Increased levels of proinflammatory cytokines and nitric oxide metabolites in neuropsychiatric lupus erythematosus

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Abstract:

Objective—To investigate systemic and intrathecal production of proinflammatory cytokines in relation to cerebrospinal fluid (CSF) nitric oxide (NO) release in patients with neuropsychiatric lupus erythematosus (NPLE).

Methods—Thirty patients with NPLE rated as mild, moderate, or severe were studied and CSF was obtained from 21 of them. Cytokine mRNA expressing cells were detected by in situ hybridisation. Soluble cytokines were assessed by enzyme linked immunosorbent assay (ELISA). Nitrite and nitrate were determined by capillary electrophoresis.

Results—Patients with NPLE had high numbers of lymphocytes expressing mRNA for tumour necrosis factor a (TNFa), interferon г, and interleukin 10 in blood. The number of peripheral blood TNFa mRNA positive cells correlated strongly with the level of NO metabolites in the CSF (r²=0.69). Both the number of peripheral blood mononuclear cells expressing mRNA for TNFa as well as the CSF level of NO metabolites correlated with NPLE disease severity.

Conclusion—These data suggest that increased peripheral production of proinflammatory cytokines such as TNFa may contribute both to an increased production of NO in the central nervous system and to generation of clinical NPLE. The data also support the possibility that measurements of NO metabolites in CSF may be of value in the diagnosis of neurological symptoms related to SLE.

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Neuropsychiatric manifestations of systemic lupus are common and may affect up to two thirds of patients with systemic lupus erythematosus (SLE).1-7 There is a diversity of manifestations from both the central nervous system and the peripheral nervous system, such as fatigue, headache, cognitive dysfunction, mood/anxiety syndromes, psychoses, seizures, stroke, and neuroapathies. Thus the diagnosis of neuropsychiatric lupus erythematosus (NPLE) is difficult because it may mimic other neurological diseases. Recently the American College of Rheumatology (ACR) proposed a standardised nomenclature system for neuropsychiatric symptoms of SLE, which may facilitate further studies on NPLE.8

Explanations for neurological manifestations in SLE include damage to the nervous system mediated by autoantibodies and immune complexes.9,10 There is also evidence that vascular lesions in patients with SLE, both cerebral and others, are associated with the occurrence of antiphospholipid antibodies.9,11,12 Thus several recent studies have divided patients with NPLE into two groups, one consisting of patients with focal vascular lesions and antiphospholipid antibodies, and the other of patients with diffuse neurological symptoms without antiphospholipid antibodies.9,11,13 However, there is a significant overlap between these two groups, with an overrepresentation of antiphospholipid antibody positivity among patients with diffuse NPLE as well. Furthermore, there is evidence that antiphospholipid antibodies in NPLE may have other pathogenic roles besides being thrombogenic.14,15

Another possible pathophysiological mechanism in NPLE is related to the direct or indirect effects of inflammatory mediators on the nervous system. Proinflammatory cytokines produced both outside and inside the central nervous system are known to have dramatic effects on the nervous system, perhaps best described in infections,16 but local production of cytokines has also been shown to be of importance in primary aseptic neuroinflammatory diseases.17 Earlier studies have reported increased levels of interleukin 1 (IL1), interleukin 6 (IL6), and interferon u (IFNу) in the cerebrospinal fluid (CSF) of patients with NPLE.18-20 Although free cytokines can be measured in body fluids, the results may be deceptive because cytokines have short half lives and are often bound to circulating receptors. Studies of cellular production may better reflect the in vivo activity of a particular cytokine.20 We therefore analysed the number of mRNA expressing cells for selected cytokines in blood and CSF as well as soluble levels of cytokines in the CSF.

Nitric oxide (NO) is an important inflammatory mediator with a potential role in NPLE. It has a short half life and is usually measured by its more stable oxidation products nitrite and nitrate. We recently reported that a small group of patients with NPLE had high levels of NO metabolites in the CSF.20,21 Cytokines such as tumour necrosis factor u (TNFу) and IFNу are potent inducers of inducible nitric oxide synthase (NOS) in cerebral cell types, including human astrocytes,22 and may thus trigger the production of excessive and toxic NO levels with the level of NO metabolites.20,21 TNFу increased peripheral production of proinflammatory cytokines such as TNFα, interferon γ, and interleukin 10 in blood. The number of peripheral blood TNFα mRNA positive cells correlated strongly with the level of NO metabolites in the CSF (r²=0.69). Both the number of peripheral blood mononuclear cells expressing mRNA for TNFα as well as the CSF level of NO metabolites correlated with NPLE disease severity. The data also support the possibility that measurements of NO metabolites in CSF may be of value in the diagnosis of neurological symptoms related to SLE.

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within the central nervous system. Therefore we measured the levels of CSF NO metabolites and determined correlations between CSF NO metabolites, soluble cytokine levels, and the number of cytokine mRNA expressing cells in blood and CSF. We also investigated whether the severity of neurological symptoms in patients with NPLE corresponded with CSF NO levels or with production of proinflammatory cytokines in blood or CSF.

Patients and methods

PATIENT GROUP

During 1996 and 1997 patients with SLE at the rheumatology unit, Karolinska Hospital in Stockholm Sweden, were examined and interviewed for neurological symptoms. Thirty patients with signs of NPLE were included (25 outpatient, five inpatients). Table 1 gives the patients' characteristics. The patients were all women, aged 20-74 years (mean SD) 46 (13). All patients fulfilled four or more of the 1982 ACR revised criteria for SLE. Patients with explanations for the neuropsychiatric symptoms other than SLE or the antiphospholipid syndrome were excluded. All patients were examined at inclusion by both a rheumatologist (ES) and a neurologist (MA). The local ethics committee approved the study.

SLE disease activity was measured using the SLE Disease Activity Index (SLEDAI). To assess neurological SLE involvement, the patients' NPLE was scored at inclusion jointly by the rheumatologist and the neurologist after discussing each case as mild (patients who only reported subjective symptoms such as mild cognitive impairment but able to work, mood/
Table 3 Patients’ neuropsychological symptoms, according to 1999 ACR case definitions for neuropsychiatric lupus erythematosus. Results are given as median (range)

Table 2 Comparison between SLEDAI score and doctors’ assessment of neuropsychiatric lupus erythematosus.

SLEDAI score Mild Moderate Severe

Mild

Total SLEDAI score 8 (0–25) 12 (8–26) 18 (8–24)

Neuropsychological SLEDAI score 8 (0–8) 8 (0–16) 16 (8–24)

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Table 4 Number of mRNA expressing cells in peripheral blood in patients with neuropsychiatric lupus erythematosus (NPLE) and controls. Results are expressed as the number of mRNA expressing cells/100 000 mononuclear cells (mean range) from June 27, 2021 by guest. Protected by copyright.
washing and blocking with bovine serum albu-
min, samples and standards (recombinant
human IFNγ; Genzyme Novakemi) were incu-
bated for two hours at 37°C. Plates were
washed and incubated with biotinylated anti-
human IFNγ (Kabi Diagnostica) followed by
extra-avidin alkaline phosphatase and substrate.
The colour developed with p-nitrophenyl
phosphatase was registered at 405 nm in an
ELISA plate reader and compared with known
standards.

CSF TNFs was measured by ELISA on plates coated with monoclonal antibody to
TNFs (Endogen, MA) with recombinant human TNFs (Endogen, MA) as standard and a
second biotin labelled antihuman TNF monoclonal antibody (Endogen MA). A simi-
lar ELISA assay was used to measure levels of
IL4 and IL10, using monoclonal antibody
IL4-I (IL4-82, Mabtech), recombinant human
IL4, (Genzyme), and biotinylated monoclonal
antibody IL4-II (IL4-42, Mabtech), mono-
clonal antibody IL10 (Pharmingen), recom-
binant human IL10 (R&D), and biotin labelled antihuman IL10 monoclonal antibodies
(Pharmingen), respectively.

IL6 activity in CSF diluted 1/50 was
measured by a proliferation assay using the IL6
dependent B9 murine cell line. After dilution,
samples were inactivated by 30 minutes’
incubation at 56°C. Samples and standards
(recombinant human IL6, Genzyme) were
incubated for 72 hours at 37°C. After the addi-
tion of [3H]thymidine (Amersham) the cells
were harvested and incorporation was meas-
ured with a β counter.

ROUTINE CSF STUDIES
In CSF white blood cells were counted, the
IgG index was obtained, albumin fractions
were calculated, and isoelectric focusing was
performed to detect oligoclonal bands.23

STATISTICAL ANALYSIS
The Wilcoxon non-parametric rank sum test,
Fisher’s exact test, and linear correlation were
calculated using “JMP” software ( SAS Insti-
<0.05 was considered significant.

Results
CLINICAL AND LABORATORY DATA
Table 1 lists the clinical and laboratory data of
the 30 patients with NPLE. Twelve patients
were classified as having mild, 11 as having
moderate, and seven as having severe NPLE.
SLEDAI scores ranged from 0 to 26 with a
median SLEDAI of 13. When SLEDAI scores
were analysed separately for neuropsychiatric and non-neuropsychiatric items, it was noted
that the overall SLEDAI scores represented, to
a large extent, neuropsychiatric rather than
non-neuropsychiatric types of disease activity
(table 2). At the time of inclusion 19/30
patients were being treated with prednisone,
with doses ranging from 3.75 to 40 mg/day.
Eleven of 30 patients had detectable
anti-dsDNA antibodies and 23/30 had anti-
phospholipid antibodies (positive test for anti-
cardiolipin antibodies (IgG or IgM) or positive
lupus anticoagulant test). Six of 21 patients had
a moderately raised CSF albumin fraction,
indicating some degree of blood-brain barrier
damage. Pleocytosis of clinical significance
for each patient group compared with healthy controls. The group with mild disease di-
and 21 patients with mild (1), moderate (2), or severe (3) neuropsychiatric lupus.
p<0.004 concentration of nitric oxide oxidation products nitrite and nitrate in six healthy controls
Figure 3 Box plot showing 10th, 25th, 50th, 75th, and 90th centiles of cerebrospinal fluid
Nitrite and nitrate (µmol/l)
0 5 10 15 20 25 30
FIGURE 3 Box plot showing 10th, 25th, 50th, 75th, and 90th centiles of cerebrospinal fluid
was not present in any patient. Five patients had oligoclonal bands. The IgG index was
MRI INVESTIGATIONS
Thirteen of 28 patients had normal findings on MRI. In the two patients examined by CT one
Increased levels of IFNγ and IL10 were found in the CSF of patients with NPLE compared
with controls (figs 2A and B). Thus the IFNγ concentration in patients with NPLE was 24.7
NO METABOLITES
Patients with NPLE had increased levels of NO metabolites (the sum of the two metabolites
number of cytokine expressing cells
Patients with SLE had significantly more peripheral blood lymphocytes containing
metabolites between each of the three severity subgroups and controls (p<0.004 for all
groups). Also, the group with mild CNS disease differed significantly from the group
SOLUBLE CYTOKINE LEVELS
Increased levels of IFNγ and IL10 were found in the CSF of patients with NPLE compared
with controls (figs 2A and B). Thus the IFNγ concentration in patients with NPLE was 24.7
(4.3) U/ml, whereas controls all had values below the detection level of 16 U/ml. CSF IL10
for patients with NPLE was 84.2 (16.6) pg/ml, controls all had values below the detection level
of 60 pg/ml. CSF levels of soluble TNFα, IL4, and IL6 did not differ significantly between
patients and controls (data not shown). There were no correlations between soluble cytokine
levels and NPLE severity or SLE activity as measured with SLEDAI.
INTERFERON GAMMA EXPRESSION
Patients with SLE had significantly more mRNA for TNFα, IFNγ, and IL10 than age
and sex matched controls (table 4). There was no positive correlation between the number of
TNFα mRNA expressing cells and neurological disease severity (fig 1A) and, also, a correla-
tion of borderline significance with the neurological items of the SLEDAI (p=0.057). In
contrast there was no correlation between TNFα mRNA expressing cells and total
SLEDAI score. Furthermore, there was no relation between IFNγ and IL10 mRNA
expressing cells and disease severity (figs 1B and C). We could not detect mRNA for IFNγ,
TNFα, IL4, or IL10 in circulating CSF lymphocytes. Of note, none of these patients
had CSF pleocytosis at the time of this study.
was not present in any patient. Five patients had oligoclonal bands. The IgG index was
slightly raised in one patient. Table 3 provides a retrospective classification of the patients
according to the ACR case definitions for neuropsychiatric syndromes seen in SLE.1
Magnetic Resonance Imaging
Thirteen of 14 MRI examinations disclosed any aneurysm in this patient.
Selective cerebral angiography did not disclose any aneurysm in this patient. There was a strong positive correlation in the patients who underwent both investiga-
tions there was a strong positive correlation in the patients who underwent both investiga-
tions.
Figure 2 Box plot showing 10th, 25th, 50th, 75th, and 90th centiles for (A) soluble interferon
interleukin 10 (IL10) in the cerebrospinal fluid of 16 patients with neuropsychiatric lupus and 23 healthy controls who all had levels under the detection level (dashed line). Patients v controls: IFNγ, p<0.001; IL10, p<0.001 by Fisher’s exact

Table 1 presents the MRI findings.
Neuropsychiatric lupus erythematosus

(Received 0.69, p=0.007) between the number of TNFα mRNA expressing cells in peripheral blood and the level of NO metabolites in the CSF. Steroid dose, immunosuppressive drugs, and SLEDAI score did not correlate with the CSF NO metabolites (data not shown).

Discussion

To our knowledge this is the first study to investigate inflammatory mediators in patients with NPLE in conjunction with markers of NO metabolites. Despite the fact that none of our patients had CSF pleocytosis we found that most patients with NPLE had evidence for enhanced immunological and inflammatory activity in both the intrathecal and systemic compartments. We extend our previous report that patients with NPLE have increased levels of CSF NO oxidation products and show that these patients also have high numbers of TNFα, IFNγ, and IL10 mRNA expressing cells in the peripheral blood.

A new finding in this study is the observation that patients with NPLE not only have high levels of CSF NO metabolites and increased numbers of TNFα producing peripheral lymphocytes but also that there is a correlation between the number of TNFα mRNA expressing cells, CSF NO metabolites, and severity of NPLE symptoms, suggesting that TNFα may be of pathogenic importance in NPLE. Previous studies of the role of TNFα in the pathogenesis of SLE are conflicting. In mouse models as well as in human studies this cytokine has been assigned both an inductive and a protective role.24 There are reports of increased local production of TNFα at sites of SLE inflammatory activity, as in active lupus nephritis where mesangial cells have been shown to produce TNFα. However, in accordance with our results, circulating levels of TNFα do not differ between patients with SLE and controls in most studies.25 Otherwise, measurements of free circulating TNFα may be misleading because TNFα in the blood. We also found that patients with NPLE had high levels of intrathecal IL10. To our knowledge there are no previous reports on intrathecal IL10 in patients with SLE. Whether this is yet another manifestation of constitutively high IL10 production or a specific marker for neuropsychiatric disease in SLE needs to be considered in future studies.

Previously, Ohga et al described high levels of CSF IFNγ and TNFα in a single case of active lupus meningoencephalitis where increased numbers of IFNγ and TNFα producing cells were found using the same technique as here.26 The six patients with abnormal albumin fractions, this abnormality did not correlate with CSF cytokine levels. This finding argues against the possibility that either passive diffusion or passage over a damaged blood-brain barrier is the cause of high intrathecal cytokine levels. Thus our results raise the possibility that peripheral inflammatory mediators such as TNFα induce intrathecal inflammation.

In a few earlier investigations cytokines were reported to be present in the CSF in acute cases of cerebral SLE. Thus Hirohata and Miyamoto reported increases of IL6. Alcocer-Varela et al found increases in both IL6 and IL1 activity, and Shiozawa et al showed increased levels of IFNγ in lupus psychoses. In our patients we did not find general increases of IL6. This may be accounted for by the more chronic presentation in our patients. It may be of relevance that one of six patients with acute psychoses, did have CSF IL6 in the same increased range as that reported by other groups.

Both an increased number of peripheral blood mononuclear cells producing IL10 and high systemic levels of IL6 were found in our patients with NPLE. However, in accordance with our results, circulating levels of TNFα do not differ between patients with NPLE and controls in most studies. Otherwise, measurements of free circulating TNFα may be misleading because TNFα in the blood. We also found that patients with NPLE had high levels of intrathecal IL10. To our knowledge there are no previous reports on intrathecal IL10 in patients with SLE. Whether this is yet another manifestation of constitutively high IL10 production or a specific marker for neuropsychiatric disease in SLE needs to be considered in future studies.

Previously, Ohga et al described high levels of CSF IFNγ and TNFα in a single case of active lupus meningoencephalitis where the CSF cytokine levels declined as symptoms subsided. Al-Janadi et al have previously reported high systemic levels of IFNγ in patients with SLE, including 10 patients with severe NPLE symptoms. In this study we show that patients with NPLE have increased IFNγ activity in both peripheral blood and CSF. IFNγ can induce the synthesis of NO by astrocytes. Thus IFNγ produced intrathecally may trigger expression of iNOS and increase NO production in the central nervous system, which over time may cause toxic damage to neural tissue. A causal relation of this kind was shown in mice, where intraperitoneal lipopolysaccharide...
injection caused systemic inflammation and induced iNOS expression in the brain. Therefore, it was of interest to measure NO metabolites directly.

The level of NO metabolites in the CSF of our patients was raised and was found to correlate with neuropsychiatric disease severity. Thus CSF NO metabolites may serve as a marker for NPLE and, possibly, as a tool to evaluate treatment efficacy and monitor treatment effects. It is especially interesting to note that some patients with SLE have significantly raised levels of NO metabolites in the CSF. Previously it has been shown that patients with SLE with pulmonary symptoms have increased levels of NO in expired air. There is also a report of systemically high levels of NO metabolites in patients with SLE which correlated with disease activity. However, measurements of systemic NO levels are difficult to interpret because they are influenced by dietary factors. NO in the CSF may also have pathological significance in that some NO metabolites are extremely toxic and can cause tissue damage, which in turn may be the direct cause of diffuse symptoms in NPLE.

MRI findings in this study are in agreement with previous studies on patients with NPLE. A majority of the patients had MRI changes such as T2-hyperintense brain atrophy, which is consistent with disease activity and severity, cytokines, or CSF NO levels.

A major limitation in this study is the lack of a control group of patients with active SLE without neuropsychiatric disease. However, it should be noted that our group of patients as a whole had relatively little non-neuropsychiatric SLE activity (table 2), making it less likely that the finding of increased cytokine expression and NO metabolites could be explained by the non-neuropsychiatric disease activity. Another interesting control group would be patients without SLE but with otherwise comparable neuropsychiatric or psychiatric disease. Although the relations between disease activity, cytokine expression, and NO metabolites in this study are suggestive, they cannot be attributed to proven causality. The overall findings in this paper suggest that better understanding of the relations between cytokines, inflammatory mediators, and disease activity may have both diagnostic and therapeutic implications. Thus measurement of NO metabolites in the CSF may suggest that the production of NO may play a role in the pathogenesis and severity of neuropsychiatric symptoms.

This supports our hypothesis that inflammatory mediators are important in the pathogenesis of NPLE and that these substances are candidate targets for future treatment.

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