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*Neurol Neuroimmunol
Neuroinflamm*
2014;1:e39; doi: 10.1212/
NXI.0000000000000039

AUTOIMMUNE ENCEPHALITIS WITH GABA_B ANTIBODIES, THYMOMA, AND GABA_B RECEPTOR THYMIC EXPRESSION

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Antibody-mediated autoimmune encephalopathies comprise a group of severe conditions with a varying degree of motor and cognitive symptoms that respond to immunotherapies.¹ The associated antibodies are directed against intracellular targets, such as the classic paraneoplastic autoantigens Hu, Yo, Ri, CV2, Ma2/Ta, or the enzyme GAD, or against cell surface antigens such as receptors and ion channels.

A newly described pathogenic antibody in autoimmune encephalopathies is directed against the γ -aminobutyric acid receptor B (GABA_B), a G-protein-coupled receptor.² In 2 large series of more than 35 GABA_B-seropositive patients, the most common symptoms were limbic encephalitis, seizures, ataxia, and opsoclonus myoclonus^{3,4}; only a single case presented with brainstem encephalitis.⁵ The GABA_B-associated syndrome can be also paraneoplastic up to 35% of the cases, most often associated with small-cell lung cancer and quite often in conjunction with another paraneoplastic antibody.

We present a case of GABA_B-associated autoimmune encephalopathy with brainstem manifestations, thymoma, and co-occurrence of anti-Hu and anti-CV2 antibodies. The main novelty of the case is GABA_B expression in the thymus, implicating reactivity against the same antigen between thymus and brain.

Case presentation. A 25-year-old man was admitted for evaluation of recurrent episodes of vertigo, dizziness, persistent hiccups, nausea, and vomiting that started 3 months prior to admission. On admission he had normal cognitive functions, horizontal nystagmus intensified to the left, brisk tendon reflexes in the lower limbs, positive Romberg sign, and tongue myoclonus. After 1 week he developed right sixth nerve palsy with diplopia, and 2 weeks later he developed left third nerve palsy.

Routine blood tests were normal. CSF analysis yielded high protein (85 mg/dL), 10 white cells per mm³, and normal glucose. CSF viral serology and Gram stain were negative. Oligoclonal immunoglobulin G bands were detected. Visual evoked potentials,

somatosensory evoked potentials, EMG, and serial EEGs were normal. A noncontrast CT scan and MRI of the brain and cervical spine were normal. A chest-abdominal CT revealed a mass in the anterior mediastinum suspicious for thymoma. A complete thymectomy was performed and pathology revealed a type B1 (lymphocyte-rich) thymoma.

His symptoms improved after surgery but worsened 2 weeks later, and he was started on oral methylprednisolone for 5 days. He again improved, but his symptoms reappeared 7 months later with the addition of excessive startle. Screening of serum for classic paraneoplastic autoantibodies by Western blot (Euroimmun, Lübeck, Germany) and autoimmune encephalitis autoantibodies by cell-based assays (Euroimmun) showed positivity for anti-Hu, anti-CV2, and anti-GABA_B receptor but negativity for anti-Ri, anti-Yo, anti-Ma2/Ta, anti-amphiphysin, anti-LGI1, anti-CASPR2, anti-NMDAR, anti-GAD, anti-glycine receptor, anti-AQP4, anti-AChR (tested with the most sensitive radioimmunoassay), and anti-MuSK. Therefore, the diagnosis of paraneoplastic brainstem autoimmune encephalitis was established.

Immunocytochemistry. To probe for GABA_B thymic expression, 5- μ m tissue sections from the extracted thymoma were incubated following deparaffinization with an antibody against GABA_B (1:100 rabbit polyclonal; Abcam, Cambridge, United Kingdom) in combination with an antibody that detects epithelial cells (1:60 pan-cytokeratin mouse monoclonal; Dako, Glostrup, Denmark). For secondary detection, a goat-anti-rabbit Alexa Fluor-568 (1:200; Life Technologies, Carlsbad, CA) and a goat-anti-mouse Alexa Fluor-488 (1:200; Life Technologies) were used. Patient serum was applied in sections (1:20) following deparaffinization and antigen retrieval combined with the pan-cytokeratin antibody. Secondary detection was performed with the combination of goat-anti-human Alexa Fluor-568 (1:200) and goat-anti-mouse Alexa Fluor-488 (1:200). A strong expression of GABA_B was noted in the thymic epithelial cells (figure 1, A–C). When serum was applied, the staining pattern was identical, suggesting that serum antibodies recognize GABA_B antigen in the thymus (figure 1, D–F). Healthy serum (1:20) and diluent (secondary only) were applied to the tissue as controls in combination with the pan-cytokeratin antibodies. No nonspecific

Supplemental data
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Figure 1 GABA_B expression in the thymus

(A–C) Staining of thymus tissue with anti-cytokeratin (A) and anti-GABA_B antibody (B, C double immunofluorescence). Cytokeratin-positive epithelial cells express GABA_B receptor (arrows). In addition, GABA_B is expressed in other cell types of the thymus, most likely T cells (arrowheads). (D–F) Staining of thymus tissue with anti-cytokeratin (D) and patient sera (E, F double immunofluorescence). Patient antibodies clearly co-localize with cytokeratin-positive epithelial cells. The pattern of staining and co-localization in these areas is identical to panels A, B, and C. Due to antibody combination and tissue availability restrictions, we were unable to triple stain for cytokeratin–GABA_B–serum. From our results it can be safely inferred that the GABA_B serum autoantibodies bind GABA_B that is expressed in thymic epithelial cells. GABA_B = γ -aminobutyric acid receptor B.

staining was observed. The University of Athens Ethics Committee granted ethical approval and patient informed consent was received.

Patient's follow-up. Because his symptomatology worsened, the patient underwent a 7-day course of plasmapheresis followed by 1 g of IV methylprednisolone for 5 days. All symptoms rapidly disappeared except for slight dizziness and vertigo in the supine position. He was discharged on methylprednisolone by mouth. Azathioprine was later added. Now, 18 months after his discharge, he remains stable.

Discussion. This case presents several novelties. It extends the clinical phenotype of GABA_B receptor-associated encephalitis to include brainstem encephalitis.

It is also the first case associated with thymoma and co-occurrence with 2 other paraneoplastic antibodies, namely anti-Hu and anti-CV2. Although these antibodies can coexist in some patients and anti-CV2-related encephalitis with thymoma has also been reported, CV2-positive patients do not develop brainstem signs. Their clinical symptomatology is different and includes cerebellar ataxia, chorea, uvea/retinal symptoms, and myasthenic syndrome.^{6,7} In our case, the rapid response to plasmapheresis after symptom reoccurrence strongly suggests (although does not prove) that the main causative factor was humoral immunity associated with GABA_B antibodies rather than T-cell immunity implicated in CV2 and Hu paraneoplastic syndromes. The key role of GABA_B is further highlighted by finding GABA_B expression in the

thymomatous epithelial cells and the temporal association of improvement onset and thymectomy.

GABA_B expression has not been previously demonstrated in a thymoma or in any of the tumors associated with GABA_B-positive autoimmune encephalitis. It is likely that the demonstration of GABA_B receptor expression in the thymic epithelial cells has triggered a pathogenetic mechanism of autoimmunization through reactivity with the same antigen in the thymus and brain.

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Author contributions: Dr. Alexopoulos, Dr. Bostantjopoulou, Dr. Dagklis, Dr. Dalakas: study concept and design. Dr. Dagklis, Dr. Alexopoulos, Ms. Akrivou: acquisition of data. Dr. Alexopoulos, Ms. Akrivou, Dr. Dagklis: analysis and interpretation. Dr. Alexopoulos, Dr. Dagklis, Dr. Bostantjopoulou, Dr. Dalakas: drafting and critical revision of the manuscript.

Study funding: No targeted funding reported.

Disclosure: H. Alexopoulos, I.E. Dagklis, and S. Akrivou report no disclosures. S. Bostantjopoulou has received funding for travel from Novartis and UCB. M.C. Dalakas is on the scientific advisory board for Novartis, Grifols, and Baxter; has received honoraria from Baxter, Novartis, Grifols, CSL, Octapharma, Dysimmune Diseases Foundation, and Therapath; is on the editorial board for Neurology, BMC Neurology, Neuropathology and Applied Neurobiology, Acta Neurologica Scandinavica, and Therapeutic Advances in Neurology; has consulted for Therapath, Grifols, Novartis, Baxter, Octapharma, and Dysimmune Diseases Foundation; is on the speakers' bureau for Talecris; and received support from Talecris, IVIG America, Biogen, Serono, Genzyme, Novartis, Teva, and CSL. Go to Neurology.org/nn for full disclosures. The Article

Processing Charge was paid by the Special Research Account, University of Athens.

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Received June 9, 2014. Accepted in final form September 9, 2014.

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Neurol Neuroimmunol Neuroinflamm 2014;1;

DOI 10.1212/NXI.0000000000000039

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