

Neuropsychiatric Lupus and Association with Cerebrospinal Fluid immunoglobulins: A Pilot Study

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Abstract

Background—Recent experimental evidence points to brain-reactive antibodies as a key factor in the pathogenesis of neuropsychiatric SLE (central nervous system-SLE1). However, clinical studies in which circulating (serum) autoantibodies were correlated with neuropsychiatric manifestations did not produce consistent findings.

Objectives—To test the hypothesis that autoantibodies in the CSF are more reflective of functional brain damage.

Methods—We compared the behavioral profiles of 12 NP-SLE2 patients, some of whom had immunoglobulin G in their CSF3.

Results—Western blotting revealed heavy and light-chain IgG bands in six patients similar in age to the subgroup of CSF IgG-free patients. A series of serological measures did not differ between the subgroups, but SLEDAI scores and daily steroid doses were higher in patients with IgG in their CSF. All three patients with severe deficits in verbal and executive functions were positive for the CSF IgG, while three other patients with psychosis were CSF IgG-negative.

Conclusions—Although the present sample size is relatively small, the results support the relationship between disease severity and central manifestations of autoimmunity. They also emphasize the importance of clinical studies that compare subpopulations of NP-SLE patients and justify development of animal models in which controlled immune mechanisms induce specific deficits in behavior.

¹SLE = systemic lupus erythematosus

²NP-SLE = neuropsychiatric SLE

³CSF = cerebrospinal fluid

Keywords

neuropsychiatric lupus; cerebrospinal fluid; immunoglobulins; brain-reactive antibodies; cognitive deficits

Diverse neurological and psychiatric manifestations are common and serious complications of systemic lupus erythematosus. They range from diffuse central nervous system disorders (such as acute confusional states, psychosis, anxiety, affective disorders, and cognitive deficits) to focal CNS⁴ syndromes (such as seizures, chorea, and myelopathy, transverse myelitis, and headaches). This behavioral and neurological heterogeneity, different onset, and fluctuating severity suggest multifactorial mechanisms, which likely overlap during the progress of SLE. Together with direct effects of autoimmune/inflammatory factors, important factors in CNS pathogenesis include the damage of peripheral tissues, opportunistic infection, and adverse effects of chronic immunosuppressive treatments. Despite a complex etiology, a significant number of clinical and experimental studies point to soluble messengers as key players in a cascade of harmful neuroimmunological events. Autoantibodies reactive with brain antigens were the focus of intensive research for several decades [1]. Current literature reviews point to the role of circulating anti-ribosomal, anticardiolipin, antiphospholipid and, more recently, anti-NR2 antibodies [2,3]. Clinical evidence, however, did not unequivocally support the relationship between antibodies in serum and psychiatric manifestations [4,5], thus leading to the possibility that intrathecally synthesized antibodies are a better predictor of disturbed brain morphology and function [6].

In particular, increased intrathecal synthesis of immunoglobulins, as revealed by an elevated IgG⁵ index and oligoclonal banding [7] and antigen-specific autoantibodies in the cerebrospinal fluid [8] seem to better correlate with CNS involvement in general (e.g., brain lesions, seizures, cranial neuropathy), and NP manifestations in particular. We presently address the above hypothesis by comparing neurological, psychiatric and immunological profiles in a cohort of NP-SLE patients who differ in the presence of IgG proteins in their CSF. The overall expectation was that protein signals in their CSF would identify behavioral variables that can distinguish two subgroups.

Patients and Methods

The study group consisted of 10 female and 2 male patients (mean age 48.6 ± 10.1 years), who fulfilled classification criteria for NP-SLE [9]. All patients underwent a detailed medical interview and routine physical examination by a qualified rheumatologist, neurologist and psychiatrist prior to inclusion in the study. Further data were obtained from patients' medical files regarding various clinical manifestations of the disease, demographic parameters, and laboratory results. Cross-sectional and Doppler color flow echocardiographic examination was performed in all patients to determine cardiac abnormalities. Pericarditis was diagnosed at disease onset. At the time of CSF evaluation none of the patients showed clinical or echocardiographic manifestations. Disease activity

⁴CSN = central nervous system

⁵Ig = immunoglobulin

was assessed according to the SLEDAI index. Laboratory investigations included a complete blood count, liver function tests, serum creatinine, and urine analysis to detect proteinuria, hematuria and cellular casts. Antinuclear antibodies were detected by indirect immunofluorescence using HEp-2 cells as substrate; anti-DNA antibodies were detected by radioimmunoassay; rheumatoid factor and total C3 and C4 levels were measured by laser nephelometry; antiphospholipid antibodies, anticardiolipin-IgG and IgM, and beta-2 glycoprotein-1-IgG and IgM were measured by enzyme-linked immunosorbent assay (Bindazyme, Binding Site, United Kingdom); and lupus anticoagulant was tested by the diluted Russel Viper Venom Test, phospholipid-sensitive partial thromboplastin time reagent and platelet neutralization procedure, as described earlier [10]. Immunosuppressive therapy in all patients consisted of the steroid drug Pronison® (prednisolone, daily maintenance dose 15 mg/kg twice a week) and chemotherapy with Endoxan® (cyclophosphamide) in bolus infusions [10]. All patients were taking aspirin (100 mg/day) and Plaquenil® (200 mg/day) during the study; none of them was receiving warfarin.

Assessment of CNS involvement

The CNS involvement in all NP-SLE patients was confirmed with digital electroencephalography (Galileo System, Italy); visual, somatosensory and acoustic evoked potentials (Medilog-Sensor, Vickers Medical, United Kingdom); electromyoneurography and magnetic resonance imaging (Gyrosan, Philips T5-NT, The Netherlands). Initial scout MRI (performed on cross-sectional, frontal and sagittal planes) was followed by the assessment of cross-sectional planes by T-1 and T-2 relaxation. The following parameters were measured: signals from cerebral and cerebellar parenchyma, ventricles, basal cistern, and subarachnoidal spaces of brain convexity.

Neuropsychological testing involved Folstein's mini-mental test, standardized Wechsler adult intelligence scale, Boston naming test, figure drawing test, speech fluency, computerized Cambridge neuropsychological test automated battery (CANTAB), finger-tapping test, and Purdue's Pegboard test. Cognitive function was assessed according to five established categories: a) General IQ, assessed from total, verbal and non-verbal IQ; b) Speech, assessed by Boston naming test and correct responses with and without semantic support; c) Attention, assessed by tests of total, verbal and non-verbal summation; d) Memory, assessed by tests of visual, spatial, and complex memory, recognition time, visual-associative memory and its time span; and e) Executive functions, which reflect "frontal" cognitive functions, such as decision making, planning, and problem solving. Standard reference values were used for comparison purposes in the assessments of IQ, speech, and executive functions. Cognitive and memory function were compared to the performance of 25 control patients who were comparable in age but showed no signs or symptoms of systemic disease.

Upon exclusion of common contraindications, CSF was obtained by lumbar puncture of the intravertebral space between the third and fourth lumbar vertebrae. Blood-free samples were used exclusively, stored at -20°C and small volumes were shipped overseas by courier service for further analysis. The CSF samples from six age-matched, healthy females were obtained from Norway and used as a negative control.

SDS-PAGE and protein detection

To assess total protein levels, CSF samples were denatured at 100°C for 4 minutes and briefly spun before loading (5 µl/lane) onto a precast 10% SDS-PAGE gel (BioRad, Mississauga, ON, Canada). After electrophoresis, gels were stained using the standard Coomassie blue method.

For the Western blotting, samples were run at 100 V for 60 min and transferred to nitrocellulose membranes (100 V for 2 hours). Resolved proteins were blocked for 1 hr with 5% skim milk prior to incubation overnight at 4°C with mouse anti-human IgG antibody (1:5,000; Chemicon, Temecula, CA, USA). Membranes were washed three times in phosphate-buffered saline and incubated for 1 hr at room temperature with a horseradish peroxidase-conjugated anti-mouse secondary antibody (Amersham Pharmacia Biotech, UK). Antigens were visualized using enhanced chemiluminescence and the signals were detected by autoradiography film exposed for 60 sec.

Statistics

The differences in frequencies and mean values between two subgroups of NP-SLE patients were analyzed by Pearson's chi-square test and Student's *t*-test, respectively. The significance level was set at $P < 0.05$; all computations were performed using the SPSS 13 statistical package.

Results

From the cohort of 12 NP-SLE patients, 6 CSF samples showed increased levels of albumin (~66 kDa region) and proteins of a lower molecular weight. As confirmed by Western blotting, distinct bands in the ~25 kDa and ~55 kDa regions pointed to light and heavy IgG chains. Such bands were not seen in the other half of the NP-SLE group or in healthy controls. No significant differences between the CSF IgG-positive and CSF IgG-negative subgroups were observed with respect to age or other demographic measures. However, CSF IgG-positive patients received significantly higher daily doses of the steroid drug Pronison® [Table 1], likely due to more severe disease manifestations, as reflected by a higher SLEDAI score [Table 2]. Interestingly, no cases with lupus anticoagulant, pericarditis or psychosis were seen in this group. Conversely, all three patients with profound dysfunctions in executive and verbal functioning showed the heavy chain IgG band in their CSF [Table 3]. No other dependent variables significantly discriminated the two subgroups of NP-SLE patients.

Discussion

The present study revealed that CNS-SLE can be accompanied by either presence or absence of IgG molecules in patients' CSF. The presence of IgGs was associated with deficits in complex memory and verbal function, more severe symptomatology, and aggressive steroid treatment. These observations are consistent with some earlier clinical studies. In particular, it was reported that NP-SLE patients with a diffuse or complex presentation have abnormal CSF IgG index/oligoclonal bands and elevated CSF antineuronal antibodies [11]. In addition, McLaurin and colleagues [12] reported a significant relationship between serum

antiphospholipid antibodies, steroid treatment, and declining cognitive function. However, due to the overlapping of therapeutic factors and IgG bands in CSF, the present results do not resolve the dilemma whether steroids or brain-reactive antibodies induce cognitive impairments. The absence of IgG bands in CSF was associated with psychosis, increased lupus anticoagulant and inflammatory manifestations (e.g., pericarditis and arthritis). This raises the possibility that disturbed perception of reality is not induced by autoantibodies but rather related to other mechanisms. Due to a relatively small sample size in the present study other important associations might be undetectable. Despite these concerns, the obtained data point to the necessity for subclassification of NP-SLE patients in search for neuropathogenic immune factors. This pilot study is our first step in designing projects with larger cohorts of NP-SLE patients free of steroid therapy, and in biochemical characterization of human CSF immunoglobulins with respect to their *in vitro* and *in vivo* pathogenicity.

In NP-SLE the blood-brain barrier can be (at least transiently) damaged and autoantibodies can be synthesized within the brain (intrathecally), as suggested by an increased immunoglobulin index [13]. It is, however, still not clear whether a subset of pathogenic brain-reactive antibodies passively diffuse from the peripheral blood or are synthesized by leukocytes which, when activated, can enter the CNS [14]. One may assume that both mechanisms are operational when the blood-brain barrier integrity is compromised by immune complex deposition and inflammation of the basal membrane [15]. The importance of a breached blood-brain barrier in the etiology of NP-SLE has been recently confirmed in an animal model where active immunization with NR2 antigen and formation of anti-NR2 receptor antibodies led to learning deficits, but only when permeability of the blood-brain barrier was increased by systemic administration of lipopolysaccharide [16].

The issue of cellular targets in the brain is of particular importance and for many years it has been proposed that brain-reactive antibodies cause neurological dysfunction by binding to CNS antigens and altering cell function [17]. A series of studies have demonstrated that brain-reactive antibodies are more frequent in the serum and CSF of NP-SLE patients [18–20]. With respect to brain antigen specificity, the CSF of a NP-SLE patient showed a subtype of anti-DNA antibodies that also reacted with an NMDA receptor (NR2). They were capable of inducing excitotoxic neuronal death, both *in vitro* and in the mouse hippocampus [21]. From these data, it was proposed that some lupus patients may have circulating anti-NMDA receptor antibodies that are capable of causing neuronal damage and memory deficits if the blood-brain barrier is breached [22]. We presently did not examine whether the CSF IgG antibodies are reactive to brain antigens, but the present association between CSF IgG and cognitive dysfunction justifies further clinical and experimental efforts in this direction.

The association between CSF IgGs and cognitive dysfunction is consistent with the findings of a recent experimental study; namely, that the detrimental effect of anti-NR2 antibodies on cognitive function can be seen exclusively when the blood-brain barrier is breached [16] and they show up in the CSF [21]. Conversely, the lack of association between autoantibodies and psychosis may indicate other mechanisms (e.g., inflammatory and/or endocrine). However, further studies with larger cohorts are required to characterize binding specificity

of CSF immunoglobulins and examine the cause-effect relationship between antibodies in CSF and aberrant behavior.

In summary, the above pilot results point to the importance of clinical studies that compare subpopulations of NP-SLE patients and justify use of lupus-prone mice [23] and experimental studies in which immune mechanisms (not confounded by therapeutic treatments) induce specific deficits in behavior [2,24,25].

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Table 1

Demographic and therapeutic profile of NP-SLE patients distinguished on the basis of IgG bands in their CSF (n = 6 patients/group)

Variable	IgG negative	IgG positive	χ^2 or <i>t</i> -test	<i>p</i>
Females	83.3%	83.3%	0	NS
Mean age (yrs)	51.3 ± 4.8	43.5 ± 4.7	1.157	NS
Smokers	33.3%	33.3%	0	NS
SLE duration (yrs)	5.5 ± 0.4	3.7 ± 1.6	1.117	NS
Cum. dose of CY (g)	11 ± 2.1	6.1 ± 2.9	1.337	NS
Pronison® (mg/day)	7.5 ± 0	16.2 ± 4	2.15	0.05

CY = cyclophosphamide

Table 2

Selected variables in NP-SLE patients distinguished on the basis of IgG bands in their CSF (n = 6 patients/group)

Variable	IgG negative	IgG positive	χ^2 or <i>t</i> -test	<i>P</i>
SLEDAI	9.3 ± 1.7	19. ± 4.0	2.264	0.047
Anti-DNA	36.1 ± 16.7	27.5 ± 17.8	0.353	NS
LA	66.7%	0%	6.0	0.014
aCL-IgG	11.1 ± 5.4	12.5 ± 9.4	0.125	NS
Arthritis	66.7%	16.7%	3.086	0.079
Pericarditis	83.3%	0%	8.571	0.003

LA = lupus anticoagulant, aCL = anticardiolipin antibodies

Table 3

Psychiatric manifestations in NP-SLE patients distinguished on the basis of IgG bands in their CSF (n = 6 patients/group)

Variable	IgG negative	IgG positive	χ^2 or <i>t</i> -test	<i>P</i>
Depression	33.3%	0%	2.4	NS
Cognit. dysfunction	0%	50%	4.0	0.046
Psychosis	50%	0%	4.0	0.046
Anxiety	33.3%	33.3%	0	NS