the synthesis of GABA, resulting in low GABA levels in the brain and cerebrospinal fluid (CSF).[2,3] To determine the clinical relevance of anti-GAD antibodies in patients with SPS, we correlated their intrathecal production and serum titers with the severity of symptoms, using validated clinical scales.

METHODS

We examined serum and CSF from 16 patients with SPS, 6 men and 10 women, aged 37 to 62 years (mean, 52 years), studied under institutional review board–approved protocols at

the Neuromuscular Diseases Section, National Institutes of Health, Bethesda, Md, from 1997 to 2003. All patients had elevated titers of serum anti-GAD antibodies and fulfilled the established diagnostic criteria for SPS.[1,3] The serum and CSF were examined for total immunoglobulins, immunofixation electrophoresis, IgG index, and intrathecal synthesis of IgG-specific anti-GAD antibodies. The integrity of the blood-brain barrier was assessed by the ratio of serum albumin to CSF albumin (normal, >130). The CSF IgG index was calculated according to the following formula:

(CSF IgG/Serum IgG)/(CSF Albumin/Serum Albumin),

in which CSF IgG and CSF albumin are measured in nanograms per milliliter and serum IgG and serum albumin are measured in milligrams per milliliter.

The CSF IgG index is considered normal when it is below 0.67.[2,7] For detection and quantification of anti-GAD antibodies in serum and CSF, we used a radioimmunoassay kit (Kronus, Boise, Idaho). The results are reported in manufacturer's units per milliliter (normal, <1 U/mL). For the calculation of IgG GAD–specific index, the following formula was used in paired serum and CSF specimens:

(CSF IgG Anti-GAD Titer/Serum IgG Anti-GAD Titer)/(CSF Albumin/Serum Albumin),

in which the CSF IgG and serum IgG anti-GAD titers are measured in units per milliliter, CSF albumin is measured in nanograms per milliliter, and serum albumin is measured in milligrams per milliliter.

The final concentration of antibodies was obtained after normalization of the different dilutions to the same standard curve. Antibody titers were blindly analyzed and compared with the stiffness index and heightened sensitivity scores, determined for each patient at the time of antibody draw.

The following scales that measure disease severity were used, as previously described.[1,2,6] For *heightened sensitivity,* the following were rated as present or absent: noise-induced stiffness and cramps, visual stimuli–induced stiffness and cramps, somatosensory-induced stiffness and cramps, voluntary activity–induced spasms, emotional upset and "stress"-induced spasms, awakenings due to nocturnal spasms, and untriggered cramps and spasms. The presence of each item added 1 point (maximum score, 7). For *distribution of stiffness and cramps,* the presence of each of the following was given 1 point: stiffness in the face, stiffness in the arms, stiffness in the upper trunk, stiffness in the abdomen, stiffness in the lower trunk, and stiffness in the legs (maximum score, 6).

RESULTS

CLINICAL DATA

The duration of disease ranged from 5 to 30 years (mean, 11 years); the time from onset of symptoms to diagnosis ranged from 1 to 13 years (mean, 4 years). The most prevalent initial symptom was lower back muscle stiffness (8 patients), followed by proximal leg stiffness (7 patients). Single-limb stiffness was the initial symptom in 1 patient, and stiffness confined to respiratory and abdominal muscles in another. The median score on the stiffness index was 3.3 (range, 1-6) (**Figure 1**). The median score for heightened sensitivity was 3.8 (range, 2-7). At the time of GAD antibody determination, all studied patients were symptomatic or incapacitated, despite receiving GABA-enhancing drugs such as diazepam, clonazepam, or baclofen. None of the study patients were taking

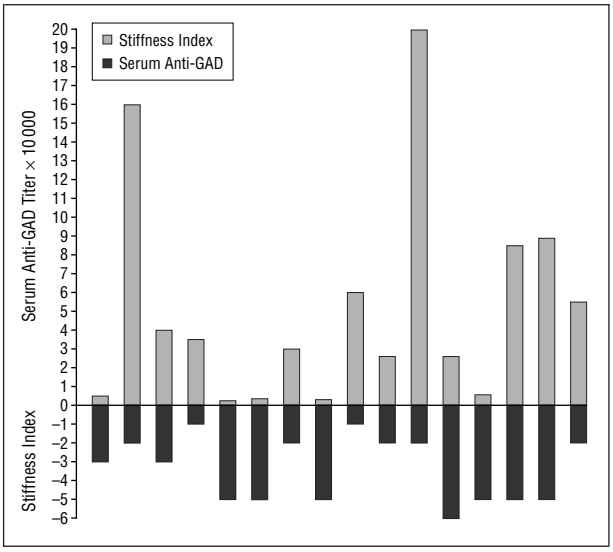

Figure 1. Relationship between serum anti–glutamic acid decarboxylase (GAD) antibodies and stiffness index. Each black bar represents the degree of stiffness for each patient rated on a scale from 0 to 6 (shown as 0 to –6), while the corresponding gray bar shows the patient's serum anti-GAD antibody titer (×10 000). No consistent correlation was found between serum anti-GAD antibody titers and disease severity.

immunomodulating therapies, but 1 patient had received intravenous immunoglobulin 4 months before determination of GAD antibodies. Seven patients also had insulin-dependent diabetes mellitus, 6 had immune-mediated thyroiditis, and 2 had pernicious anemia. Five patients had strongly positive antinuclear antibodies, 3 had thyroid microsomal antibodies, and 2 others had anti-islet or anti-Jo antibodies.

Neuropsychiatric symptoms were ubiquitous, with anxiety being most prevalent in 9 patients and depressed mood in 3. Five patients had seizures.

ANTIBODY TITERS AND CORRELATION WITH CLINICAL SEVERITY

No consistent correlation was found between the serum anti-GAD antibody titers and disease severity; the titers were high in some patients with mild disease and low in some others with severe disease (Figure 1).

The mean anti-GAD antibody titer in the CSF was 181 U/mL (range, 30-400 U/mL) and the mean serum titer was 51 500 U/mL (range, 24 000-200 000) (normal, <1 U/mL). The mean ratio of the CSF to serum anti-GAD antibody titer was 0.02 (range, 0.001-0.166), compared with the mean ratio of 0.002 for the CSF to serum IgG (range, 0.001-0.004), reflecting a 10-fold increased production of GAD-specific IgG antibodies in the CSF.

The CSF to serum albumin ratio was normal in all patients, indicating intact blood-brain barrier. No correlation was found between the CSF anti-GAD antibody titers and their intrathecal production with severity of clinical symptoms (**Figure 2**). Although 6 patients with high intrathecal anti-GAD antibody production had clinically severe disease, others with equally severe disease had low GAD-specific CSF IgG index.

The level of anti-GAD antibodies in serum or CSF did not correlate with the presence of other autoantibodies or

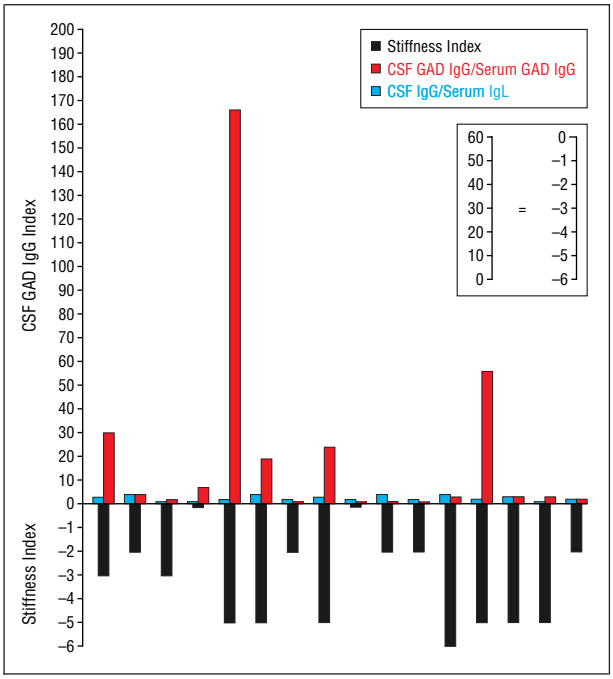

Figure 2. Ratio between cerebrospinal fluid (CSF) glutamic acid decarboxylase (GAD)–specific IgG index and stiffness index. Each black bar represents an individual patient's stiffness score rated on a scale from 0 to 6 (shown as 0 to –6). Corresponding blue bars show the ratio between CSF and serum IgG (indicative of intact blood-brain barrier), while the corresponding red bars show the ratio between CSF GAD-specific IgG and serum GAD-specific IgG. High CSF GAD-specific IgG index reflects increased intrathecal anti-GAD antibody production, which does not always correlate with severity of clinical symptoms.

the patients' subsequent response to intravenous immune globulin.[6] Preliminary results in 4 patients who had anti-GAD antibodies measured serially during a 2-year period demonstrated that titers did not correlate with the clinical fluctuations of their disease (data not shown).

COMMENT

High anti-GAD antibodies appear to be specific markers for SPS; only rare patients with cerebellar degeneration and diabetes have high antibody titers.[8] It has been proposed that these antibodies may be pathogenic because (1) GAD activity and GABA synthesis are directly inhibited in vitro by the anti–GAD-specific IgG from patients with SPS[9] and (2) anti-GAD antibodies are produced intrathecally and may suppress GABA level, as supported by the finding of reduced GABA in the brain and CSF.[2,3] Because GABA is the brain's predominant inhibitory neurotransmitter, reduction of GABA could explain the patient's symptoms of muscle hyperactivity, as well as the motor cortex hyperexcitability noted by the electrophysiologic studies.[4] Our finding that the anti-GAD antibodies, which recognize a cytoplasmic antigen, do not correlate with the clinical symptoms casts doubt on their direct pathogenic role in affecting GABA level. Whether GABA levels correlate with disease severity remains unclear.

Glutamic acid decarboxylase is a cytoplasmic antigen, not easily recognized by the immune system, indicating that another surface protein associated with GAD or GABA may be the target of the autoimmune attack. Although GAD antibodies may cross-react with GABA binding sites on the neuronal surface and GAD peptide fragments may be expressed at the cell surface during exocytosis of GABA, there is no sufficient evidence to prove their pathogenic role in SPS. Alternatively, cytotoxic T cells may be the main pathogenic effectors if cytoplasmic GAD peptides, bound to major histocompatibility complex I molecules, are recognized by the $CD8^+$ T cells, as proposed for paraneoplastic neuronal antibodies.[10]

Until the specific surface antigen responsible for the initiation of self-sensitization is demonstrated, the anti-GAD antibodies in SPS should be considered only as excellent disease markers. Our present findings indicate that there is no practical value in observing anti-GAD antibodies serially during the various stages of the disease or to monitor response to therapies. The observation is consistent with the significance of autoantibody levels in other autoimmune disorders such as myasthenia gravis or Lambert-Eaton myasthenic syndrome.

Accepted for publication November 24, 2003.

Author contributions: *Study concept and design* (Drs Rakocevic and Dalakas); *acquisition of data* (Drs Rakocevic and Raju); *analysis and interpretation of data* (Drs Rakocevic, Raju, and Dalakas); *drafting of the manuscript* (Dr Rakocevic); *critical revision of the manuscript for important intellectual content* (Drs Rakocevic, Raju, and Dalakas); *statistical expertise* (Dr Rakocevic); *obtained funding* (Dr Dalakas); *administrative, technical, and material support* (Drs Rakocevic and Dalakas); *study supervision* (Drs Raju and Dalakas).

This study was funded through the intramural program of the National Institute of Neurological Disroders and Stroke (Dr Dalakas).

Corresponding author and reprints: Marinos C. Dalakas, MD, Neuromuscular Diseases Section, NINDS, NIH, Bldg 10, Room 4N248, 10 Center Dr, MSC 1382, Bethesda, MD 20892-1382 (e-mail: dalakasm@ninds.nih.gov).

REFERENCES

1. Dalakas MC, Fujii M, Li M, McElroy B. The clinical spectrum of anti-GAD antibody–positive patients with stiff-person syndrome. *Neurology.* 2000;55:1531-1535.
2. Dalakas MC, Li M, Fujii M, Jacobowitz DM. Stiff person syndrome: quantification, specificity, and intrathecal synthesis of GAD65 antibodies. *Neurology.* 2001; 57:780-784.
3. Levy LM, Dalakas MC, Floeter MK. The stiff-person syndrome: an autoimmune disorder affecting neurotransmission of γ-aminobutyric acid. *Ann Intern Med.* 1999;131:522-530.
4. Kissel JT, Elble RJ. Stiff-person syndrome: stiff opposition to a simple explanation. *Neurology.* 1998;51:11-14.
5. Amato AA, Cornman EW, Kissel JT. Treatment of stiff-man syndrome with intravenous immunoglobulin. *Neurology.* 1994;44:1652-1654.
6. Dalakas MC, Fujii M, Li M, Lutfi B, Kyhos J, McElroy B. High-dose intravenous immune globulin for stiff-person syndrome. *N Engl J Med.* 2001;345:1870-1876.
7. Link H, Tibbling G. Principles of albumin and IgG analysis in neurological disorders, III: evaluation of IgG synthesis within the central nervous system in multiple sclerosis. *Scand J Clin Lab Invest.* 1977;37:397-401.
8. Vianello M, Tavolato B, Giometto B. Glutamic acid decarboxylase autoantibodies and neurological disorders. *Neurol Sci.* 2002;23:145-151.
9. Dinkel K, Meinck HM, Jury KM, Karges W, Richter W. Inhibition of gamma-aminobutyric acid synthesis by glutamic acid decarboxylase autoantibodies in stiff-man syndrome. *Ann Neurol.* 1998;44:194-201.
10. Benyahia B, Liblau R, Merle-Beral H, Tourani JM, Dalmau J, Delattre J-Y. Cell-mediated auto-immunity in paraneoplastic neurologic syndromes with anti-Hu antibodies. *Ann Neurol.* 1999;45:162-167.