Glycine receptor antibodies in stiff-person syndrome and other GAD-positive CNS disorders

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Clinical/Scientific Notes

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GLYCINE RECEPTOR ANTIBODIES IN STIFF-PERSON SYNDROME AND OTHER GAD-POSITIVE CNS DISORDERS

Stiff-person syndrome (SPS) is characterized by stiffness in trunk and limb muscles, phobic anxiety, and sudden spasms. A disturbance in inhibitory GABAergic pathways, presumably by autoantibodies against GAD (the main GABA-synthesizing enzyme), is considered fundamental for SPS pathogenesis.[1] As the precise role of anti-GAD antibodies in SPS remains unclear,[2,3] several other candidate disease-specific autoantibodies associated with inhibitory pathways have been explored[3,4] or are actively pursued. Recently, McKeon et al.[5] described anti-glycine-α1 receptor (GlyR) antibodies in a subset of patients with SPS. Glycine is a neurotransmitter in spinal inhibitory interneurons and GlyR are primarily expressed in the spinal cord, brainstem, and cerebellum. Anti-GlyR antibodies have been associated with progressive encephalomyelitis with rigidity and myoclonus (PERM), a syndrome resembling SPS.[6] Our aims were to search for GlyR antibodies in a large number of patients with well-characterized SPS and other CNS autoimmune controls and other GAD-positive disorders; and to correlate anti-GlyR titers with clinical symptomatology using quantitative scales of stiffness and spasms.[7]

Methods and results. We tested sera from the following groups: 1) 62 patients with typical SPS, determined by standard diagnostic criteria, high titer (>20,000 units) of anti-GAD antibodies, and information on disease severity based on Stiffness Index and Heightened Sensitivity Scales[7]; 2) 7 patients with other high GAD antibody–associated CNS disorders, including epilepsy,[3] cerebellar ataxia,[2] limbic encephalitis,[1] and impaired eye movements[1]; 3) 7 patients with low GAD antibody–associated diseases (3 neurologic, 4 with type 1 diabetes); 4) 14 patients with GAD-negative autoimmune encephalitis; 5) 20 patients with relapsing-remitting multiple sclerosis (RRMS); and 6) 20 healthy controls.

Standard protocol approvals, registrations, and patient consents. A total of 50/62 SPS samples were from previously reported patients[3,4,7] studied at the NIH Clinical Center under approved clinical protocols (Marinos Dalakas, Principal Investigator). All other samples were prospectively collected (University of Athens under the University's Ethics Committee approval).

All sera were tested concurrently for anti-GAD using ELISA (Euroimmun, Lubeck, Germany). We established a cell-based assay by transfecting HEK293T cells with the glycine receptor-α1 cDNA (GlyR-green fluorescent protein clone and anti-GlyR positive serum were a gift from Prof. Vincent FRS, Oxford). We used live cells, which were incubated with patient sera for 1 hour, then fixed with 4% paraformaldehyde–phosphate-buffered saline and incubated with an anti-human secondary antibody (goat-anti-human AlexaFluor 568, Invitrogen, Carlsbad, California).

We found that 9/62 (15%) patients with typical SPS were positive for anti-GlyR autoimmunity (figure, A). One patient with SPS with a low anti-GAD titer (<50 U/mL) and 1 GAD-negative patient with RRMS were also positive. The remaining 66 sera from the other autoimmune neurologic diseases or controls were negative. Representative data are presented from patient 5 (GlyR+) and patient 9 (GlyR+++) (figure, B). The staining protocol as described by McKeon et al.[5] that utilized fixed cells was also tested but gave negative results.

Discussion. We found anti-GlyR antibodies in 15% of high anti-GAD–positive typical patients with SPS, confirming the recent finding that these antibodies are present in a subset of patients with SPS.[5] No correlation was observed between GAD titers (range 26,000–1,547,235 U/mL) and GlyR semiquantitative titers (range + to +++). Additionally, no correlation was noticed between the overall disease severity status and the degree of GlyR staining, casting doubt on the direct pathogenic role of these antibodies. It is possible, however, that anti-GlyR antibodies might significantly contribute to or identify a subset of patients with SPS with anxiety or hyperexcitability, similar to patients with hereditary hyperekplexia, a condition characterized by hyperexcitability due to mutations in the glycine receptor gene. Of interest, 7/9 of our anti-GlyR antibody–positive patients had prominent anxiety and hyperexcitability, as determined by the Heightened Sensitivity Scales.

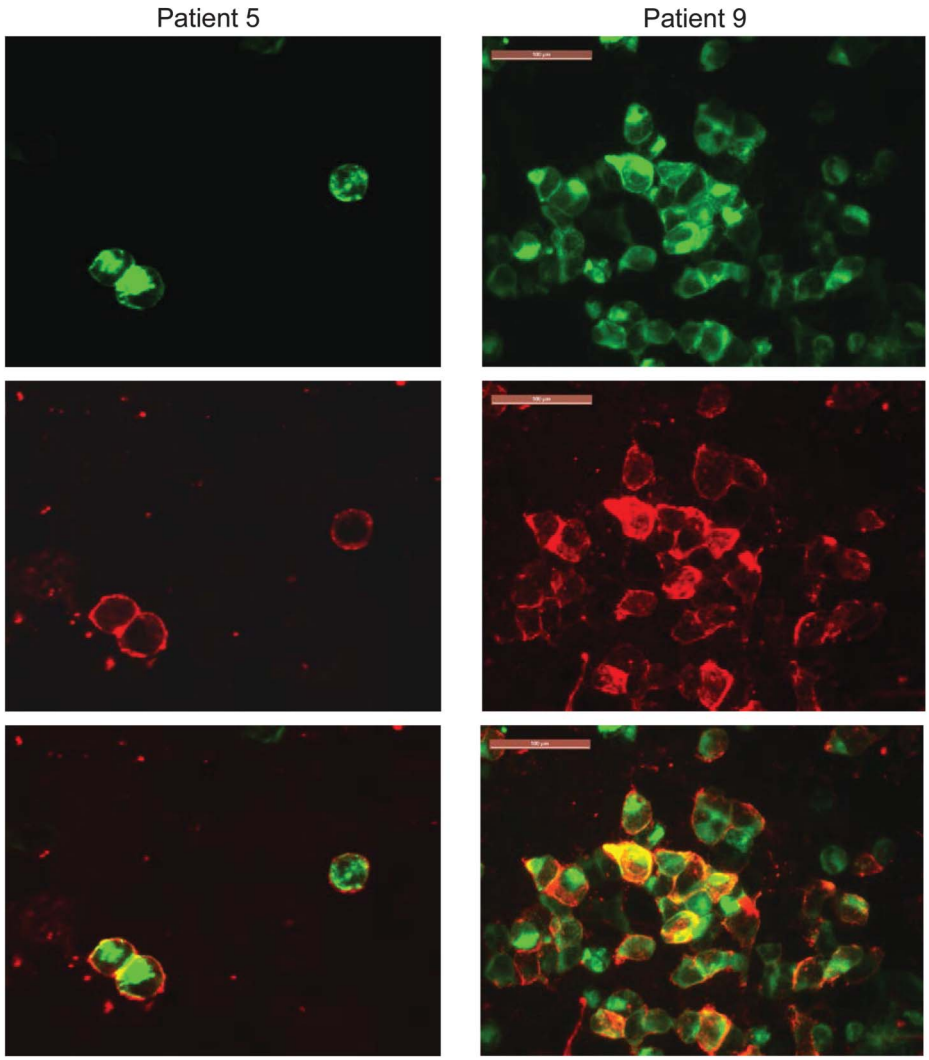
Anti-GlyR antibodies appear to be SPS-specific as they were absent in all other diseases studied, including other high GAD antibody–positive patients. Their

Figure **Anti-glycine receptor positivity in patients with SPS and clinical associations**

A

Patient #	Diagnosis	Disease status	Anxiety and hyperexcitability	GLYR	GAD (serum)
1	SPS	Severe	+	+	670,808 U/mL
2	SPS	Severe	+++	+	468,740 U/ml
3	SPS	Mild	-	++	1,547,235 U/mL
4	SPS	Severe	+++	+	216,839 U/mL
5	SPS	Severe	+	+	143,265 U/mL
6	SPS	Moderate	-	+	124,073 U/mL
7	SPS	Moderate	++	+	26,000 U/mL
8	SPS	Severe	++	++	130,858 U/mL
9	SPS	Mild	+	+++	74,818 U/mL

B



(A) A summary of clinical and laboratory features of anti-glycine receptor (GlyR)-positive patients with stiff-person syndrome (SPS). Disease status, according to Stiffness Index and Heightened Sensitivity Scales, was characterized from mild to severe. Hyperexcitability was scored from negative to +++. Anti-GlyR degree of staining was semiquantitatively assessed and scored + to +++. Finally, GAD titers were measured using an anti-GAD ELISA assay. (B) Representative images of anti-GlyR staining in patient 5 (+) and patient 9 (+++). Green stain corresponds to green fluorescent protein expression; red stain corresponds to human serum reactivity visualized with an anti-human fluorescent secondary antibody. Scale bar = 100 μm.

presence in 1 patient with RRMS is perplexing and may be attributed to a nonspecific polyclonal antibody response, although it is unclear whether this patient exhibited features of stiffness or spinal hyperexcitability.

The finding of anti-GlyR antibodies in patients with SPS, as demonstrated by 2 independent laboratories, is important; their presence may denote disease subsets or highlight pathogenetic relevance. Despite

methodologic variance between the 2 studies, the percentage of positivity among patients with SPS was similar, demonstrating the need for functional studies. Previous efforts to transfer disease using high GAD titer sera were partially successful, as only certain aspects of the syndrome were replicated in rats.[3] The possibility that anti-GlyR can transfer some SPS symptomatology is intriguing and remains to be tested.

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