

Original article

Identification of novel autoantibodies to GABA_B receptors in patients with neuropsychiatric systemic lupus erythematosusHaruka Tsuchiya^{1,2}, Shiori Haga², Yuko Takahashi¹, Toshikazu Kano¹,
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Abstract

Objective. The gamma-aminobutyric acid type B receptors (GABA_B) are G-protein coupled receptors for GABA, the main inhibitory neurotransmitter in the brain. We identified GABA_B subunits as candidate antigens in patients with SLE using a random peptide display library. The aim of this study was to investigate the possible link between anti-GABA_B antibodies and disease activity and NPSLE.

Methods. ELISA was performed with recombinant proteins of GABA_{B1b} and GABA_{B2} on serum samples from patients with SLE ($n=88$), scleroderma ($n=20$), myositis ($n=20$) or vasculitis ($n=20$) as well as healthy subjects ($n=20$). Cerebrospinal fluid (CSF) from 23 patients with SLE was also examined.

Results. Autoantibodies to GABA_Bs were exclusive to patients with SLE ($P<0.001$) and positively associated with SLEDAI (anti-GABA_{B1b}, $P=0.001$; anti-GABA_{B2}, $P<0.001$). Of note, autoantibodies were positively linked with NPSLE (anti-GABA_{B1b}, $P=0.02$; anti-GABA_{B2}, $P=0.03$). Moreover, anti-GABA_Bs was detected in 61.5% of CSF samples from patients with active NPSLE, a frequency that was significantly higher than that for patients with non-SLE syndromes.

Conclusion. Anti-GABA_B antibodies could represent novel candidate markers for disease activity and NPSLE.

Key words: systemic lupus erythematosus, neuropsychiatric systemic lupus erythematosus, disease activity, GABA_B receptor, autoantibody.

Introduction

SLE is arguably the most clinically and serologically diverse autoimmune disease, with >100 autoantibodies (anti-double-stranded DNA antibodies and anti-Sm antibodies) found in patients and disease spectra ranging from subtle symptoms to life-threatening multiorgan failure. The hallmark characteristics of SLE, including production of autoantibodies, immune complex depositions and excessive complement activation, are generally

thought to be consequences of immune dysregulation [1–3].

Besides clinical manifestations such as arthritis, nephritis, serositis, blood cytopenias and thrombosis [4], approximately 10–80% of patients with SLE suffer from neuropsychiatric symptoms [5–7]. NPSLE is probably mediated by autoantibodies, microvasculopathy and intracranial production of inflammatory mediators, often in combination. Although several studies have shown a positive correlation between NPSLE and autoantibodies (aPLs, anti-ribosomal P antibodies and autoantibodies that bind to neuronal antigens such as *N*-methyl-D-aspartate glutamate receptor), little is known about how these autoantibodies are involved in disease pathogenesis [8–10].

The gamma-aminobutyric acid type B receptors (GABA_Bs) are G-protein coupled receptors for GABA, the main inhibitory neurotransmitter in the brain. GABA_B is composed of GABA_{B1} and GABA_{B2}

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subunits, sharing a high degree of identity. The GABAR_{B1} subunit, existing as splice variants GABAR_{B1a} and GABAR_{B1b}, differs in two Sushi domains at the N-terminus. GABAR_B is widely expressed in the brain and spinal cord, most highly in the hippocampus, thalamus and cerebellum [11, 12], and GABAergic signalling is related to anxiety-related behaviour, cognitive processing, discrimination of information and sensorimotor gating. Depending on their subcellular localization, GABAR_B exerts distinct regulatory effects on synaptic transmission. Pre-synaptically, GABAR_B inhibits calcium influx by closing voltage-gated calcium channels, thereby regulating neurotransmitter release [13, 14]. Post-synaptically, GABAR_B activates inwardly rectifying potassium channels, leading to hyperpolarization of the postsynaptic membrane [15] and inducing a slow inhibitory postsynaptic current [16]. GABAR_B limits the duration of network high-activity states, thereby preventing excessive neuronal synchronization and allowing new stimuli to break synchronous activity [17, 18].

In this study, using a random peptide display library (RPDL), we identified GABAR_B subunits as candidate antigens in patients with SLE. Based on reactivity with serum and cerebrospinal fluid (CSF) samples from patients with SLE, we looked at the possible link between anti-GABAR_B antibodies and disease activity and NPSLE.

Methods

Patients

All 88 enrolled patients were adults admitted to the National Center for Global Health and Medicine (NCGM) hospital from January 2002 to December 2012. Diagnosis of SLE was based on ACR classification criteria [19]. Clinical data were obtained from medical records. Consent was obtained from the patients for storage of the residual CSF and serum samples that had been originally collected for clinical purposes. Written informed consent was newly obtained from the patients prior to use of the samples for research purposes. The study was approved by the Institutional Review Board of the NCGM, Tokyo (NCGM-G-001292-00).

Data collection

Disease activity was assessed using the SLEDAI [20]. Nephritis was defined as class III, IV or V (proliferative, membranous or membranoproliferative) according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification [21, 22]. NPSLE was divided into 19 neuropsychiatric syndromes classified according to the ACR [23] and diagnosed based on neurological examination, brain/spinal cord MRI, electroencephalogram, CSF, nerve conduction examination, psychiatric interviews and a short battery of neuropsychological tests recommended by the ACR committee [23]. Cognitive function was evaluated in all patients except those with a disturbance of consciousness or poor general condition. Other possible aetiologies of NPSLE, such as infection and drugs, were excluded. APS was

diagnosed based on the Sydney criteria [24]. Laboratory tests included evaluation of complete blood count and immunological markers [complement component 3 (C3)]. Anti-dsDNA antibody titres (normal values <20 IU/ml) and autoantibodies to SSA, Sm and U1-RNP were measured by enzyme immunoassay.

RPDL screening

The FliTrx Random Peptide Library was amplified according to the manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). Briefly, *Escherichia coli* that possesses an RPDL inserted into its flagellar protein was grown at 25°C in IMC medium [1 × M9 salts (6 g Na₂HPO₄, 3 g KH₂PO₄, 0.5 g NaCl and 1 g NH₄Cl for 1 l), 0.2% casamino acids, 0.5% glucose, 1 mM MgCl₂] in the presence of 100 µg/ml ampicillin and then fusion peptides were expressed in the same medium supplemented with 100 µg/ml tryptophan. After washing with a blocking buffer composed of 1% skimmed milk, 150 mM NaCl and 1% α-methyl mannoside, bacteria were reacted for 1 h at room temperature with 150 µl of SLE patients' serum, which was pre-cleaned three times with a bacterial clone that expressed a truncated version of FliTrx protein. Then, protein G sepharose was incubated with pre-cleaned serum for 1 h at room temperature. After centrifugation at 120 g, the pellet was rinsed five times with a washing buffer composed of the IMC medium with 1% α-methyl mannoside and recovered bacteria were vigorously agitated then inoculated into the IMC medium with ampicillin (100 µg/ml). After overnight culture, the same procedures were carried out using the same volume of the patients' serum. After five rounds of biopanning procedures, the enriched library was grown. Then, ~1000 single colonies were picked up, and plasmid DNA from each clone was purified and sequenced (QIAprep Spin Miniprep Kit, Qiagen, Leusden, The Netherlands). After sequencing, the deduced amino acid sequence of each clone was applied to a Basic Local Alignment Search Tool (BLAST) program for searching the homology of proteins. As candidate antigens of GABAR autoantibodies in patients with SLE, we identified peptides composed of amino acids (aa) GGWPGG (aa 63–68), RKLRH (aa 336–340) and GGRSGVR (aa 371–377) for GABAR_{B1b} and VCPSVT (aa 134–139), LSFAAT (aa 154–159), YQWKRVG (aa 193–199), ASSRHQR (aa 378–384) and VGEYNAV (aa 439–445) for GABAR_{B2}.

Preparation of recombinant proteins of GABAR_Bs

We prepared recombinant proteins of the amino-terminal (N) region of GABAR_Bs, where epitopes of candidate autoantibodies were identified by RPDL.

Full-length human GABAR_{B1b} cDNA was purchased from the National Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Chiba, Japan. An amino-terminal (N) region of the extracellular domain (aa 1–475) was amplified by PCR and cloned into pcDNA3.1 (–) vector (Life Technologies). The FLAG sequence, consisting of eight aa (DYKDDDDK), was inserted just after the C-terminus. For the GABAR_{B2}

N-terminal extracellular domain (aa 1–480), cDNA with the FLAG sequence inserted just after its N-terminal signal sequence was synthesized using Eurofins MWG Operon (Huntsville, AL, USA) and cloned into pcDNA3.1 (–) vector. FreeStyle 293 cells (Life Technologies) were transfected with plasmid DNA encoding GABAR_{B1b}-FLAG or FLAG-GABAR_{B2} according to the manufacturer's protocol. On day 2 after transfection, supernatants were collected and purified by column chromatography using diethylaminoethyl cellulose.

Detection of anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies in patients' serum and CSF samples

ELISA was performed as previously described [25] and the researchers performing these assays were blinded to the underlying clinical information. Briefly, 96-well plates (Immuno MaxiSorp, Nunc, Roskilde, Denmark) were coated with 250 ng of GABAR_{B1b}-FLAG or FLAG-GABAR_{B2} in bicarbonate buffer (pH 9.6) overnight at 4°C. Wells were washed and blocked with 5% skim milk in Tris-buffered saline Tween 20 [TBS-T; 20 mM Tris-HCl (pH 7.5), 150 mM NaCl and 0.1% Tween 20]. Sera were diluted 1000-fold with 0.25% skim milk in TBS-T and undiluted CSF was added. After incubation for 1 h at room temperature, the wells were rinsed five times with TBS-T and developed with horseradish peroxidase (HRP)-conjugated anti-human IgG antibody. SureBlue TMB was used as an HRP substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) and optical density (OD) at 450 nm was measured. All samples were independently assayed twice. OD values were normalized to samples from one patient. The intra-assay coefficients of variation for anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies were 9.0% and 9.2%, respectively. The cut-off values for positive and negative assignments were determined as mean values + 3 s.d. compared with controls. We defined serum samples as positive when OD values were >0.39 (anti-GABAR_{B1b}) or >0.31 (anti-GABAR_{B2}). We defined CSF samples as positive when OD values were >0.47 (anti-GABAR_{B1b}) or >0.46 (anti-GABAR_{B2}).

Statistical analysis

Statistical analyses were performed using Fisher's exact test to compare frequencies and the Mann-Whitney *U*-test to compare median values. Correlation coefficients (*r_s*) were calculated as Spearman's rank correlation coefficients. Logistic regression analysis was performed to evaluate the association between anti-GABAR_{B1b} or anti-GABAR_{B2} antibodies and various organ involvements using a backwards stepwise model. Data were analysed using JMP software (SAS Institute, Cary, NC, USA). Statistical significance was defined at *P* < 0.05.

Results

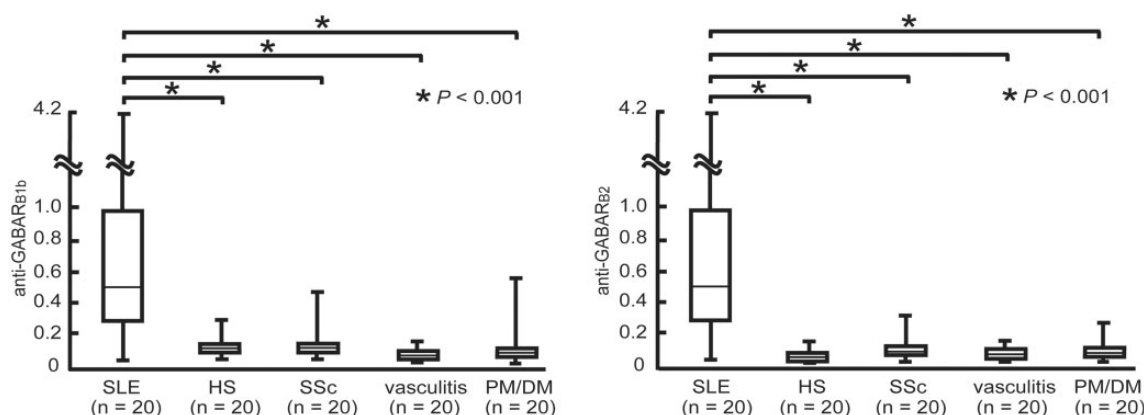
Patient demographics

The clinical characteristics of 88 patients with SLE are summarized in supplementary Table S1, available at

Rheumatology Online. The mean age of all patients with SLE was 47 years (s.d. 18) and 90% were female. Fifty patients suffered from symptoms associated with SLE on admission (26 patients were admitted for the first treatment, whereas 24 patients were recurrent and received low- or maintenance-dose steroids and/or immunosuppressants; active SLE). The other 38 patients had no symptoms or abnormal clinical results related to SLE and were being treated with or without low- or maintenance-dose steroids and/or immunosuppressants (chronic SLE). The median SLEDAI score was 10 [interquartile range (IQR) 2–17]. The frequency of each clinical feature was as follows: malar erythema, 22%; arthritis, 16%; serositis, 11%; nephritis, 28%; and NPSLE, 17% [including aseptic meningitis (*n* = 4), cerebrovascular disease (*n* = 1), headache (*n* = 2), movement disorder (*n* = 1), myelopathy (*n* = 3), seizure (*n* = 1), acute confusional state (*n* = 2), other psychiatric syndromes (*n* = 2) and peripheral nervous system disorders (*n* = 2)]. Combined neuropsychiatric forms were observed in two patients with SLE. APS coexisted in 7% of patients. The median dosage of prednisolone was 9 mg/day (IQR 1.8–15) and concurrent immunosuppressant use was reported in 32% of patients. The positive frequency of each autoantibody was as follows: anti-dsDNA, 53%; anti-SSA, 48%; anti-Sm, 48%; and anti-U1 RNP, 55%. Patients with non-SLE syndromes included those with SSc, vasculitis and PM/DM diagnosed using established criteria [26–28]. All patients with non-SLE syndromes were newly diagnosed and had never been treated with steroids and/or immunosuppressants. The mean age and frequency of female patients with non-SLE syndromes were matched with those of patients with SLE.

Measurement of serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies in patients with SLE and non-SLE syndrome

First, we analysed the presence of anti-GABAR_B antibodies in 20 randomly selected serum samples from patients who were either active SLE or non-SLE (Fig. 1). The median OD values of serum anti-GABAR_{B1b} antibodies in samples of patients with SLE, SSc, vasculitis and PM/DM were 0.512 (IQR 0.324–0.985), 0.078 (0.063–0.107), 0.051 (0.036–0.061) and 0.07 (0.054–0.09), respectively; the mean OD value of healthy subjects was 0.07 (0.063–0.13). The values for serum anti-GABAR_{B2} antibodies in patients with SLE, SSc, vasculitis and PM/DM were 0.48 (IQR 0.307–0.995), 0.086 (0.066–0.109), 0.055 (0.043–0.071) and 0.079 (0.061–0.118), respectively; the value in healthy subjects was 0.064 (0.046–0.102). The OD values of autoantibodies were significantly different between patients with SLE and non-SLE syndromes (*P* < 0.001), and specificity was confirmed by excluding the FLAG-tag reactivity (supplementary Fig. S1, available at *Rheumatology* Online). These results indicated that autoantibodies against GABAR_Bs were uniquely detectable in patients with SLE.

Fig. 1 Serum antibodies in SLE and non-SLE patients were measured for anti-GABAR_{B1b} (left) and anti-GABAR_{B2} (right) antibodies* $P < 0.001$.

Comparison of clinical characteristics between serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibody-positive and -negative SLE patients

Next we compared clinical characteristics of patients who were positive or negative for autoantibodies (Table 1). The positive association of the SLEDAI with anti-GABAR_B antibodies was marked (anti-GABAR_{B1b}, $P < 0.001$; anti-GABAR_{B2}, $P < 0.001$). Of note, the frequency of patients with NPSLE was also greater in the autoantibody-positive groups (anti-GABAR_{B1b}, $P < 0.001$; anti-GABAR_{B2}, $P < 0.001$). In the autoantibody-positive groups, the value of anti-dsDNA antibodies increased (anti-GABAR_{B1b}, $P = 0.03$; anti-GABAR_{B2}, $P = 0.002$) and the haemoglobin content decreased (anti-GABAR_{B1b}, $P = 0.007$; anti-GABAR_{B2}, $P < 0.001$). In the anti-GABAR_{B2}-positive group, C3 abundance decreased ($P = 0.01$). Mean age; gender; frequencies of malar erythema, arthritis, serositis, nephritis and APS; leucocyte and platelet counts; median prednisolone dosage; frequency of concurrent immunosuppressant use and antibody positivity rates of anti-SSA, anti-Sm and anti-U1 RNP showed no significant differences between the autoantibody-positive and -negative groups. There were four patients in whom the anti-GABAR_{B2} antibody was the only antibody that could be demonstrated. There were no patients with peripheral nervous system disorders or diffuse psychiatric syndromes except for acute confusional states in the autoantibody-positive groups. No significant differences in other clinical characteristics (age, gender, SLEDAI, treatment at baseline, complete blood cell count, C3, anti-dsDNA antibody, anti-SSA antibody, anti-Sm antibody or anti-U1 RNP antibody) were found between autoantibody-positive and -negative patients with NPSLE (data not shown).

The presence of autoantibodies was assayed in active and chronic patients and titres were significantly higher in patients with active SLE than in chronic patients ($P < 0.001$; supplementary Fig. S2, available at

Rheumatology Online). The titres markedly decreased after immunosuppressive treatment in all eight pairs examined (anti-GABAR_{B1b}, $P = 0.008$; anti-GABAR_{B2}, $P = 0.006$; supplementary Fig. S3, available at *Rheumatology* Online).

Correlations between clinical parameters and serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies

Next we determined correlations among autoantibody titres and clinical parameters in 88 patients with SLE (Table 2). A strong correlation was detected between anti-GABAR_{B1b} and anti-GABAR_{B2} antibody titres ($r_s = 0.87$, $P < 0.001$). A moderate correlation was identified between anti-GABAR_B and anti-dsDNA antibodies (anti-GABAR_{B1b}, $r_s = 0.46$; anti-GABAR_{B2}, $r_s = 0.58$; $P < 0.001$). Of note, a significant correlation was detected between anti-GABAR_B antibodies and the SLEDAI (anti-GABAR_{B1b}, $r_s = 0.51$; anti-GABAR_{B2}, $r_s = 0.72$; $P < 0.001$) and between anti-dsDNA antibodies and the SLEDAI ($r_s = 0.65$, $P < 0.001$). A moderate correlation was detected between C3 and anti-GABAR_{B2} ($r_s = -0.45$, $P < 0.001$) or anti-dsDNA ($r_s = -0.58$, $P < 0.001$) and a significant but weak correlation between C3 and anti-GABAR_{B1b} antibodies was detected ($r_s = -0.33$, $P = 0.002$). No strong correlation was found between either of the antibodies and other laboratory markers tested.

Association between serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies and various organ involvements and clinical parameters

Logistic regression analysis was performed to evaluate associations between autoantibodies, organ involvements and clinical parameters. Titres of anti-GABAR_B antibodies were the dependent variables. Independent variables included the SLEDAI, NPSLE, leucocytes, haemoglobin, C3 and anti-dsDNA antibodies. The SLEDAI showed the best association with anti-GABAR_B antibodies, with an odds ratio of 1.14 (95% CI 1.05, 1.26, $P = 0.001$) for anti-GABAR_{B1b} antibodies and 1.13 (1.05, 1.24, $P < 0.001$) for

TABLE 1 Comparison of clinical characteristics between serum anti-GABAR_B antibody-positive and -negative SLE patients

Variables	Anti-GABAR _{B1b} antibody			Anti-GABAR _{B2} antibody		
	Positive (n = 14)	Negative (n = 74)	P-value ^a	Positive (n = 18)	Negative (n = 70)	P-value ^a
Age, mean (s.d.), years	41 (12)	48 (19)	0.21	41 (13)	48 (19)	0.12
Gender, n (%), female	14 (100)	65 (88)	0.17	18 (100)	61 (87)	0.11
SLEDAI, median (IQR)	20 (13–30)	7 (1–15)	<0.001**	19 (13–27)	6 (1–15)	<0.001**
Malar erythema, n (%)	4 (29)	15 (20)	0.49	5 (28)	14 (20)	0.47
Arthritis, n (%)	4 (29)	10 (14)	0.16	5 (28)	9 (13)	0.12
Serositis, n (%)	3 (21)	8 (11)	0.27	3 (17)	8 (11)	0.55
Nephritis, n (%)	7 (50)	18 (24)	0.05	8 (44)	17 (24)	0.09
NPSLE, n (%)	8 (57)	7 (9)	<0.001**	9 (50)	6 (9)	<0.001**
Neurological syndromes, n						
Aseptic meningitis	2	2		2	2	
Cerebrovascular disease	1	0		1	0	
Demyelinating syndrome	0	0		0	0	
Headache	2	0		2	0	
Movement disorder	0	1		0	1	
Myelopathy	2	1		3	0	
Seizure	1	0		1	0	
Diffuse psychiatric syndromes, n						
Acute confusional state	2	0		2	0	
Other psychiatric syndromes	0	2		0	2	
Peripheral nervous system disorders						
APS, n (%)	1 (7)	5 (7)	>0.99	1 (6)	5 (7)	>0.99
Prednisolone, median (IQR), mg/day	8 (1.3–17.5)	9 (2.5–15)	0.89	6.5 (0–10)	9.5 (5–15)	0.28
Concurrent immunosuppressant, n (%)	2 (14)	26 (35)	0.21	3 (17)	25 (36)	0.16
Leucocytes, median (IQR), × 10 ³ /μl	4.4 (3.3–7.7)	6.0 (4.0–7.5)	0.47	4.0 (3.3–7.3)	6.2 (4.2–7.6)	0.14
Haemoglobin, median (IQR), g/dl	10.3 (9.2–11.1)	12.2 (10.7–12.9)	0.007**	10.8 (9.2–11.1)	12.3 (10.7–13)	<0.001**
Platelets, median (IQR), × 10 ⁷ /μl	16.2 (12.9–18.1)	18.5 (13.6–23.9)	0.25	16.5 (14.1–20.3)	18.5 (13.1–23.6)	0.52
C3, median (IQR), mg/dl	49.1 (35.3–86.5)	76.3 (58.3–101)	0.07	49.1 (34–83.2)	77.3 (60.2–102.5)	0.01*
Anti-dsDNA antibody, median (IQR), IU/ml	63.1 (16.9–328.8)	21.6 (5.1–56.9)	0.03*	111 (22.3–320.5)	17.2 (4.9–48.9)	0.002**
Antibody positivity, n (%)						
Anti-SSA	8 (57)	34 (46)	0.56	10 (56)	32 (46)	0.60
Anti-Sm	10 (71)	32 (43)	0.08	12 (67)	30 (43)	0.11
Anti-U1 RNP	9 (64)	39 (53)	0.56	11 (61)	37 (53)	0.60

^aMann-Whitney U-test P-value. *P < 0.05 and **P < 0.01 indicate significant differences in the positive and negative groups, respectively.

TABLE 2 Correlation coefficients between the OD values of anti-GABAR_B and anti-dsDNA antibodies and clinical parameters

Variables	Anti-GABAR _{B1b} antibody		Anti-GABAR _{B2} antibody		Anti-dsDNA antibody	
	r_s^a	<i>P</i> -value	r_s^a	<i>P</i> -value	r_s^a	<i>P</i> -value
vs SLEDAI	0.51	<0.001**	0.72	<0.001**	0.65	<0.001**
vs anti-dsDNA	0.46	<0.001**	0.58	<0.001**	—	—
vs anti-GABAR _{B1b}	—	—	0.87	<0.001**	0.46	<0.001**
vs anti-GABAR _{B2}	0.87	<0.001**	—	—	0.58	<0.001**
vs C3 value	−0.33	0.002**	−0.45	<0.001**	−0.58	<0.001**
vs leucocyte count	−0.21	0.046*	−0.36	0.001**	−0.4	<0.001**
vs haemoglobin count	−0.25	0.02*	−0.34	0.001**	−0.29	0.007**
vs platelet count	−0.14	0.2	−0.15	0.16	−0.09	0.41

^aCorrelation coefficient estimated by Spearman's rank correlation coefficient. **P* < 0.05 and ***P* < 0.01 indicate significant differences between the autoantibody titres with clinical parameters.

anti-GABAR_{B2} antibodies. For the association of NPSLE with antibodies the odds ratio was 5.51 (95% CI 1.26, 24.74, *P* = 0.02) for anti-GABAR_{B1b} antibodies and 4.64 (1.16, 19.13, *P* = 0.03) for anti-GABAR_{B2} antibodies.

Detection of anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies in the CSF of patients with NPSLE

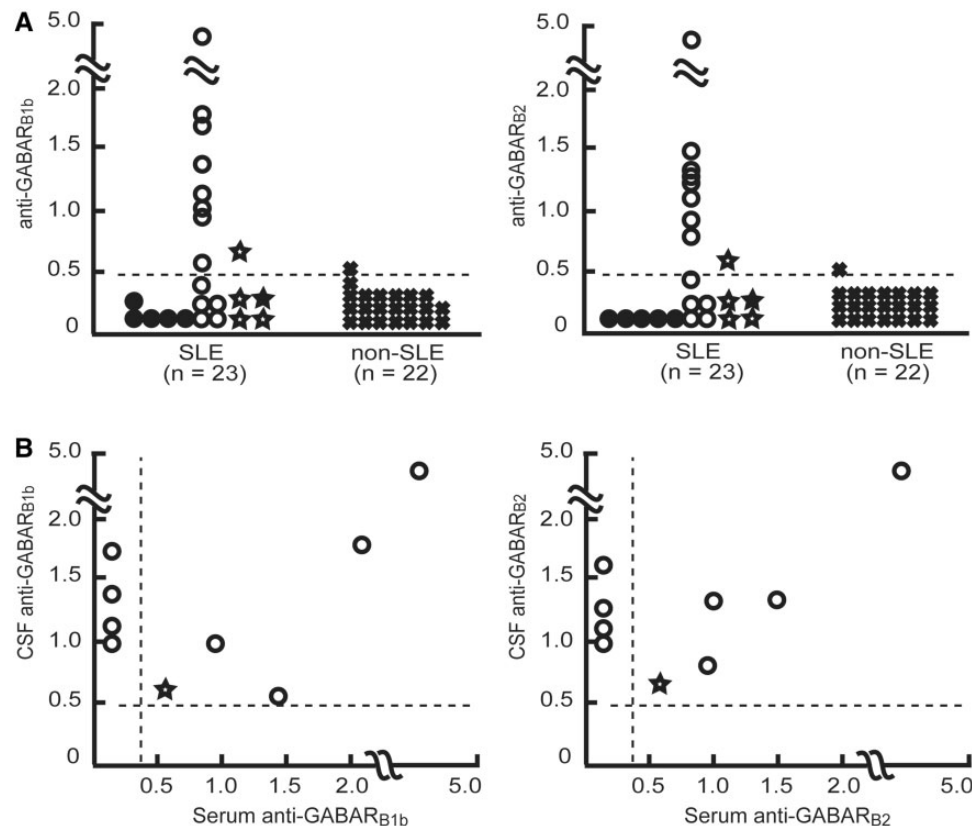
Thirteen of the 23 SLE samples examined were from patients with active NPSLE (Fig. 2A), while 22 CSF control samples from patients with non-SLE syndromes, including SSc (*n* = 5), vasculitis (*n* = 12) and PM/DM (*n* = 5), were examined. The mean age and frequency of females in the non-SLE group were matched with those of patients in the SLE group. Nine samples in the SLE group, including eight samples from patients with active NPSLE, were positive for anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies (39.1%), whereas only one sample in the non-SLE group was positive (4.5%). CSF anti-GABAR_B antibodies and the clinical characteristics of 13 patients with active NPSLE are summarized in Table 3. Neuropsychiatric symptoms in patients with anti-GABAR_B antibodies included acute confusional state (patient 1), visual field defect (patient 2), meningitis (patients 3, 4, 5 and 8), myelitis (patients 4 and 5), brainstem findings (patient 6), chorea (patient 7) and aphasia (patient 7). Patients without anti-GABAR_B antibodies presented with cranial neuropathy (patient 9), psychosis (patients 10 and 12) and mononeuropathy (patients 11 and 13). The IgG index, which is one of the markers of IgG production in the central nervous system, increased (>0.8) in seven of eight patients with CSF anti-GABAR_B antibodies. Interestingly, we observed that four of nine patients with SLE (patients 5–8 in Table 3) were positive for anti-GABAR_B antibodies in CSF but not in serum (Fig. 2B).

Discussion

In 15–20% of SLE patients we detected serum autoantibodies against GABAR_Bs that were associated with disease activity. First, the SLEDAI in the patients who were

positive for serum anti-GABAR_B antibodies significantly increased (Table 1). Second, the SLEDAI was the most significantly associated clinical parameter with serum anti-GABAR_B antibodies, and anti-dsDNA antibodies, which have been reported as both diagnostic and disease activity markers for SLE [29], significantly correlated with serum anti-GABAR_B antibodies (Table 2). Finally, therapeutic intervention significantly decreased serum anti-GABAR_B antibody titres in all eight patient pairs examined (supplementary Fig. S3, available at *Rheumatology* Online). Taken together with observations of a significant difference in serum anti-GABAR_B antibody titres in patients with active and chronic SLE (supplementary Fig. S2, available at *Rheumatology* Online), our data suggest that serum anti-GABAR_B antibodies could be a novel biomarker for the disease activity of SLE. Although anti-GABAR_B antibodies have been detected in patients with limbic encephalitis and have been reported to block receptor function [30, 31], our study is the first to show that serum anti-GABAR_B antibodies are present in patients with SLE and have significant correlations with disease activity.

Although GABA is known purely as an inhibitory neurotransmitter, receptors and enzymes involved in GABA metabolism and catabolism have been shown to be widely distributed outside the brain, notably in the immune system. GABAR_B is expressed on haematopoietic progenitor and stem cells (HSPCs), neutrophil granulocytes and peripheral blood mononuclear cells (PBMCs) and has recently been reported to influence neutrophil chemotaxis, HSPC locomotion and cytokine production by PBMCs [32]. GABA primarily has an inhibitory effect on the immune system, although the effect of activating GABAR_B seems to be more complex and includes immune stimulation [33]. One plausible explanation of our findings that serum anti-GABAR_B antibodies significantly correlated with disease activity of SLE is that autoantibodies have a disruptive effect on GABA-mediated immune response.

Fig. 2 CSF from SLE and non-SLE patients were measured for anti-GABAR_{B1b} (left) and anti-GABAR_{B2} (right) antibodies

Bars indicate cut-off values. **(A)** The non-SLE subjects included 22 patients with SSc, vasculitis and PM/DM. Open circles (○) represent patients with active NPSLE; filled circles (●) represent patients with chronic NPSLE; open stars (☆) represent patients with active SLE (except for NPSLE); filled stars (★) represent patients with chronic SLE (except for NPSLE), and cross marks (×) represent patients with non-SLE. Active SLE (NPSLE) patients were defined as patients admitted for a first treatment or recurrence. Chronic SLE (NPSLE) patients were defined as patients who had no symptoms or abnormal clinical results related to SLE, treated with or without low-dose steroids and/or immunosuppressants. **(B)** Comparison between serum and CSF anti-GABAR_B antibodies in nine patients who were positive for the CSF autoantibodies.

Intriguingly, the current study demonstrated that serum anti-GABAR_B antibodies are positively linked with NPSLE and that autoantibodies were present in 61.5% of CSF samples from patients with active NPSLE. Although our sample size was small and further study will be required, our results suggest that anti-GABAR_B antibodies have specificity for some neuropsychiatric manifestations. For instance, in serum or CSF anti-GABAR_B antibody-positive groups there were no patients with peripheral nervous system disorders or diffuse psychiatric syndromes except for acute confusional states.

Blood-brain barrier (BBB) permeability increases in patients with SLE, particularly in those with neuropsychiatric manifestations [34, 35]. Abbott *et al.* [36] described the following two main mechanisms for BBB damage in SLE: microthrombi in cerebral vessels and immune-mediated attack of the endothelium. Thus the presence of anti-GABAR_B antibodies in CSF may be the result of a

transfer of antibodies from the blood into intrathecal regions. However, four patients with NPSLE were positive for anti-GABAR_B antibodies in the CSF but negative in serum, and the IgG index increased in those patients. These results imply that anti-GABAR_B antibodies could be produced within the central nervous system, consistent with previous studies showing that intrathecal IgG production increased in patients with NPSLE [34, 37].

Given the wide variety of clinical manifestations of NPSLE, it is unlikely that a single pathogenic mechanism is responsible for all of them. In addition to autoantibodies, there is evidence to support the notion that proinflammatory cytokines and chemokines, which have been identified in the CSF of patients in the SLE group with neuropsychiatric disease, have a pathogenic role [38–40]. With respect to autoantibodies, the present data suggest that aPL antibodies cause focal neuropsychiatric diseases (stroke or seizures) by promoting

TABLE 3 CSF anti-GABAR_B antibodies and clinical characteristics of 13 patients with active NPSLE

Patient	Age/gender	Neuropsychiatric symptoms	CSF Anti-GABAR _{B1b} / GABAR _{B2} Anti-GABAR _{B2}	Serum Anti-GABAR _{B1b} / GABAR _{B2}	IgG index
CSF anti-GABAR _B positive					
1	31/F	Acute confusional state	4.99/4.70	3.57 ^a /2.72 ^a	1.09 ^b
2	31/F	Visual field defect	0.50/0.67	1.46 ^a /0.96 ^a	0.2
3	36/F	Meningitis	0.93/1.29	1.00 ^a /1.00 ^a	1.06 ^b
4	40/F	Meningitis, myelitis	1.67/1.28	2.35 ^a /1.54 ^a	2.08 ^b
5	49/M	Meningitis, myelitis	1.15/0.93	0.07/0.10	1.26 ^b
6	18/F	Brainstem findings— impaired consciousness, diplopia, nystagmus, ataxia	1.38/1.28	0.15/0.22	1.22 ^b
7	33/F	Chorea, aphasia	1.75/1.47	0.10/0.08	0.97 ^b
8	35/F	Meningitis	1.00/1.08	0.09/0.10	1.11 ^b
CSF anti-GABAR _B negative					
9	66/F	Cranial neuropathy	0.10/0.12	0.06/0.08	
10	45/F	Psychosis	0.12/0.09	0.04/0.05	
11	32/F	Mononeuropathy	0.37/0.19	0.12/0.10	
12	24/F	Psychosis	0.26/0.23	0.08/0.12	
13	67/F	Mononeuropathy	0.08/0.06	0.28/0.30	

IgG index = (CSF IgG/CSF albumin)/(serum IgG/serum albumin). ^aSerum anti-GABAR_B antibodies positive. ^bIgG index >0.8.

intravascular thrombosis [6]. In contrast, anti-ribosomal P antibodies [41, 42], and possibly anti-NR2 antibodies [43, 44], cause diffuse neuropsychiatric events (psychosis, depression and cognitive impairment) via a direct effect on neuronal cells. It is possible that a combination of biomarkers reflecting different components of the pathogenic model of NPSLE will best explain clinical events. Anti-GABAR_B antibodies could play a pathogenic role in NPSLE.

Despite the novel aspects of our study, several limitations should be considered. First, a limited number of patients with NPSLE were included, which could raise concerns about the generalization of our results. In addition, access to CSF samples in our study was infrequent and restricted to situations when a lumbar puncture was clinically indicated. Second, clinical data were retrospectively obtained, meaning that some underestimation in the clinical manifestations may be present. Third, we did not perform an in-depth screening for neuropsychiatric manifestations among patients lacking histories and could not exclude that they had mild or subclinical neuropsychiatric manifestations. Finally, the procedure detected autoantibodies towards only the ectodomain of GABAR_Bs and may accordingly have excluded antibodies against epitopes on the GABAR_Bs.

Nevertheless, as far as we know, this is the first study that demonstrates the presence of anti-GABAR_B antibodies in serum and CSF from SLE patients and suggests the positive link between anti-GABAR_B antibodies and disease activity and NPSLE.

Conclusions

In conclusion, anti-GABAR_B antibodies could represent novel candidate markers for disease activity and NPSLE.

Further studies will shed light on the biological activity of anti-GABAR_B antibodies on GABA-mediated signalling neuronal cells.

Rheumatology key messages

- Anti-gamma-aminobutyric acid type B receptor (anti-GABAR_B) antibodies are present in serum and cerebrospinal fluid from SLE patients.
- Anti-GABAR_B antibodies could represent novel candidate markers for disease activity and NPSLE.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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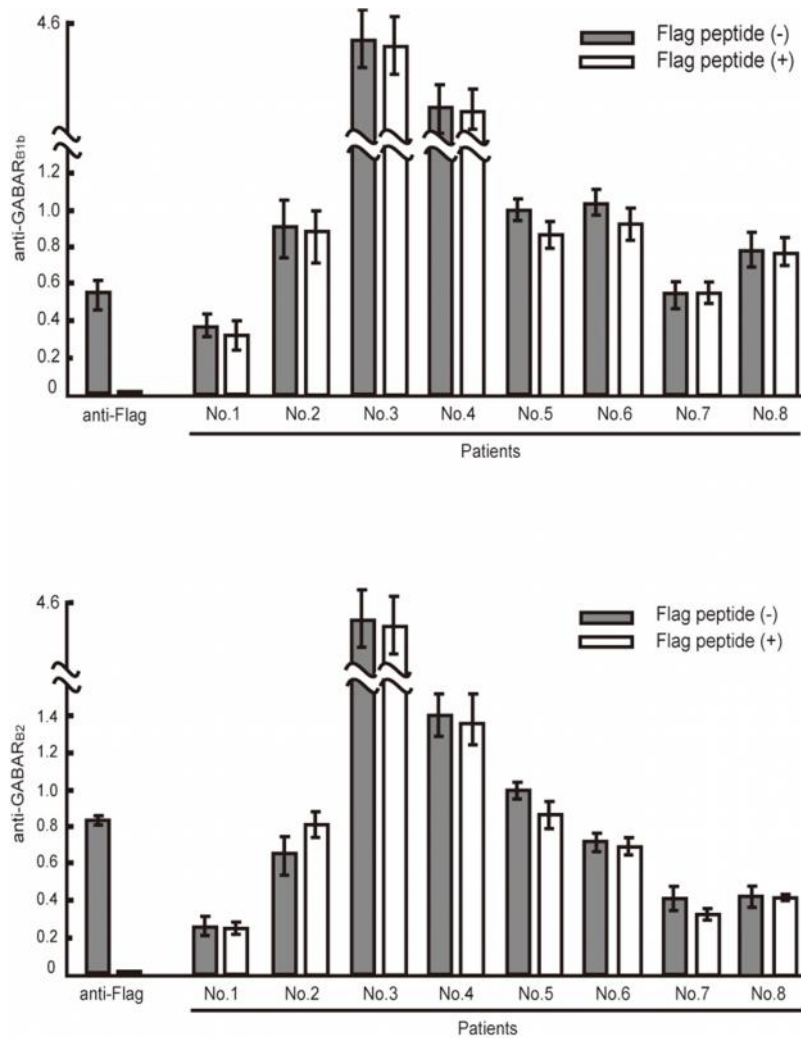
Supplementary material

Supplementary Table S1. Demographic and clinical characteristics of 88 patients with SLE

Age, mean (S.D.), years	47 (18)
Gender: female, n (%)	79 (90)
Active SLE, n (%)	50 (57)
first treatment ,n	26
recurrence ,n	24
Chronic SLE, n (%)	38 (43)
SLEDAI, median (interquartile range)	10 (2-17)
Malar erythema, n (%)	19 (22)
Arthritis, n (%)	14 (16)
Serositis, n (%)	10 (11)
Nephritis, n (%)	25 (28)
NPSLE, n (%)	17 (19)
APS, n (%)	6 (7)
Prednisolone, median (interquartile range), mg/day	9 (1.8-15)
Concurrent immunosuppressant, n (%)	28 (32)
Antibody positive, n (%)	
anti-dsDNA	47 (53)
anti-SSA	42 (48)
anti-Sm	42 (48)
anti-U1 RNP	48 (55)

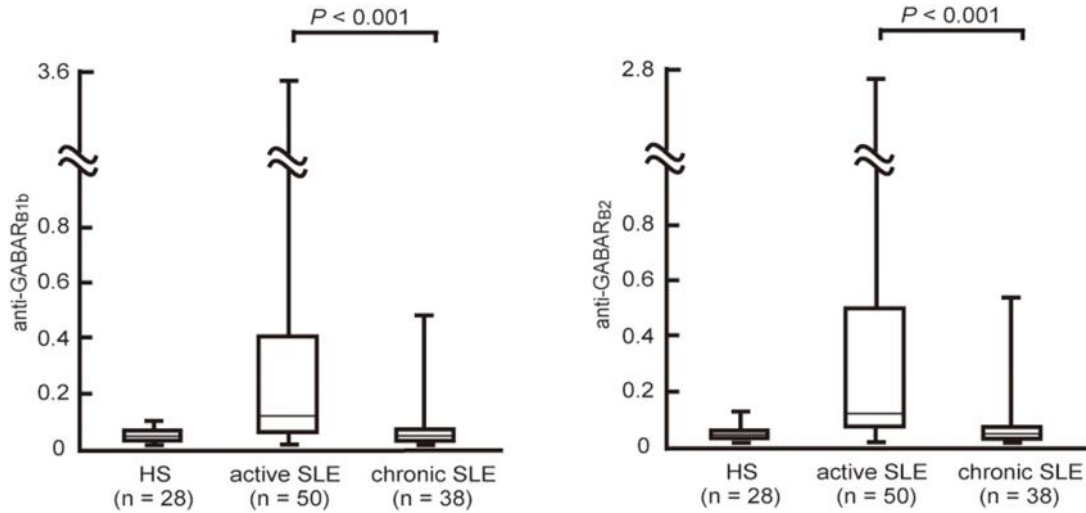
Active SLE, patients suffered from symptoms associated with SLE and admitted for a first treatment or recurrence on low/maintenance-dose steroid and/or immunosuppressants. Chronic SLE, patients who had no symptoms or abnormal clinical results related to SLE, treated with or without low-dose steroids and/or immunosuppressants. SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; NPSLE, neuropsychiatric SLE; APS, antiphospholipid syndrome.

Supplementary Figure S1. Peptide absorption assay



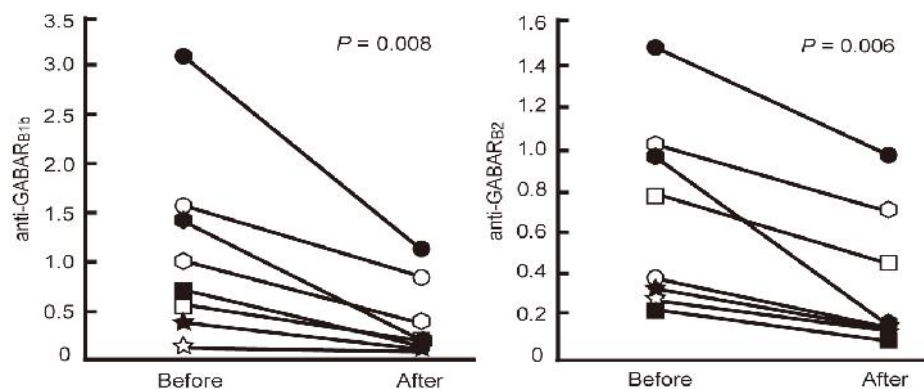
Sera (1:1000) and Flag antibodies (1:500, a specificity control) were absorbed with Flag peptides at a 1:100 Flag-tag antibody to peptide molar-ratio. Antibody titers of anti-GABAR_{B1b} (the upper graph) and anti-GABAR_{B2} (the lower graph) were measured by ELISA. OD values were similarly detected after absorption with Flag peptides. Although sera of No. 3, 4 and 5 patients were incubated with an excess of Flag peptides (1:7500 moles), antibody titers did not change (data not shown).

Supplementary Figure S2. Autoantibodies in active and chronic patients



Median OD values of serum anti-GABAR_{B1b} antibodies in the active and chronic SLE patients were 0.106 (0.005–3.567) and 0.056 (0.012–0.415), respectively, and values of serum anti-GABAR_{B2} antibodies in the active and chronic SLE patients were 0.115 (0.079–0.45) and 0.051 (0.034–0.081), respectively. Titers were strikingly different between the active and chronic SLE patients ($P < 0.001$).

Supplementary Figure S3. Autoantibodies before and after immunosuppressive treatment



Titers of both autoantibodies decreased remarkably after treatment in all pairs examined ($P = 0.008$ for anti-GABAR_{B1b}, 0.006 for anti-GABAR_{B2}).