Role of serum autoantibodies in blood brain barrier damages in neuropsychiatric systemic lupus erythematosus

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Abstract

Objective

The present study was carried out to elucidate the roles of serum autoantibodies in the development of blood-brain barrier (BBB) damages in neuropsychiatric systemic lupus erythematosus (NPSLE).

Methods

Paired serum and CSF samples were obtained from 101 SLE patients when they presented active neuropsychiatric manifestations (69 patients with diffuse psychiatric/neuropsychological syndromes [diffuse NPSLE] and 32 patients with neurologic syndromes or peripheral neuropathy [focal NPSLE]). IgG anti-NR2 subunit of NMDA receptor (anti-NR2), anti-Sm, anti-ribosomal P and IgG anti-cardiolipin in sera and albumin in CSF and sera were measured by ELISA. Blood-brain barrier (BBB) function was evaluated by Q albumin (CSF/serum albumin quotient x 1,000).

Results

Q albumin was significantly higher in acute confusional state (ACS) than in non-ACS diffuse NPSLE (anxiety disorder, cognitive dysfunction, mood disorder and psychosis) or in focal NPSLE. Anti-Sm, but not anti-NR2, anti-P or anti-cardiolipin, was significantly elevated in ACS compared with the other 2 groups of NPSLE, although serum anti-NR2 was significantly higher in ACS than that in focal NPSLE. Multiple regression analysis confirmed the significant contribution of anti-Sm (p=0.0040), but not anti-NR2 (p=0.5023), anti-P (p=0.2651), or anti-cardiolipin (p=0.6769) in the elevation of Q albumin.

Conclusion

The data demonstrate that serum anti-Sm antibodies play a most important role in the disruption of BBB in NPSLE.

Key words

NPSLE, blood-brain barrier, autoantibodies, anti-Sm

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Introduction

Neuropsychiatric systemic lupus erythematosus (NPSLE) is one of the recalcitrant complications of the disease, leading to substantial impairment of quality of life as well as disability (1). Among a variety of manifestations in NPSLE, acute confusional state (ACS) in diffuse psychiatric neuropsychological syndromes (diffuse NPSLE) is the most serious one, requiring extensive immunosuppressive therapy and sometimes resulting in poor prognosis (1, 2). N-methyl-D-aspartate (NMDA) receptor is one of the glutamate receptor families and its stimulation has been shown to cause excitatory synaptic transmission in the central nervous system (CNS) (3). Previous studies showed that antibodies to NMDA receptor NR2 subunit (anti-NR2) in cerebrospinal fluid (CSF), but not those in sera, were closely associated with the development of diffuse psychiatric/neuropsychological manifestation in SLE (diffuse NPSLE) (4). Notably, CSF anti-NR2 levels were significantly higher in ACS than in non-ACS diffuse NPSLE (anxiety disorder, cognitive dysfunction, mood disorder and psychosis) or in focal NPSLE (5). In addition, we have also demonstrated that anti-Sm antibodies are one of anti-neuronal antibodies and were significantly elevated in CSF as well as in sera from patients with ACS (6). More importantly, the elevation of anti-Sm as well as anti-NR2 in CSF from patients with ACS has been shown to result from the damage of blood- brain barriers (BBB), but not from the increased intrathecal production thereof (5, 6). However, the mechanism of the BBB breakdown in SLE remained unclear.

The expression of a variety of autoantibodies is a hallmark of SLE. It is thus possible that several autoantibodies found in sera from patients with NPSLE might be involved in the damages of BBB. The current studies were therefore designed in order to elucidate the roles of such autoantibodies in the development of the disruption of BBB in NPSLE.

Materials and methods

Patients and samples

One hundred and one patients with SLE were included in the present study. All patients fulfilled the American College of Rheumatology 1982 revised criteria for the classification of SLE (7). Of the 101 SLE patients, 69 showed diffuse NPSLE according to the 1999 ACR definition of NPSLE (1), whereas 32 patients showed neuropsychiatric manifestations other than diffuse NPSLE, including neurologic syndromes and peripheral nervous system involvement (focal NPSLE) (Table I). Nineteen of the 69 patients with diffuse NPSLE were also complicated with seizures. All the patients with NPSLE were hospitalised in Teikyo University Hospital, Kitasato University Hospital or other correlated Hospitals between 1993 and 2015. As for cognitive dysfunction, only patients with clinically apparent cognitive deficits, who needed steroid therapy with improvement, were included. Attribution of NP events, especially diffuse NPSLE, including mood disorder, were performed using CSF IL-6 as was described in previous studies (8) in addition to the exclusion of causes other than SLE. Thus, all the patients with mood disorder showed elevation of CSF IL-6. There were no patients with lupus nephritis among those with positive anti-Sm, although some patients displayed transient proteinuria which disappeared after steroid therapy. All the 101 patients gave informed consent, and the study was approved by the institutional ethics committee of Teikyo University School of Medicine and that of Kitasato University School of Medicine. CSF specimens were obtained from the patients by a lumbar puncture on the same day serum samples were obtained, when the diagnosis of NPSLE was made by neurologists and rheumatologists. These samples were kept frozen at -30ºC until they were assayed. All assays were performed without knowledge of the diagnosis or clinical presentations. Furthermore, upon entering the present study, the diagnosis of the 101 patients with NPSLE and its classification was reconfirmed by hospital case records.

Measurement of albumin and serum autoantibodies

Albumin in CSF and sera was measured by ELISA using Human Albumin ELISA Quantitation Set (Bethyl Laboratories,

Table I. Profiles of patients with neuropsychiatric systemic lupus erythematosus (NPSLE).

Diagnosis	No. of patients	Gender (male/female)	Age (mean ± SD)
Total NPSLE	**101**	12/101	39.4±14.6
Diffuse NPSLE	69	8/61	38.1±14.4
Acute confusional state	34[§]	5/34	37.8±15.5
Anxiety disorder	4		
Cognitive dysfunction	9*		
Mood disorder	12	3/35	38.5±13.5
Psychosis	10		
Focal NPSLE	32	4/32	42.2±14.9
Cerebrovascular disease	10		
Demyelinating syndrome	1		
Headache	3		
Meningitis	1		
Movement disorder	2		
Myelitis	1		
Seizure disorder	12		
Polyneuropathy	2		

[§]Three patients also presented myelitis. *Two patients also presented mood disorder.

Montgomery, Tx). BBB function was evaluated by Q albumin (CSF albumin x 1000 / serum albumin) (5, 6).

Anti-Sm levels and IgG anti-cardiolipin (CL) antibody were measured using ELISA kits, MESACUP®-3 test Sm (MBL, Nagano, Japan) and MESACUP cardiolipin test (MBL), respectively. Arbitrary unit was designated according to the manufacture's direction. Anti-NR2 levels were determined by specific ELISA using the highly purified synthetic 10 amino-acid peptide DWEYSVWLSN, conjugated to human serum albumin (HSA) as previously described (5). Anti-ribosomal P (anti-P) antibodies were determined by ELISA as previously described (9).

Statistical analysis

Differences in various parameters among various groups of NPSLE were analysed by Kruskal-Wallis test with Dunn's multiple comparison test, using GraphPad Prism 6 for Mac OS X v. 6.0b, GraphPad Software, Inc., San Diego, CA. Correlation of various antibodies with Q albumin was evaluated by multiple-linear regression test, using JMP v. 9.0.2, SAS Institute, Inc., Cary, NC.

Results

BBB function and serum autoantibodies in various groups of NPSLE

Initial experiments compared BBB function in various groups of NPSLE. As shown in Figure 1, Q albumin (CSF/serum albumin quotient x 1000) was significantly higher in ACS than in non-ACS diffuse NPSLE (anxiety disorder, cognitive dysfunction, mood disorder and psychosis) or in focal NPSLE. As for serum autoantibodies, only serum anti-Sm was significantly elevated in ACS compared with the other 2 groups of NPSLE, although serum anti-NR2 was significantly elevated in ACS compared with that in

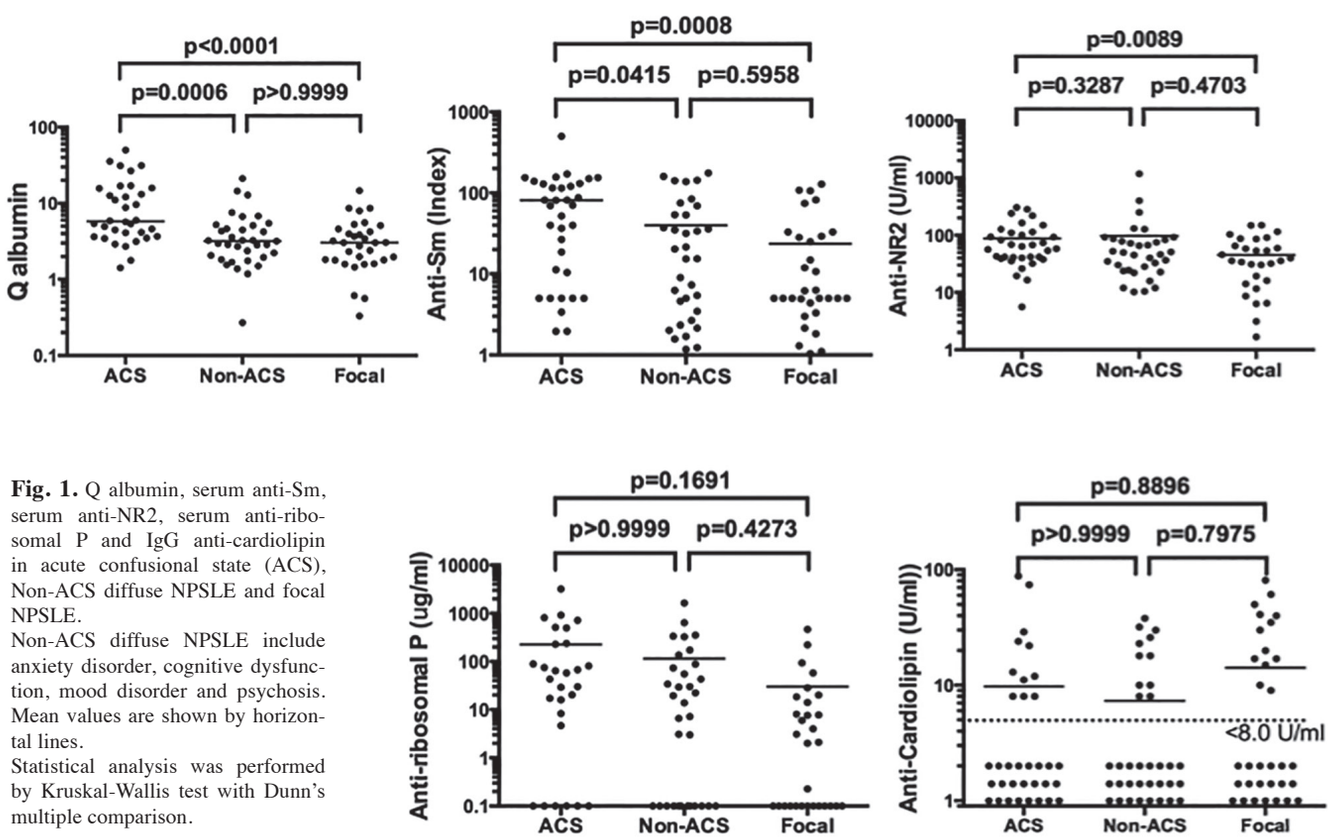

Fig. 1. Q albumin, serum anti-Sm, serum anti-NR2, serum anti-ribosomal P and IgG anti-cardiolipin in acute confusional state (ACS), Non-ACS diffuse NPSLE and focal NPSLE.

Non-ACS diffuse NPSLE include anxiety disorder, cognitive dysfunction, mood disorder and psychosis. Mean values are shown by horizontal lines.

Statistical analysis was performed by Kruskal-Wallis test with Dunn's multiple comparison.

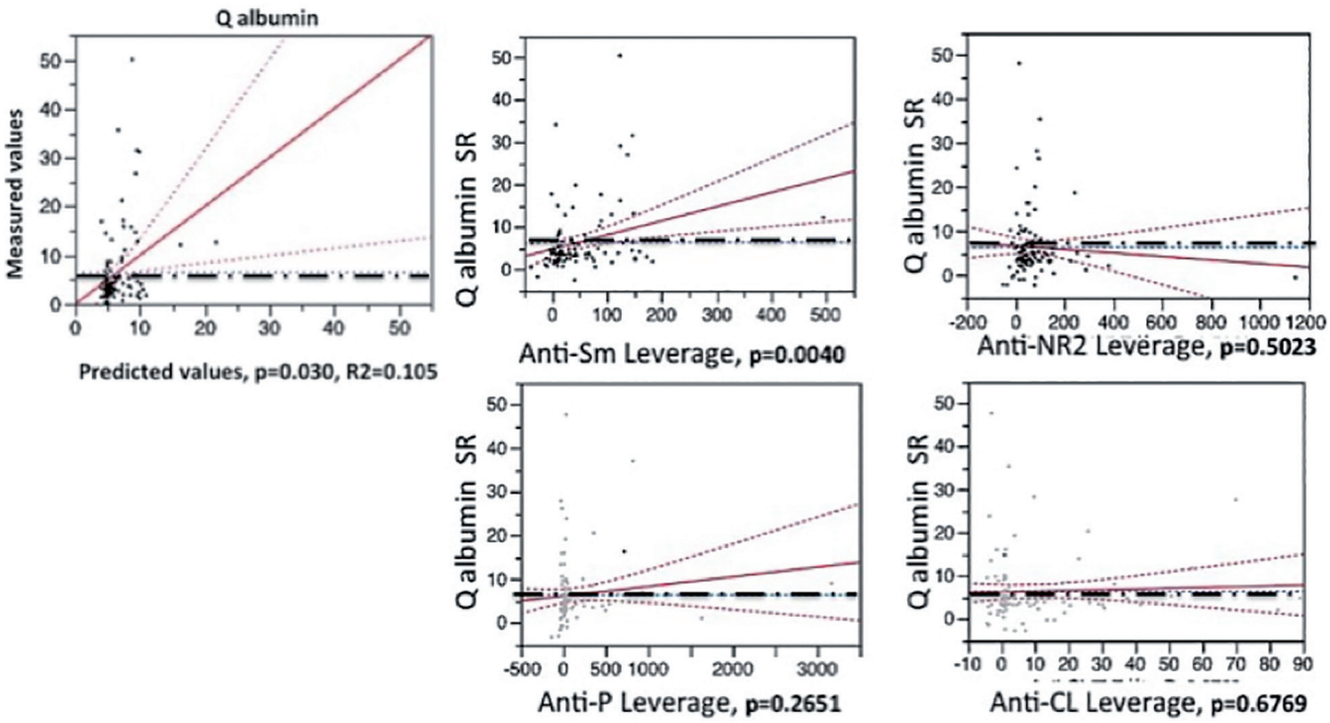

Fig. 2. Multiple-linear regression analysis on the correlation of various serum autoantibodies with blood-brain barrier (BBB) function in NPSLE. Correlation of serum anti-Sm, anti-NR2, anti-ribosomal P (anti-P) and IgG anti-cardiolipin (anti-CL) with Q albumin as examined in a model of multiple-linear regression in 101 patients with NPSLE. Left: relationship between predicted values and measured values for Q albumin in this model. Upper middle, Upper right, Lower left, Lower right: relationship of leverages of serum autoantibodies with Q albumin standardised residuals (SR), highlighting the effects of each autoantibody on Q albumin. Dotted lines indicate 95% confidence intervals for each regression line (solid lines). Horizontal alternate long and short dash lines indicate the mean value of Q albumin (6.49).

focal NPSLE (Fig. 1). The results suggest that serum anti-Sm might play a most important role in the development of ACS in NPSLE, presumably through contributing to the damages of BBB.

Multiple-linear regression analysis on the correlation of various serum autoantibodies with BBB function

In order to explore the influences of various autoantibodies on BBB function in 101 patients with NPSLE, multiple-linear regression analysis was carried out. For the analysis, we included anti-Sm, anti-NR2, anti-P and anti-CL as explanatory variables for the response variable Q albumin. In this model, a significant regression equation was found (F=2.8099, p=0.0297), with an R2 of 0.104808). As can be seen in Figure 2, the relationship of leverage of each explanatory variable with Q albumin standardised residuals indicate that serum anti-Sm (p=0.0040), but not anti-NR2 (p=0.5023), anti-P (p=0.2651) or anti-CL (p=0.6769), was a significant predictor of Q albumin. Thus, the data indicate that serum anti-Sm, but not anti-NR2, anti-P or anti-cardiolipin, plays a significant role in the damages of BBB in patients with NPSLE.

Discussion

The importance of breakdown of BBB in the development of central nervous system involvement in SLE has been appreciated in mouse (3) as well as in human (5, 6). The results in the present study have demonstrated that anti-Sm antibodies in the systemic circulation play a pivotal role in the damages of BBB in human NPSLE. Accordingly, serum anti-Sm levels were significantly higher in severer forms of NPSLE in the present study as well as in the previous studies (6, 10). In contrast with anti-RNP, anti-Sm usually decrease after successful immunosuppressive treatment including steroids (6). Moreover, it has been demonstrated that the presence of serum anti-Sm antibodies significantly increased the risk for mortality in patients with ACS of diffuse NPSLE on multivariate analysis (11). Taken together, the data indicate anti-Sm are prognostic factor for ACS in SLE.

How anti-Sm affect the BBB function is currently unknown. In this regard, anti-Sm have been shown to react with neuronal cells (6). Similarly, we have found that anti-Sm react with a human hemangiosarcoma cell line (ISOHAS) (data not shown). Since ISOHAS has characteristics of endothelial cells, it is possible that anti-Sm might also react brain endothelial cells to influence their function. Further studies are required to confirm this point.

Since both anti-NR2 and anti-P have been shown to react with endothelial cells (12, 13), they might affect the integrity of BBB. Moreover, it has been revealed that IgG fractions from patients with anti-phospholipid (aPL) syndrome and human monoclonal aPL antibody modulate the function of human umbilical vein endothelial cells to express IL-8, MCP-1 and ICAM-1 *in vitro* (14). In fact, it has been also reported that aPL antibodies are involved in the disruption of BBB in murine model (15). However, the results of multivariate

analysis in the present study failed to show any significant influence of anti-NR2, anti-P and IgG anti-CL antibodies on the damages of BBB in patients with NPSLE. It is possible that such mechanisms other than direct interactions with endothelial cells might be more profoundly involved in the damages of BBB in NPSLE. In this regard, recent studies have demonstrated that a complement split product C5a is one of the important factors that can cause BBB damages (16). Moreover, it has been disclosed that C5a alters BBB integrity in experimental lupus (17). Of note, previous studies have demonstrated the ability of anti-Sm to activate complement (18). Therefore, it is possible that anti-Sm might cause the damages of BBB through accelerated generation of C5a following complement activation.

The limitation of the present study is that the R2 value (0.104808) of our model was not enough large. Thus, anti-Sm, anti-NR2 and anti-P explained approximately 10% of the elevation of Q albumin. Since a variety of autoantibodies are expressed in SLE, it is possible that there might be such autoantibodies, other than anti-Sm, anti-NR2 anti-P and anti-CL, that might cause the damages of BBB. In this regard, recent studies have demonstrated that autoantibody to glucose-regulated protein 78 causes disruption of BBB in patients with neuromyelitis optica (19). Further studies would be interesting to explore the presence of such an autoantibody in patients with NPSLE.

Another limitation is that our study is just descriptive without any demonstration of the pathogenic role of anti-Sm on BBB. However, our data have demonstrated the positive correlation of anti-Sm, but not other autoantibodies, with BBB dysfunction (Q albumin elevation). Therefore our data warrant further investigation of the mechanism of anti-Sm to damage BBB, which is under way (almost completed) to provide novel aspects.

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