Evidence of a pathogenic role for CD8⁺ T cells in anti-GABA_B receptor limbic encephalitis

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Kristin S. Golombek, MD*
Kathrin Bönnte*
Constanze Mönig, MD
Karen M. van Loo, PhD
Marvin Hartwig
Wolfram Schwindt, MD
Guido Widman, MD
Matthias Lindenau, MD
Albert J. Becker, MD
Markus Glatzel, MD
Christian E. Elger, MD
Heinz Wiendl, MD
Sven G. Meuth, MD, PhD
Hubertus Lohmann, PhD
Catharina C. Gross, PhD
Nico Melzer, MD

Correspondence to
Dr. Melzer:
nico.melzer@ukmuenster.de

ABSTRACT

Objectives: To characterize the cellular autoimmune response in patients with γ -aminobutyric acid (GABA)_B receptor antibody-associated limbic encephalitis (GABA_B-R LE).

Methods: Patients underwent MRI, extensive neuropsychological assessment, and multiparameter flow cytometry of peripheral blood and CSF.

Results: We identified a series of 3 cases of nonparaneoplastic GABA_B-R LE and one case of paraneoplastic GABA_B-R LE associated with small cell lung cancer. All patients exhibited temporal lobe epilepsy, neuropsychological deficits, and MRI findings typical of LE. Absolute numbers of CD19⁺ B cells, CD138⁺ CD19⁺ plasma cells, CD4⁺ T cells, activated HLADR⁺ CD4⁺ T cells, as well as CD8⁺ T cells and HLADR⁺ CD8⁺ T cells did not differ in peripheral blood but were elevated in CSF of patients with GABA_B-R LE compared to controls. Augmented absolute numbers of CD138⁺ CD19⁺ plasma cells and activated HLADR⁺ CD8⁺ T cells in CSF corresponded to higher overall neuropsychological and memory deficits in patients with GABA_B-R LE. A histologic specimen of one patient following selective amygdalohippocampectomy revealed perivascular infiltrates of CD138⁺ plasma cells and CD4⁺ T cells, whereas cytotoxic CD8⁺ T cells were detected within the brain parenchyma in close contact to neurons.

Conclusion: Our data suggest a pathogenic role for CD8⁺ T cells in addition to the established role of plasma cell-derived autoantibodies in GABA_B-R LE. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e232; doi: 10.1212/NXI.0000000000000232

GLOSSARY

AVLT = Auditory Verbal Learning Test; **FLAIR** = fluid-attenuated inversion recovery; **GABA** = γ -aminobutyric acid; **GABA_B-R** = γ -aminobutyric acid B receptor; **GAD65** = glutamic acid decarboxylase, 65-kDa isoform; **Ig** = immunoglobulin; **LE** = limbic encephalitis; **PB** = peripheral blood; **SCLC** = small cell lung cancer.

Limbic encephalitis with autoantibodies against synaptic and extrasynaptic neuronal γ -aminobutyric acid (GABA)_B receptors (GABA_B-R LE) is associated with small cell lung cancer (SCLC) in about 50% of all patients.^{1–4} Clinically, patients present with mesial temporal lobe epilepsy, memory disturbance, and a variety of neuropsychiatric symptoms.^{1–4} MRI in about two-thirds of all cases reveals volume increase and hyperintense signals in one or both mesial temporal lobes on T2-weighted and fluid-attenuated inversion recovery (FLAIR)-weighted sequences suggesting temporomesial encephalitis.^{1–4} EEG usually shows slowing and epileptic activity in one or both anterior temporal lobes.^{1–4} Autoantibodies can be detected in serum, CSF, or both and recognize an extracellular domain of the B1-subunit of the GABA_B receptor mainly expressed in hippocampus, amygdala, thalamus, and cerebellum.⁴ GABA_B receptors mediate pre- and postsynaptic GABAergic inhibition and thereby suppress high-activity states with excessive synchronization and thus seizure generation in neuronal networks. Genetic disruption or

*These authors contributed equally to this work.

From the Departments of Neurology (K.S.G., K.B., C.M., M.H., H.W., S.G.M., H.L., C.C.G., N.M.) and Clinical Radiology (W.S.), and Institute of Physiology I–Neuropathophysiology (S.G.M.), University of Münster; Departments of Epileptology (G.W., C.E.E.) and Neuropathology (K.M.v.L., A.J.B.), University of Bonn; Epilepsy Center Hamburg (M.L.), Evangelisches Krankenhaus Alsterdorf, Hamburg; and Department of Neuropathology (M.G.), University of Hamburg, Germany.

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Supplemental data
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pharmacologic blockade of the GABA_B receptor in animal models causes excessive neuronal excitability, as well as disturbance of learning, memory, and behavior⁴ resembling the clinical phenotype of human GABA_B-R LE. Moreover, patients with GABA_B-R LE have been reported to respond at least partially to antibody-depleting immunotherapies and tumor therapy.^{1–4} Hence, autoantibodies are considered pathogenic. In a subset of patients, GABA_B-R antibodies have been reported to occur together with antibodies to the 65-kDa isoform of glutamic acid decarboxylase (GAD₆₅) and classic onconeural proteins (Hu, Ri, amphiphysin, SOX1), suggesting the presence of an autoimmune response to both intracellular and plasma membrane neuronal antigens in these patients.^{1–6} However, except for the presence of autoantibodies in serum and CSF, little is known on the (auto)immune response in this novel CNS disorder.

Herein, we provide a detailed description of the composition of peripheral blood (PB) and CSF-infiltrating immune cells and confirm their presence in the inflamed limbic system and their relation to neuropsychological deficits in 4 cases of GABA_B-R LE.

METHODS Patients and controls. Patients with GABA_B-R LE and controls were recruited at the Department of Neurology, University of Münster, Germany, or the Department of Epileptology, University of Bonn, Germany, and analyzed using flow cytometry at the Department of Neurology, University of Münster, Germany. Patients and controls underwent physical, neurologic, and psychiatric examination by a trained physician. PB and CSF were taken from patients and controls and analyzed by routine and multiparameter flow cytometry. In addition, routine EEG using the 10-20 system and/or video-EEG monitoring, MRI of the brain at 1.5 or 3.0 tesla, and a comprehensive neuropsychological test battery were conducted.

Standard protocol approvals, registrations, and patient consents. The study was approved by the local ethics committee of the medical faculty of the University of Münster, Germany (Az 2013-350-f-S). All participants or their nearest relatives gave written informed consent to the study including scientific evaluation and publication of all clinical and paraclinical data obtained.

Multiparameter flow cytometry of PB and CSF. PB and CSF of all patients were immediately obtained after admission. PB and CSF of healthy controls were obtained from 26 individuals with suspected presence of a neurologic disorder who retrospectively were determined to have somatization disorders.⁷ In addition to the clinical classification, patients included in the control group also fulfilled the following laboratory criteria defining a noninflammatory CSF: <5 cells/μL, <500 mg protein/mL, <2 mM lactate, no disruption of the PB–CSF barrier (defined by

the CSF/serum albumin ratio), no intrathecal immunoglobulin (Ig)G, IgA, or IgM synthesis (Reiber criteria),^{7,8} and no CSF-specific oligoclonal bands on isoelectric focusing.

Potential disease-related changes in the cellular composition of both PB and CSF compartments were analyzed using multiparameter flow cytometry.⁹ CSF samples were obtained by lumbar puncture, collected in polypropylene tubes, and were processed within 30 minutes. Cells were obtained from EDTA blood by erythrocyte lysis using VersaLyse buffer (Beckman Coulter, Germany) following the manufacturer's instructions. Cells were obtained from CSF by centrifugation (15 minutes, 290g, 4°C) and incubation in VersaLyse buffer. Cells were stained using the following fluorochrome-conjugated antibodies: CD14-FITC, CD138-PE, HLA-DR-ECD, CD3-PC5.5, CD56-PC7, CD4-APC, CD19-APCAlexafluor700, CD16-APCAlexafluor750, CD8-PacificBlue, and CD45-KromeOrange (all Beckman Coulter) and analyzed by 2 blinded authors (M.H., C.C.G.) using the Navios (Beckman Coulter). The gating strategy to determine leukocyte subsets in PB and CSF cells was performed as described.⁷ Absolute cell numbers were analyzed using Flow-Count Fluorospheres (Beckman Coulter) according to the manufacturer's instructions.

Antineuronal antibody testing. Serum and CSF were tested for the presence of IgG antibodies against intracellular neuronal antigens (ANNA1 [Hu], ANNA2 [Ri], ANNA3, PCA1 [Yo], PCA2, Tr/DNER, Ma1/2, CV2/CRMP5, amphiphysin, SOX1, GAD₆₅) and neuronal surface membrane antigens (NMDA receptor, AMPA receptor, GABA_A receptor, GABA_B receptor, glycine receptor, CASPR2, LGI1, and VGKC) using established assays (EUROIMMUN, Lübeck, Germany^{10,11}).

Neuropsychological assessment. All patients were assessed by a comprehensive neuropsychological test battery conducted by experienced neuropsychologists (H.L., K.B.). The test battery assesses the full range of neuropsychological domains as presented in table e-1 at Neurology.org/nn. The test scores represent the individual percentile rank in comparison to neurologically healthy adults matched for age and level of education.

Magnetic resonance imaging. Standard MRI was performed on 1.5- or 3.0-tesla scanners. Diffusion-weighted imaging with calculation of apparent diffusion coefficient map, axial and coronal T1 spin-echo before and after application of gadolinium, axial and coronal FLAIR, T2-weighted fast-field echo, and T2-weighted turbo spin-echo sequences were performed and evaluated by an experienced neuroradiologist (W.S.).

Histopathologic analysis. For histopathologic analysis, sections from selective amygdalohippocampectomy specimen were incubated with antibodies against CD138, CD3, and CD8 antigen using standard methods, counterstained with hematoxylin & eosin.

For immunohistochemical analysis, slides were incubated 2 × 10 minutes in xylene, ethanol (100%-95%-70%-50%), and citrate buffer (10 mM, pH 6.0) and blocked for 2 hours at 37°C in blocking buffer consisting of 10% normal goat serum (Invitrogen) and 1% fetal calf serum (Invitrogen) in PBS. Slides were incubated with primary antibodies against CD8 (1:50, SP16 MA5-14548; Thermo Fisher) and Granzyme B Ab-1 (1:50, GZB01 MS-1157-S1; Thermo Fisher) overnight at room temperature in blocking buffer. After washing with PBS, slides were incubated with secondary antibodies (1:200 Alexa Fluor 488 donkey anti-mouse and 1:200 Alexa Fluor 568 goat anti-rabbit; both from Thermo Fisher) and with 0.1 μg/mL DAPI (4',6-diamidino-2-phenylindole) (Life Technologies) for 2 hours

at room temperature in blocking buffer. After washing with PBS, sections were coverslipped in fluorescein mounting medium (VECTASHIELD, Vector laboratories) and imaged with a confocal laser scanning microscope (Eclipse Ti, Nikon).

Statistics. If not explicitly stated otherwise, all statistical analyses were performed using Sigma Plot 11 (Systat Software, Germany). Data were tested for normality using the Shapiro–Wilk test. All normally distributed data are given as mean together with the SD. All not normally distributed data are given as median together with the interquartile range. If not explicitly stated otherwise, the prechosen significance level for all confirmatory tests was set to $p < 0.05$. Levels of significance are indicated by n.s. (not significant) for all $p > 0.05$, * for all $p < 0.05$ and ** for all $p < 0.01$.

RESULTS We identified 3 cases of nonparaneoplastic and one case of paraneoplastic GABA_B-R LE associated with SCLC (3 women, 1 man, aged 42–60 years, 2 patients with a smoking history). A detailed case description is provided in appendix e-1. GABA_B-receptor antibodies were detected in sera and/or CSF of all patients. One patient (patient 2) also exhibited GAD₆₅ antibodies in serum but not CSF. All patients exhibited temporal lobe seizures, neuropsychological deficits (tables e-1 and e-2), and MRI (figure e-1, A and B) findings typical of LE. A tumor search was performed using whole-body ¹⁸F-fluorodeoxyglucose PET/CT together with urologic or gynecologic examination. Except for patient 4, all patients had normal white blood cell

counts (appendix e-1) in the CSF. Time intervals between clinical disease onset and CSF analysis were short (2–8 weeks) in patients 1, 3, and 4 but long (5 years) in patient 2 (patient 1, 2 weeks; patient 2, about 5 years; patient 3, 8 weeks; patient 4, 4 weeks). At the time of CSF analysis, patients 1, 3, and 4 were immunotherapy-naïve, whereas patient 2 had received several preceding immunotherapies all with marginal clinical effect suggesting persisting disease activity (see appendix e-1).

All patients underwent multiparameter flow cytometry analysis of PB and CSF to characterize the autoimmune response in GABA_B-R LE in more detail (figures 1 and 2, table 1). A representative multiparameter flow cytometry analysis of PB and CSF in GABA_B-R LE (patient 4) illustrating the gating strategy is given in figure e-2 (PB upper panels, CSF lower panels). Twenty-six patients who received CSF analysis for suspected neurologic disease but were found to have somatization disorders served as controls.

Absolute numbers of CD19⁺ B cells (figure 1A, table 1) as well as CD4⁺ T cells (figure 2A, table 1) and CD8⁺ T cells (figure 2D, table 1) did not differ in PB but were elevated in CSF of patients with GABA_B-R LE compared to controls, illustrating an intrathecal accumulation of B and T lymphocytes in GABA_B-R LE.

Moreover, absolute numbers of activated CD138⁺ CD19⁺ B cells, i.e., plasma cells (figure 1B, table 1) as well as activated HLADR⁺ CD4⁺ (figure 2B, table 1) and HLADR⁺ CD8⁺ (figure 2D, table 1) T cells did not differ in PB but were elevated in CSF of patients with GABA_B-R LE compared to controls.

Histopathologic assessment of selective amygdalohippocampectomy specimen from patient 2 (figure 3, A–D) revealed perivascular infiltrates of CD138⁺ plasma cells (figure 3A) and CD3⁺ T cells (figure 3B). CD8⁺ T cells (figure 3C) were predominantly detected within the brain parenchyma in close contact to neurons but not in perivascular regions, suggesting that perivascular CD3⁺ T cells are mainly CD4⁺ T cells. Parenchymal CD8⁺ T cells were activated expressing cytotoxic effector molecules (granzyme B; figure 3D).

The results of the neuropsychological assessment (tables e-1 and e-2) represent the cognitive functions of 3 of the 4 patients with GABA_B-R LE. Because of the clinical condition, detailed neuropsychological assessment was impossible in patient 4. All tested patients had deficits in verbal working memory (Auditory Verbal Learning Test [AVLT] trial 1), learning ability (AVLT trial 5, AVLT trials 1–5), and retrieval performance (AVLT trial 6, AVLT trial 7) and some also in visuospatial ability (Rey Complex Figure Test) as well as processing speed and set shifting (Trail Making Test).

Multiparameter flow cytometry analysis of absolute numbers of CD19⁺ B cells (A) and CD138⁺ CD19⁺ plasma cells (B) within PB and CSF in 4 patients with GABA_B receptor antibody-associated LE and controls. Data are given as whisker plots. Levels of significance are indicated by n.s. (not significant) for all $p > 0.05$, * for all $p < 0.05$, and ** for all $p < 0.01$. For details, refer to the main text and table 1. GABA_B = γ -aminobutyric acid B; LE = limbic encephalitis; PB = peripheral blood; Pt. = patient.

Figure 2 CD4⁺ and CD8⁺ T cells accumulate in the CSF in GABA_B receptor antibody-associated LE

Multiparameter flow cytometry analysis of absolute numbers of CD4⁺ T cells (A) and CD8⁺ T cells (C), activated HLADR⁺ CD4⁺ T cells (B), and HLADR⁺ CD8⁺ T cells (D) within PB and CSF in 4 patients with GABA_B receptor antibody-associated LE and controls. Data are given as whisker plots. Levels of significance are indicated by n.s. (not significant) for all $p > 0.05$, * for all $p < 0.05$, and ** for all $p < 0.01$. For details, refer to the main text and table 1. GABA_B = γ -aminobutyric acid B; LE = limbic encephalitis; PB = peripheral blood; Pt. = patient.

Hence, we tried to relate the neuropsychological deficits detected in patients with GABA_B-R LE to immune cell infiltrates in their CSF (figure 4), which we consider closely reflect those present in the inflamed limbic system.

Indeed, higher absolute numbers of HLADR⁺ CD4⁺ T cells in CSF corresponded well to lower overall neuropsychological deficits quantified as the total neuropsychological percentile rank sum as well as to lower deficits in verbal memory retrieval performance quantified as the respective percentile rank

sum (figure 4B). In contrast, higher absolute numbers of HLADR⁺ CD8⁺ T cells in CSF corresponded even stronger to higher overall neuropsychological deficits as well as to higher deficits in verbal memory retrieval performance (figure 4C). This suggests a pathogenic role of activated HLADR⁺ CD8⁺ T cells and a more regulatory function of activated HLADR⁺ CD4⁺ T cells in the adaptive autoimmune inflammation of the limbic system in GABA_B-R LE.

Likewise, although on a lower level in terms of absolute cell numbers and less strongly, higher absolute CD138⁺ CD19⁺ plasma cell numbers in CSF were associated with more severe overall neuropsychological impairment as well as with more severe deficits in verbal memory retrieval performance (figure 4A) consistent with the known pathogenic role of autoantibodies in GABA_B-R LE.

DISCUSSION We used multiparameter flow cytometry of PB and CSF in combination with clinical and neuropsychological characterization and MRI to characterize the cellular autoimmune response in 3 cases of nonparaneoplastic GABA_B-R LE (patients 1–3) and one case of paraneoplastic GABA_B-R LE (patient 4) associated with SCLC in comparison to patients with somatization disorders as controls. All patients with GABA_B-R LE exhibited neuropsychological deficits and MRI findings typical of LE.^{1,3,4} Three of them were studied very early in the disease course (4–8 weeks after disease onset) in a therapy-naïve state (patients 1, 3, and 4) and one was in a non-therapy-naïve state (patient 2) with a disease course of about 5 years necessitating amygdalohippocampectomy for seizure control. The long-standing disease course of the latter case (patient 2) is somewhat unusual for the so far known GABA_B-R LE phenomenology. One may speculate that long-standing temporal lobe epilepsy might have triggered GABA_B-R LE in an individual obviously prone to autoimmunity as revealed by the additional presence of GAD₆₅ antibodies. Moreover, pronounced deterioration of cognitive function was observed following amygdalohippocampectomy with involvement of the contralateral temporal lobe consistent with boosting the antineuronal immune response due to liberation of neuronal antigens. All cases finally responded to immunotherapy consisting of methylprednisolone pulse therapy and immunoadsorption together with rituximab or chemotherapy in 2 of 4 cases.

As compared to controls, in patients with GABA_B-R LE, CD19⁺ B cells, CD4⁺ T cells, and CD8⁺ T cells and their activated forms were enriched in absolute numbers in CSF. Consistently, histologic specimen of patient 2 following selective amygdalohippocampectomy revealed perivascular infiltrates of CD138⁺ plasma cells and CD4⁺ T cells whereas activated

Table 1 Data of different cell types in peripheral blood and CSF in patients with limbic encephalitis compared to controls

Cell type	Peripheral blood					CSF				
	Limbic encephalitis		Control		p Value	Limbic encephalitis		Control		p Value
	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
CD19 ⁺ B cells, cells/mL	119,246	78,218	172,427	191,055	0.093	59	158	5	9	0.011 ^a
CD138 ⁺ CD19 ⁺ plasma cells, cells/mL	1,502	3,778	604	1,544	0.410	1.1	2.2	0.0	0.0	0.014 ^a
CD138 ⁺ CD19 ⁺ plasma cells, %	0.18	0.21	0.05	0.08	0.032 ^a	0.20	0.06	0.00	0.00	0.162
CD4/CD8 T cell ratio	3.4	3.4	2.6	1.8	0.647	2.7	2.7	3.4	2.5	0.410
CD4 ⁺ T cells, cells/mL	371,998	503,521	650,136	485,364	0.106	1,084	402	480	716	0.026 ^a
HLADR ⁺ CD4 ⁺ T cells, cells/mL	47,393	44,756	30,159	18,217	0.314	94	52	23	51	0.016 ^a
HLADR ⁺ CD4 ⁺ T cells, %	13.5	10.7	4.6	3.4	0.003 ^a	7.6	5.5	5.4	3.8	0.127
CD8 ⁺ T cells, cells/mL	177,398	171,584	273,242	230,753	0.072	447	320	123	235	0.035 ^a
HLADR ⁺ CD8 ⁺ T cells, cells/mL	203,64	56,125	16,530	13,285	0.259	155	104	32	54	0.013 ^a
HLADR ⁺ CD8 ⁺ T cells, %	28.6	42.3	6.4	5.0	0.010 ^a	35.5	6.1	26.1	14.4	0.022 ^a

Abbreviation: IQR = interquartile range.

Wilcoxon rank sum test was used for statistical analyses.

^a Significant.

CD8⁺ T cells expressing cytotoxic effector molecules were detected within the brain parenchyma in close contact to neurons. Moreover, higher absolute numbers of activated HLADR⁺ CD4⁺ T cells in CSF corresponded to lower neuropsychological deficits, whereas higher absolute numbers of CD138⁺

CD19⁺ plasma cells and activated HLADR⁺ CD8⁺ T cells in CSF corresponded to higher neuropsychological deficits in patients with GABA_B-R LE. This suggests a pathogenic role of CD8⁺ T cells in addition to the established role of plasma cell-derived autoantibodies⁴ as well as a more regulatory function of CD4⁺

Figure 3 A pathogenic role for CD8⁺ T cells in addition to plasma cell-derived autoantibodies in GABA_B receptor antibody-associated limbic encephalitis

Histologic specimen of one patient with GABA_B receptor antibody-associated limbic encephalitis following selective amygdalohippocampectomy revealed perivascular infiltrates of CD138⁺ plasma cells (A) and CD3⁺ T cells (B). CD8⁺ T cells (C) were predominantly detected within the brain parenchyma in close contact to neurons but not in perivascular regions, suggesting that perivascular CD3⁺ T cells are mainly CD4⁺ T cells (scale bar represents 50 μ m in A–C; insets show magnification of 2 representative neurons in close contact with CD8⁺ T cells). Representative confocal triple staining (D) for DAPI, CD8 (red), and granzyme B (green) revealed expression of granzyme B (white arrow) in parenchymal CD8⁺ T cells (scale bar represents 10 μ m in D). DAPI = 4'-6-diamidino-2-phenylindole · 2HCl; GABA_B = γ -aminobutyric acid B.

Figure 4 Relation of neuropsychological deficits and intrathecal immune cell subsets in GABA_B receptor antibody-associated limbic encephalitis

Relation of neuropsychological deficits to the absolute numbers of CD138⁺ CD19⁺ plasma cells (A), activated HLADR⁺ CD4⁺ T cells (B), and activated HLADR⁺ CD8⁺ T cells (C) and within the CSF in 3 patients with GABA_B receptor antibody-associated limbic encephalitis. The overall neuropsychological deficits are quantified as the total neuropsychological percentile rank sum, and the verbal memory retrieval performance is quantified as the respective percentile rank sum (see table e-2). For details, refer to the main text. GABA_B = γ-aminobutyric acid B; Pt. = patient.

T cells¹² in the adaptive autoimmune inflammation of the limbic system in GABA_B-R LE.

However, our data of course have several strong limitations, as (1) we only studied as few as 4 partially not therapy-naïve patients with GABA_B-R LE; (2) one patient had left amygdalohippocampectomy due to treatment-resistant temporal lobe epilepsy additionally impairing memory performance^{13,14} and potentially altering ongoing cerebral autoimmune reactions; (3) we used a restricted panel of flow cytometry parameters to analyze the cellular immune reaction in GABA_B-R LE limiting the insight gained into details of the activation and differentiation status of the immune cells involved; and (4) we did not perform analysis of the B and T cell receptor

repertoire in GABA_B-R LE, which would have allowed more precise delineation of the antigen-driven activation of the immune cells involved.

Moreover, the association between increased numbers of CD138⁺ CD19⁺ plasma cells and HLADR⁺ CD8⁺ T cells in the CSF with neuropsychological deficits in patients 1, 2, and 3 per se does not prove a causative role of these lymphocyte subsets, and lymphocyte infiltrates detected in selective amygdalohippocampectomy specimen are not very prominent compared to classic onconeural autoimmunity.¹⁵ However, as there was no evidence of an underlying tumor, and GAD₆₅ antibodies were only detected in serum but not CSF in patient 2, we do not consider this case to represent a GAD₆₅ antibody-associated LE, which might also have explained the presence of parenchymal T cells.⁶ Moreover, widespread perivascular and parenchyma T cell infiltrates were detected at autopsy in a case of paraneoplastic encephalomyelitis with SCLC in which GABA_B receptor antibodies could be detected together with onconeural antibodies against amphiphysin.^{1,16} Here, parenchymal CD8⁺ T cells were found in close apposition to neurons.^{1,16} Hence, one might speculate that occult tumors are underlying the T cell immune response in our patients.

Assuming that both the humoral as well as the cellular arm of the immune system are actively involved in the pathogenesis of GABA_B-R LE, it is unclear which is the primary and which the secondary immune mechanism, and whether cellular and humoral effectors share the same target antigens.

In one less likely scenario, a primary CD8⁺ T cell-mediated autoimmune reaction against mesial temporal lobe neurons or other parenchymal cells triggers humoral autoimmunity toward GABA_B receptors. In another, more likely scenario, expression of GABA_B receptors by peripheral tumors^{4,17} or thymus¹⁸ drives the pathogenic immune response. GABA_B receptor antibodies first bind to their receptors, disturb pre- and postsynaptic GABAergic inhibition, and promote increased activity states with excessive synchronization in mesial temporal lobe neuronal networks leading to epileptic seizures.^{4,19} Antibodies against GABA_B receptors are mainly of the IgG1 subclass⁴ and may thus promote neuronal damage directly via complement activation and antibody-dependent cell-mediated cytotoxicity²⁰ and thus trigger epitope and antigen-spreading through the release of intracellular neuronal antigens (such as GAD₆₅⁵). However, most antineuronal IgG1 autoantibodies seem to exert their pathogenic effects indirectly via crosslinking and subsequent internalization of their receptors without causing overt neuronal damage,^{21–24} and in GABA_B-R LE, direct blocking antibody effects on the receptor without internalization have recently been

suggested.²⁵ Epileptic seizures per se have been reported to turn on neuronal expression of major histocompatibility complex (MHC) class I molecules^{26–28} and other neuronal proteins (such as Hu²⁹) potentially rendering neurons targets for CD8⁺ T cells specific for a variety of neuronal antigens. Consistently, following temporal lobe seizures induced by certain chemoconvulsants in mice, a successive accumulation of CD8⁺ T cells in the mesial temporal lobe with a strong impact on epileptogenesis has been observed.³⁰ Moreover, CD8⁺ T cells have been shown to dramatically affect neuronal excitability on the single cell and network level^{31–33} and may thus well contribute to the neuronal pathology in LE. This possibility should be considered when stratifying immunotherapeutic approaches in GABA_B-R LE.

AUTHOR CONTRIBUTIONS

K.S.G. and C.M. recruited and treated the patients under the supervision of N.M., S.G.M., and H.W. and wrote the first draft of the manuscript. K.B. performed the neuropsychological testing under the supervision of H.L. and wrote the first draft of the manuscript. M.H. together with C.C.G. analyzed flow cytometry data. W.S. performed MRI analysis. M.G., A.J.B., and K.M.v.L. performed histopathologic analysis. M.L., G.W., C.E.E., and A.J.B. contributed 2 of the patients. N.M., S.G.M., and H.W. designed and supervised the project. All authors contributed to and approved the final manuscript.

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DISCLOSURE

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