Evidence of a pathogenic role for CD8\(^+\) T cells in anti-GABA\(_B\) receptor limbic encephalitis

**ABSTRACT**

**Objective:** To characterize the cellular autoimmune response in patients with \(\gamma\)-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\(^-\) 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\(^-\) 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**

| Acronym | Definition |
|---------|------------|
| AVLT    | Auditory Verbal Learning Test; |
| FLAIR   | fluid-attenuated inversion recovery; |
| GABA    | \(\gamma\)-aminobutyric acid; |
| GABA\(_B\)-R | \(\gamma\)-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 \(\gamma\)-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
pharmacologic blockade of the GABA<sub>B</sub> receptor in animal models causes excessive neuronal excitability, as well as disturbance of learning, memory, and behavior<sup>4</sup> resembling the clinical phenotype of human GABA<sub>B</sub>-R LE. Moreover, patients with GABA<sub>B</sub>-R LE have been reported to respond at least partially to antibody-depleting immunotherapies and tumor therapy.<sup>1,4</sup> Hence, autoantibodies are considered pathogenic. In a subset of patients, GABA<sub>B</sub>-R antibodies have been reported to occur together with antibodies to the 65-kDa isofrom of glutamic acid decarboxylase (GAD<sub>65</sub>) 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.<sup>1,4,6</sup> 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<sub>B</sub>-R LE.

**METHODS**  
**Patients and controls.** Patients with GABA<sub>B</sub>-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.<sup>7</sup> 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/μL, <2 mM lactate, no disruption of the PB–CSF barrier (defined by the CSF/serum albumin ratio), no intrathecal immunoglobulin (IgG, IgA, or IgM synthesis (Reiber criteria)),<sup>7,8</sup> 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.<sup>3</sup> 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.<sup>7</sup> Absolute cell numbers were analyzed using Flow-Count Fluorospheres (Beckmann Coulter) according to the manufacturer’s instructions.

**Antineuronal antibody testing.** Serum and CSF were tested for the presence of IgG antibodies against intraneuronal neuronal antigens (ANNA1 [Hu], ANNA2 [Ri], ANNA3, GAD<sub>65</sub> and neuronal surface membrane antigens (NMDA receptor, AMPA receptor, GABA<sub>A</sub> receptor, GABA<sub>B</sub> receptor, glycine receptor, CASPR2, LGI1, and VANG3) and using established assays (EUROIMMUN, Lübeck, Germany<sup>9,10</sup>).

**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/jn. 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; Thermofisher) and Granzyme B Ab-1 (1:50, GBZ01 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.
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 GAD\(_{65}\) 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 \(^{18}\)F-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-naive, whereas patient 2 had received several preceding immunotherapies all with marginal clinical effect suggesting persistent 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).
Hence, we tried to relate the neuropsychological deficits detected in patients with GABA\textsubscript{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\textsuperscript{+} CD8\textsuperscript{+} 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\textsuperscript{+} CD8\textsuperscript{+} 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\textsuperscript{+} CD8\textsuperscript{+} T cells and a more regulatory function of activated HLADR\textsuperscript{+} CD4\textsuperscript{+} T cells in the adaptive autoimmune inflammation of the limbic system in GABA\textsubscript{B}R LE.

Likewise, although on a lower level in terms of absolute cell numbers and less strongly, higher absolute CD138\textsuperscript{+} CD19\textsuperscript{+} 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\textsubscript{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\textsubscript{B}R LE (patients 1–3) and one case of paraneoplastic GABA\textsubscript{B}R LE (patient 4) associated with SCLC in comparison to patients with somatization disorders as controls. All patients with GABA\textsubscript{B}R LE exhibited neuropsychological deficits and MRI findings typical of LE.\textsuperscript{1,3,4} Three of them were studied very early in the disease course (4–8 weeks after disease onset) in a therapy-naive state (patients 1, 3, and 4) and one was in a non–therapy-naive 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\textsubscript{B}R LE phenomenology. One may speculate that long-standing temporal lobe epilepsy might have triggered GABA\textsubscript{B}R LE in an individual obviously prone to autoimmunity as revealed by the additional presence of GAD\textsubscript{65} 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\textsubscript{B}R LE, CD19\textsuperscript{+} B cells, CD4\textsuperscript{+} T cells, and CD8\textsuperscript{+} 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\textsuperscript{+} plasma cells and CD4\textsuperscript{+} T cells whereas activated...
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 GABAergic-associated limbic encephalitis. 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 GABAergic receptor antibody–associated limbic encephalitis

Histologic specimen of one patient with GABAergic 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 μm 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 μm in D). DAPI = 4’-6-diamidino-2-phenylindole·2HCl; GABAergic = g-aminobutyric acid B.

## 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 |
| **CD19+ B cells, cells/mL** | Median | IQR | Median | IQR | p Value | Median | IQR | Median | IQR | p Value |
| 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* |
| CD138+CD19+ plasma cells, % | 0.18 | 0.21 | 0.05 | 0.08 | 0.032* | 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* |
| HLADR+CD4+ T cells, cells/mL | 47,393 | 44,756 | 30,159 | 18,217 | 0.314 | 94 | 52 | 23 | 51 | 0.016* |
| HLADR+CD4+ T cells, %      | 13.5 | 10.7 | 4.6 | 3.4 | 0.003* | 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* |
| HLADR+CD8+ T cells, cells/mL | 203,64 | 56,125 | 16,530 | 13,285 | 0.259 | 155 | 104 | 32 | 54 | 0.013* |
| HLADR+CD8+ T cells, %      | 28.6 | 42.3 | 6.4 | 5.0 | 0.010* | 35.5 | 6.1 | 26.1 | 14.4 | 0.022* |

**Abbreviation:** IQR = interquartile range.

Wilcoxon rank sum test was used for statistical analyses.

*Significant.
T cells in the adaptive autoimmune inflammation of the limbic system in GABA\(\beta\)-R LE.

However, our data of course have several strong limitations, as (1) we only studied as few as 4 partially therapy-naive patients with GABA\(\beta\)-R LE; (2) one patient had left amygdalohippocampectomy due to treatment-resistant temporal lobe epilepsy additionally impairing memory performance 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\(\beta\)-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\(\beta\)-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 neurological 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 onconeuronal autoimmunity. However, as there was no evidence of an underlying tumor, and GAD\(_{65}\) antibodies were only detected in serum but not CSF in patient 2, we do not consider this case to represent a GAD\(_{65}\) antibody–associated LE, which might also have explained the presence of parenchymal T cells. Moreover, widespread perivascular and parenchyma T cell infiltrates were detected at autopsy in a case of paraneoplastic encephalomyelitis with SCLC in which GABA\(_\beta\) receptor antibodies could be detected together with onconeuronal antibodies against amphiphysin. Here, parenchymal CD8\(^+\) T cells were found in close apposition to neurons. 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\(\beta\)-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\(_\beta\) receptors. In another, more likely scenario, expression of GABA\(_\beta\) receptors by peripheral tumors or thymus drives the pathogenic immune response. GABA\(_\beta\) receptor antibodies first bind to their receptors, disturb perisynaptic GABAergic inhibition, and promote increased activity states with excessive synchronization in mesial temporal lobe neuronal networks leading to epileptic seizures. Antibodies against GABA\(_\beta\) receptors by peripheral tumors or thymus drives the pathogenic immune response. GABA\(_\beta\) receptor antibodies first bind to their receptors, disturb perisynaptic 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\(_\beta\) receptors are mainly of the IgG1 subclass and may thus promote neuronal damage directly via complement activation and antibody-dependent cell-mediated cytotoxicity and thus trigger epitepo and antigen-spreading through the release of intracellular neuronal antigens (such as GAD\(_{65}\)). 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, and in GABA\(\beta\)-R LE, direct blocking antibody effects on the receptor without internalization have recently been
suggested.\textsuperscript{25} Epileptic seizures per se have been reported to turn on neuronal expression of major histocompatibility complex (MHC) class I molecules\textsuperscript{26–28} and other neuronal proteins (such as Hu)\textsuperscript{29} potentially rendering neurons targets for CD8\textsuperscript{+} 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\textsuperscript{+} T cells in the mesial temporal lobe with a strong impact on epileptogenesis has been observed.\textsuperscript{30} Moreover, CD8\textsuperscript{+} T cells have been shown to dramatically affect neuronal excitability on the single cell and network level\textsuperscript{31–35} and may thus well contribute to the neuronal pathology in LE. This possibility should be considered when stratifying immunotherapeutic approaches in GABA\textsubscript{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.I., 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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Evidence of a pathogenic role for CD8⁺ T cells in anti-GABA_{B} receptor limbic encephalitis

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