Kinins and Cytokines in Plasma and Cerebrospinal Fluid of Patients with Neuropsychiatric Lupus

RENATA DELLALIBERA-JOVILIANO, MARINA LEMOS DOS REIS, FERNANDO DE QUEIROZ CUNHA, and EDUARDO ANTONIO DONADI

ABSTRACT. ***Objective.*** To evaluate the kinin system components and selected cytokines in plasma and cerebrospinal fluid (CSF) of patients with neuropsychiatric lupus (NPL).

Methods. We studied 29 women with active NPL and 29 healthy women matched to patients for age. Low (LKg) and high molecular weight kininogen (HKg) and cytokine concentrations [interleukin 1ß (IL-1ß), IL-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α)] were determined by ELISA. The activities of tissue kallikrein, plasma prekallikrein, and kininase II were assayed by their action on selective substrates.

Results. Compared to controls, patients with NPL presented increased plasma and CSF levels of LKg, HKg, and prekallikrein, increased activity of tissue kallikrein and kininase II, and increased levels of IL-6, IL-10, and TNF-α ($p < 0.001$ each comparison). IL-1ß levels were increased in patient plasma ($p < 0.001$), whereas plasma IL-8 levels did not differ from controls. IL-1ß and IL-8 were not detected in CSF of patients or controls.

Conclusion. The increased levels of kininogen fractions, kallikreins, and kininase II in patient plasma and CSF indicate overactivity of the kinin system, suggesting intense kinin production. Since kinins may induce the production of proinflammatory cytokines including IL-1ß, IL-6, and TNF-α, these findings support the participation of kinins and cytokines in the acute manifestations of NPL. Most of the variables evaluated in patients' CSF increased proportionally in relation to plasma levels. In contrast, the activity of tissue kallikrein in patient CSF increased out of proportion to plasma levels, appearing to be locally synthesized in response to brain involvement. (J Rheumatol 2003;30:485–92)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS NEUROPSYCHIATRIC LUPUS
KALLIKREIN KININOGEN KININOGENASE CEREBROSPINAL FLUID

Kinins are biologically active peptides originated by limited proteolysis of a complex protein interaction. Plasma prekallikrein and tissue prokallikrein are serine proteases that, upon activation, hydrolyze the high molecular weight (HKg) and low molecular weight kininogens (LKg), yielding the short-lived peptides bradykinin (Bk) and lysil-bradykinin (Lys-Bk), which are rapidly metabolized by kininases. Kininogens are multifunctional glycoproteins produced mainly by the liver, brain, kidneys, neutrophils, and platelets, and are secreted into the circulation[1-4]. As well as being kinin precursors, kininogens may act as cofactor for the intrinsic coagulation system, downregulate platelet activity induced by thrombin, inhibit cysteine proteases, and bind to endothelial cells, impairing neutrophil adhesion[5,6]. Plasma prekallikrein is synthesized to a major extent by the liver, circulates in complex with HKg, and is activated to kallikrein by negatively charged surfaces and cysteine proteases present on endothelial cell membranes[1]. Tissue prokallikreins are synthesized by salivary glands, kidneys, brain, pancreas, and neutrophils, and the mechanisms of physiological activation of these zymogens are still unknown[1,2]. Kininases I, collectively known as carboxypeptidases M and N, remove the Arg^9 residue from both kinins, generating des-Arg^9 Bk or des-Arg^9 Lys-Bk. These metabolites are specific agonists of B_1 receptors expressed only in pathological states in response to several inflammatory stimuli. Kininase II, also known as angiotensin converting enzyme, is a dipeptidylcarboxypeptidase that inactivates Bk and Lys-Bk, the agonists of B_2 receptors. These receptors

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are constitutively expressed on many cell types, mediating most of the physiological actions of kinins[1,7].

Neuropsychiatric abnormalities in patients with systemic lupus erythematosus (SLE) may occur in the absence of generalized disease and in the virtual absence of histopathologically documentable vasculitis within the brain[8,9]. Although the pathogenesis of neuropsychiatric lupus (NPL) has not been completely elucidated, the direct and indirect effects of several inflammatory mediators on the nervous system have been emphasized as possible contributors[10,11]. Proinflammatory cytokines produced outside or inside the central nervous system induce dramatic effects on the nervous system, especially in infectious conditions, and local production of cytokines has been shown to be of importance in primary aseptic neuroinflammatory disorders[12-14]. Besides proinflammatory cytokines, antiinflammatory cytokines such as interleukin 10 (IL-10) are also produced in several inflammatory conditions[12,15]. Recent studies have reported increased levels of IL-10 and interferon-γ (IFN-γ) in cerebrospinal fluid (CSF) of patients with NPL[12]. In addition to other stimuli, kinins mediate the production of proinflammatory cytokines including IL-1, IL-2, IL-6, IL-8, and tumor necrosis factor-α (TNF-α)[16-18]. We evaluated some components of the kinin system including plasma prekallikrein levels, the activity of tissue kallikrein and kininase II, the concentrations of kininogen fractions, and the concentrations of selected cytokines including IL-1ß, IL-6, IL-8, IL-10, and TNF-α in plasma and CSF of patients with lupus presenting with acute neuropsychiatric features.

MATERIALS AND METHODS

Subjects. We studied 29 women aged 19 to 45 years presenting with NPL seen at the University Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo. The diagnosis of SLE was based on the criteria of the American College of Rheumatology (ACR)[19]. The activity of disease was determined using the SLE Disease Activity Index (SLEDAI)[20], which yielded scores ranging from 12 to 28 (median 19), including neuropsychiatric features. The diagnosis of NPL was based on the standardized nomenclature and case definition of the ACR[21]. Computed tomography neuroimaging was performed in all patients, and major cerebral findings included atrophy (51%) and infarcts (41%). Total protein concentration ranged from 74.1 to 79.0 mg/ml (median 78) in plasma, and from 0.167 to 0.195 mg/ml (median 0.190) in CSF. Albumin concentration ranged from 21.1 to 24.3 mg/ml (median 22.2) in plasma, and from 0.116 to 0.131 mg/ml (median 0.123) in CSF. The albumin index (CSF/plasma albumin levels × 100) ranged from 0.49 to 0.57 (median 0.55). CSF leukocytes ranged from 1 to 8 cells/mm^3 (median 4). Table 1 shows the major clinical and laboratory findings of patients with NPL.

Patients presenting with antiphospholipid syndrome[22] or other underlying diseases that could interfere with the kinin system such as hypertension, allergic asthma/rhinitis, or diabetes were excluded. Patients were studied during the acute phase or relapse of the neuropsychiatric disease, and before the beginning of treatment with pulse methylprednisolone or immunosuppressive drugs.

Twenty-nine healthy nonsmoking women who underwent surgery to correct stress urinary incontinence and matched the patients for age were also studied. Blood and CSF were collected at the time of epidural anesthesia just before the injection of anesthetic agents. Total protein concentration ranged from 72.8 to 77.2 mg/ml (median 74) in plasma, and from 0.155 to 0.187 mg/ml (median 0.170) in CSF. Albumin concentration ranged from 27.5 to 41.4 mg/ml (median 37.7) in plasma, and from 0.059 to 0.085 mg/ml (median 0.076) in CSF. The CSF/plasma albumin index ranged from 0.12 to 0.23 (median 0.20). Control individuals presented no underlying diseases and were not taking angiotensin converting enzyme inhibitors or any other drug that interferes with the kinin system. Controls presented no family history of autoimmune disorders.

Informed consent was obtained from all individuals, and the study protocol was approved by the Ethics Committee of the University Hospital, School of Medicine of Ribeirão Preto.

Blood and CSF collections. Blood and CSF were collected with plastic syringes using 3.8% sodium citrate as anticoagulant, centrifuged at 2000 g for 15 min at room temperature, and stored in plastic tubes at –70°C until the time for use.

High and low molecular weight kininogens. The concentrations of kininogen fractions were determined in plasma and CSF previously incubated (30 min) with kaolin (1.5 mg/ml) for LKg determination, and in plasma and CSF without kaolin treatment for total kininogen determination. Treated and untreated samples were submitted to acid denaturation and trypsin hydrolysis[23]. The immunoreactivity of the released kinin was measured by ELISA using an antibody against bradykinin (Markit M., Dainippon Pharmaceutical, Osaka, Japan)[24,25]. The concentration of HKg was indirectly measured by the difference between the values of total kininogen and LKg. The results were expressed as µg Bk equivalents/ml plasma or CSF.

Plasma prekallikrein levels. Plasma prekallikrein was activated to kallikrein and then evaluated for amidase activity on the selective chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide (S-2302, Chromogenix, Molndal, Sweden). The paranitroaniline (pNA) formed in this reaction was detected spectrophotometrically at 405 nm. The results were expressed as units (U) of kallikrein/ml plasma or CSF using purified human plasma kallikrein (Chromogenix) as standard[26]. To prove that the activity observed was that of plasma kallikrein, additional reactions were performed in the presence or absence of soybean trypsin inhibitor (Sigma, St. Louis, MO, USA).

Tissue kallikrein. Tissue kallikrein-like activity in plasma and CSF was measured by its amidase activity on the selective chromogenic substrate H-D-Val-Leu-Arg-paranitroanilide (S2266, Chromogenix)[27]. Tissue kallikrein-like activity in plasma and CSF was determined in the presence or absence of aprotinin (Sigma), an inhibitor of tissue kallikrein. The pNA formed in these reactions was detected at 405 nm by spectrophotometry. The activity of tissue kallikrein corresponded to the difference between these 2 evaluations. The ratio observed between the amount of pNA formed in the presence and absence of aprotinin was roughly 0.7 for both patients and controls, i.e., about 70% of the observed amidase activity was due to tissue kallikrein. The results were expressed as µmol pNA/ml plasma or CSF.

Kininase II. Kininase II activity in plasma and CSF was determined using the selective substrate Hippuryl-His-Leu (Sigma) using a fluorimetric assay[28]. The results were expressed as µmol His-Leu/ml plasma or nmol His-Leu/ml CSF.

Cytokines. The concentrations of IL-1ß, IL-6, IL-8, IL-10, and TNF-α in plasma and CSF were determined by double-ligand ELISA[29,30]. Briefly, flat bottom 96 well microtiter plates were coated with 100 µl/well of specific antibody (Pharmingen, St. Louis, MO, USA) to one of the above cytokines at a concentration of 2 µg/ml (TNF-α, IL-1ß, IL-6, IL-8) or 1 µg/ml (IL-10) of coating buffer, and incubated overnight at 4°C. The plates were washed with appropriate buffer and incubated 120 min at 37°C with buffer containing 1% bovine serum to prevent nonspecific binding. Standard curves were performed using recombinant human IL-1ß, IL-6, IL-8, IL-10, and TNF-α (Pharmingen). Samples and standards were loaded into wells and incubated overnight at 4°C. The plates were thoroughly washed and the appropriate biotinylated polyclonal or monoclonal anticytokine antibody

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Table 1. Patients' neuropsychological symptoms classified according to the 1999 ACR case definitions for NPL. Total SLEDAI including nervous system manifestations, computed tomography neuroimaging, cerebrospinal fluid (CSF) protein/albumin levels, and CSF leukocyte counts are shown.

Patient	Age, yrs	Neuropsychiatric Syndromes	SLEDAI	Neuroimaging	CSF Protein Albumin*, mg/100 ml	CSF Leukocytes, cells/mm^3
1	19	Psychosis, seizures	19	Cerebral atrophy	14 (10.8)	6 (100% mononuclear cells)
2	25	Psychosis, cognitive dysfunction**	27	Left occipital lobe infarct	16	5 (100% mono)
3	35	Cerebrovascular disease, psychosis, cognitive dysfunction**	28	Cortical infarcts in R and L insulae	19 (11.0)	8 (100% mono)
4	42	Cranial neuropathy, headache	19	Cerebral atrophy	23 (13.2)	3 (100% mono)
5	19	Cranial neuropathy, psychosis	24	Parietal lobe infarct	19 (12.0)	6 (100% mono)
6	21	Psychosis	25	Cerebral atrophy	16	7 (100% mono)
7	27	Psychosis, cognitive dysfunction**	24	Cerebral atrophy	18	6 (100% mono)
8	33	Acute confusional state, psychosis, seizures	26	Cerebral atrophy	20 (11.2)	4 (100% mono)
9	35	Cognitive dysfunction**, seizures	23	Left parietal lobe infarct	21	2 (100% mono)
10	40	Headache, cerebrovascular disease	23	Cerebral atrophy, lacunar infarcts	23 (12.5)	3 (100% mono)
11	19	Psychosis	22	Cerebral atrophy	25 (14.5)	5 (100% mono)
12	19	Cognitive dysfunction**, psychosis, seizures	19	Cerebral atrophy, cerebral calcifications	19	4 (100% mono)
13	25	Psychosis, cognitive dysfunction**	16	Cerebral atrophy	20 (13.1)	5 (100% mono)
14	27	Psychosis, cognitive dysfunction**	20	Cerebral atrophy	17 (12.1)	4 (100% mono)
15	32	Psychosis, cognitive dysfunction**, seizures	28	Normal	19	1 (100% mono)
16	36	Cognitive dysfunction**, movement disorder	22	Cerebral atrophy, brain abscess	21 (14.0)	7 (95% mono, 5% neutrophils)
17	40	Psychosis, seizures	12	Right occipital lobe infarct	14	4 (100% mono)
18	45	Cognitive dysfunction**, movement disorder	23	Right parietal lobe infarct	18 (13.0)	2 (100% mono)
19	42	Movement disorder, psychosis	24	Cerebral atrophy	20	3 (100% mono)
20	36	Psychosis, seizures	23	Left occipital lobe infarct	17 (12.1)	4 (100% mono)
21	34	Myelopathy, cerebrovascular disease	16	Cerebral atrophy, lacunar infarcts	19 (11.0)	3 (100% mono)
22	28	Acute confusional state, seizures	26	Normal	20	2 (100% mono)
23	29	Psychosis, seizures	20	Right parietal lobe infarct	19	7 (100% mono)
24	30	Movement disorder, cognitive dysfunction**	18	Cerebral atrophy	20	2 (90% mono)
25	35	Cognitive dysfunction**, seizures	16	Left parietal lobe infarct	21	3 (100% mono)
26	39	Acute confusional state	16	Cerebral atrophy	9	2 (100% mono)
27	30	Acute confusional state	19	Cerebral atrophy	9	5 (100% mono)
28	21	Movement disorder, psychosis	18	Right parietal lobe infarct	16	4 (100% mono)
29	25	Psychosis, seizures	20	Normal	14	2(100% mono)

*Albumin: albumin CSF levels were available for only 13 patients and 13 controls. **Neuropsychometric tests to evaluate cognitive dysfunction included the Digit Span (Forward), the Wechsler Adult Intelligence Scale (WAIS-III), and the Wechsler Memory Scale, in compliance with the ACR criteria[21].

(Pharmingen) was added. After 1 h, the plates were washed 5 times with appropriate buffer followed by the addition of avidin-peroxidase (Sigma) diluted 1:5000. The plates were then incubated for 15 min and thoroughly washed again. Finally, the chromogenic substrate O-phenylenediamine (OPD; Dako, Rostrup, Denmark) (0.4 mg OPD plus 0.4 µl of H_2O_2 for 1 ml of substrate buffer) was added, and the reaction was stopped 15 min later with 1 M H_2SO_4. The intensity of the color developed was measured spectrophotometrically at 490 nm using an ELISA plate scanner (Spectra Max 250, Molecular Devices Corp., Sunnyvale, CA, USA). The results were expressed as pg cytokine/ml plasma or CSF.

Statistical analysis. Data were analyzed by the nonparametric Mann-Whitney test. Differences were considered to be significant at $p < 0.05$.

RESULTS

High and low molecular weight kininogens. Figure 1 shows HKg and LKg concentrations in plasma and CSF of patients with NPL and controls. HKg levels in patient plasma (median 3.06) and CSF (median 0.05) were significantly increased compared to control plasma (median 0.5) or control CSF (median 0.01), yielding p values < 0.001 for each comparison. HKg levels observed in CSF represented 1.6% of patient plasma levels and 2.0% of control plasma levels, as calculated by the respective median values in CSF and plasma.

LKg levels in patient plasma (median 4.20) and CSF (median 0.08) were significantly increased compared to control plasma (median 0.71) and control CSF (median 0.02) ($p < 0.0001$ for each comparison). LKg levels observed in CSF represented 1.9% of patient plasma levels and 2.8% of control plasma levels.

Plasma prekallikrein concentrations. The levels of prekallikrein were significantly increased in plasma (median

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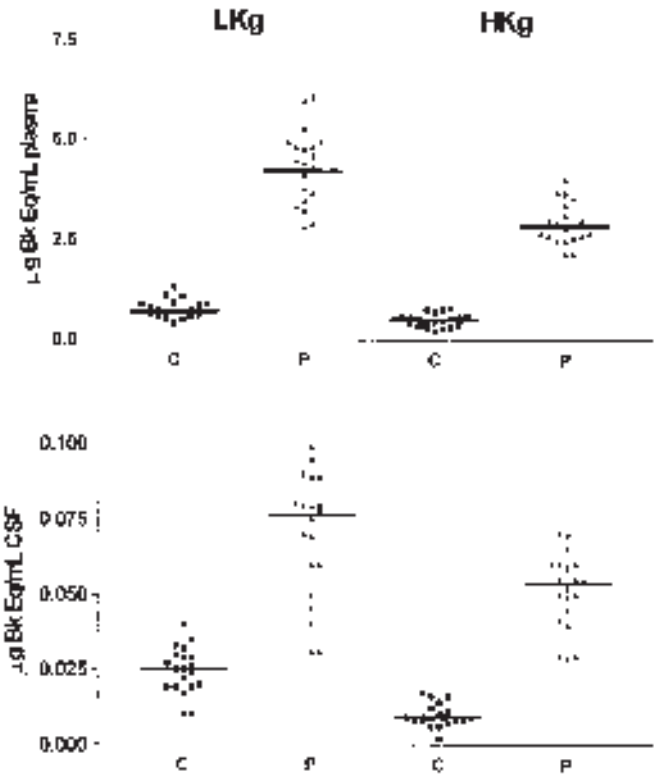

Figure 1. Low (LKg) and high molecular weight kininogen (HKg) concentrations in plasma and CSF of patients (P) with neuropsychiatric lupus and controls (C), expressed as µg bradykinin equivalents/ml plasma or CSF. LKg and HKg concentrations in plasma and CSF of patients were significantly increased compared to respective control levels ($p < 0.001$ for each comparison). LKg levels in CSF corresponded to 1.9% of patient plasma levels and 2.8% of control plasma levels. HKg levels in CSF corresponded to 1.6% of patient plasma levels and 2.0% of control plasma levels. Horizontal bars indicate median values.

4.3) and CSF (median 0.12) of patients compared to control plasma (median 1.86) or control CSF (median 0.03), with $p < 0.0001$ for each comparison. Plasma prekallikrein levels observed in CSF represented 2.8% of plasma levels for patients, and 1.6% of plasma levels for controls. Figure 2 illustrates these results.

Tissue kallikrein. The tissue kallikrein-like activity in plasma (median 2.19) or CSF (median 0.15) of patients was significantly increased compared to that in control plasma (median 1.81) or control CSF (median 0.03; $p < 0.0001$ for each comparison). The tissue kallikrein-like activity observed in CSF represented 6.8% of that seen in patient plasma and 1.6% of that in controls, i.e., the activity observed in patient CSF was roughly 4 times higher compared to controls. Figure 2 shows these results.

Kininase II. Kininase II activity in patient plasma (median 1.89) or CSF (median 0.61) was significantly increased compared to control plasma (median 0.52) or control CSF (median 0.18), with $p < 0.0001$ for each comparison. Kininase II activity observed in CSF (nmol His-Leu/ml) represented 0.03% of that seen in plasma (mmol His-Leu/ml) for both patients and controls. Figure 3 illustrates these results.

Cytokines. IL-1ß, IL-6, IL-10, and TNF-α concentrations in patient plasma were significantly increased compared to controls ($p < 0.0001$ for each comparison). In contrast, patient plasma IL-8 levels were not significantly different from controls. At the CSF level, increased concentrations of IL-6, IL-10, and TNF-α were observed in patients compared to controls ($p < 0.0001$ for each comparison). IL-6 and IL-10 levels in CSF represented roughly 8% of those detected in patient plasma and about 6% of those in controls. TNF-α levels in CSF represented 12% of those detected in plasma for both patients and controls.

IL-1ß and IL-8 concentrations in CSF of patients or controls were below the sensitivity of the ELISA used here (0.468 to 2.32 for TNF-α, 0.238 to 8.86 for IL-1ß, 0.313 to 3.0 for IL-6, 0.211 to 1.0 for IL-8, and 0.027 to 1.0 for IL-10) (Figure 4).

DISCUSSION

Among the myriad mediators involved in inflammation, kinins actively participate in almost all phases of the inflammatory response. The kinin system may interact with several other systems including complement, coagulation, fibrinolysis, prostaglandin, nitric oxide (NO), and the cytokine network. Since increased concentrations of proinflammatory cytokines have been reported to occur in the active phase of SLE[12], and since the production of proinflammatory cytokines has been reported to be induced by kinins[31], we evaluated several components of the kinin system and several cytokines simultaneously in plasma and CSF of SLE patients presenting with active neuropsychiatric features.

Compared to controls, the NPL patients of this series exhibited increased concentrations of kininogen fractions and plasma prekallikrein, and increased tissue kallikrein-like and kininase II activities in plasma and CSF, signaling the production of kinins at the systemic and CSF levels. Although patients presented increased absolute plasma and CSF values for most of the kinin components studied here, the relative values (median values expressed as percentage in relation to plasma levels) for kininogens, plasma prekallikrein, and kininase II were closely similar for patients and controls, indicating that CSF values in patients increased proportionally in relation to those seen in plasma. Increased levels of CSF proteins may occur by several mechanisms, including passive transcellular diffusion, specialized membrane transportation (carrier mediated membrane transport and pinocytosis), changes of blood–brain barrier permeability, and others[32]. In addition, proteins may be synthesized *de novo* in CSF. Although few data regarding the participation of all these mechanisms in the pathogenesis of active NPL are available, an increased permeability of the blood–brain barrier has been suggested

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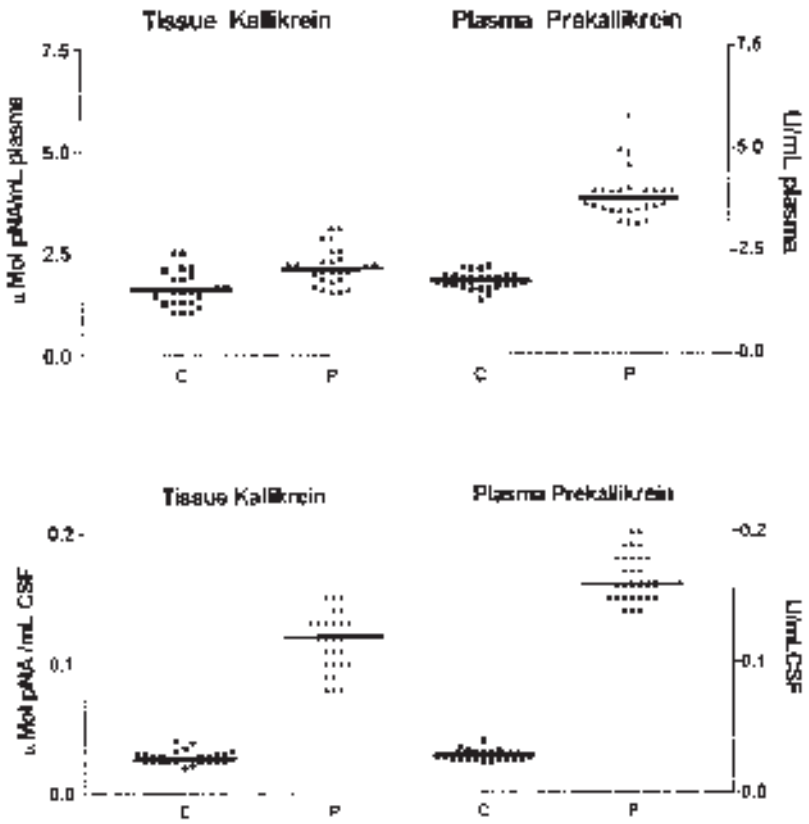

Figure 2. Plasma prekallikrein levels (Units/ml) and tissue kallikrein-like activity (µmol paranitroaniline-pNA/ml) in plasma and CSF of patients (P) with neuropsychiatric lupus and controls (C). Concentrations of plasma prekallikrein and activity of tissue kallikreins observed in plasma and CSF of patients were significantly increased compared to respective control enzymatic activity ($p < 0.001$ for each comparison). Plasma prekallikrein levels in CSF corresponded to 2.8% of patient plasma levels and 1.6% of control levels. In contrast, tissue kallikrein activity in CSF corresponded to 6.8% of patient plasma activity and 1.6% of control plasma activity. Horizontal bars indicate median values.

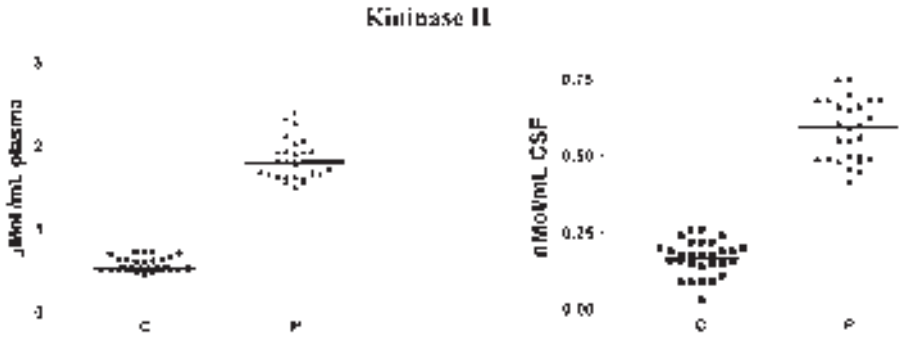

Figure 3. Kininase II activity in plasma and CSF of patients (P) with neuropsychiatric lupus and controls (C), expressed as µmol Hys-Leu/ml for plasma and as nmol Hys-Leu/ml for CSF. Activity of kininase II in plasma and CSF of patients was significantly increased compared to the respective control enzymatic activity ($p < 0.001$ for each comparison). For patients and controls, the activity of kininase II observed in CSF corresponded to 0.03% of the respective activity observed in plasma. Horizontal bars indicate median values.

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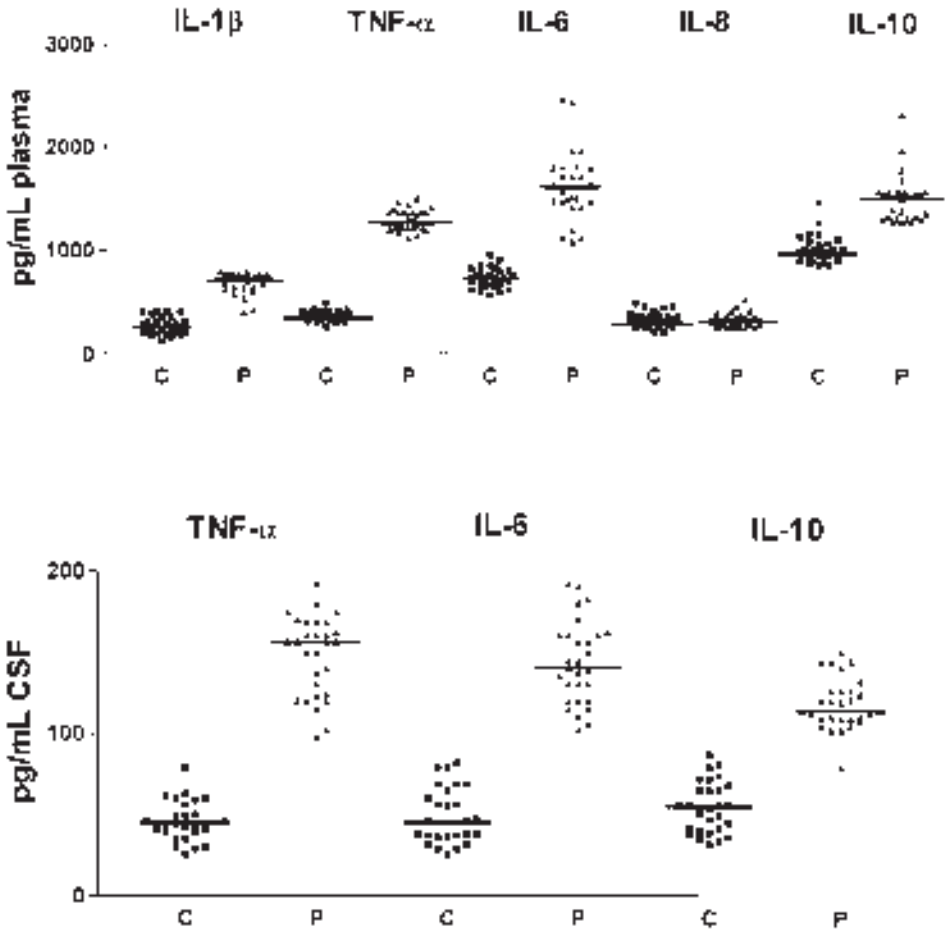

Figure 4. Cytokine levels in plasma and CSF of neuropsychiatric lupus patients (P) and controls (C), expressed as pg/ml plasma or CSF. Concentrations of IL-6, IL-10, and TNF-α in plasma and CSF of patients were significantly increased in relation to the respective control levels ($p < 0.001$ for each comparison). Higher levels of these cytokines detected in CSF were proportional to respective plasma levels. Patient IL-1ß levels were increased in relation to controls only in plasma ($p < 0.001$). Patient plasma IL-8 levels were not significantly different from controls. IL-1ß and IL-8 levels in CSF of patients and controls were below the sensitivity of the assay. Horizontal bars indicate median values.

to occur in patients with NPL[33]. Increased permeability of the blood–brain barrier has been associated with an increased CSF/plasma albumin index[34], and this ratio was higher in the NPL patients of our series compared to controls, indicating an alteration in the permeability of the blood–brain barrier. In addition, our results suggest that kinins may be one of the inflammatory mediators involved in changes of blood–brain barrier permeability, since bradykinin acting on B_2 receptors has been shown to increase this permeability[35].

Contrasting with other kinin system components evaluated in this study, the activity of tissue kallikrein in CSF of NPL patients increased in terms of absolute and relative values, suggesting that tissue kallikrein is also produced or activated or both in the central nervous system. Indeed, tissue kallikrein is synthesized in neurons of the hypothalamus, thalamus, cerebral gray matter, and reticular areas of the brain stem, as well as in cells of the anterior pituitary and epithelial cells of the choroid plexus and cerebellum[1,2,36]. Inflammatory changes in these areas may induce a synthesis *de novo* or activation of CSF prokallikrein. Additional tissue kallikrein detected in CSF of NPL patients may also originate from plasma due to increased permeability of the blood–brain barrier as well as to other specialized processes, as observed for the other kinin system components studied here.

In a study conducted on 30 patients with SLE presenting with active nephritis and no neuropsychiatric manifestations, we also observed significantly increased concentrations of plasma kininogens and plasma prekallikrein, and increased activity of tissue kallikrein and of kininase II in plasma. In addition, the activity of tissue kallikrein and kininase II observed in the urine of these patients was roughly 7 times higher than the respective enzymatic activity observed

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in plasma[37], indicating increased production and activation of these enzymes in urine. On the other hand, the evaluation of kinin system components in the urine of patients with NPL presenting no renal involvement disclosed no alterations of kallikrein or kininase II in urine (unpublished observations). In addition, lupus patients presenting with active dermatological lesions and no renal involvement presented systemic changes of the kinin system components, but no alterations of kallikreins or kininases in urine (unpublished observations). Taken together, these data indicate that several changes in the kinin system are shared at the systemic level in the different forms of SLE presentation; however, peculiar local changes occur when a specific organ or tissue is involved. Overall, the kinin system abnormalities seen in SLE patients may facilitate immune complex deposition. Particularly in NPL, the changes of the kinin system at the systemic and CSF level may also facilitate the arrival of autoreactive antibodies to the CSF and their spreading to brain structures.

Although the mechanisms of kinin system activation in CSF have not been defined, kinins may act directly on the inflammatory process or indirectly through the activation of other mediators. In inflammatory diseases, immunological and inflammatory mediators are strongly implicated in the disruption of the blood–brain barrier, primarily through the secretion of cytokines that stimulate the proliferation or metabolic activity of the barrier components[38]. We observed that plasma IL-1ß, IL-6, IL-10, and TNF-α levels were significantly increased in NPL patients compared to controls, and IL-6, IL-10, and TNF-α levels increased proportionally in CSF in relation to plasma levels. It has been described that peripheral cytokines may stimulate cytokine synthesis within the brain, and several genes encoding for cytokines and their receptors, initially identified in the peripheral immune system, are also constitutively expressed in the brain[39].

In a recent study, Svenungsson, *et al*[12] reported increased concentrations of IFN-γ and IL-10 in the CSF of lupus patients presenting with active neuropsychiatric manifestations. In addition, these authors reported other abnormalities in NPL patients, including: (1) increased number of lymphocytes expressing mRNA for TNF-α, IFN-γ, and IL-10 in peripheral blood; (2) strong correlation between concentrations of NO metabolites and the number of peripheral lymphocytes bearing mRNA for TNF-α; and (3) correlation between NO metabolites at the CSF level and the severity of NPL manifestations[12]. Bradykinin has been reported to produce NO from endothelial cells by interacting with B_2 receptors[40,41], and the finding of increased NO metabolites in the CSF of NPL patients[12] also supports the idea of kinin formation in active lupus neuropsychiatric disease, as emphasized in our study.

Kinins have been reported to induce the production of IL-1ß, IL-2, IL-6, and TNF-α[31], and increased plasma concentrations of these cytokines have been found in association with the active phase of SLE, as reported here and in other studies[12,17,42-44]. Peripheral cytokines may exert their effects on the brain by at least 3 reported mechanisms: (1) by acting on peripheral tissues innervated by the peripheral or autonomic nervous system, which can send direct signals to the brain; (2) by inducing the production of brain cytokines after crossing the blood–brain barrier; and (3) by acting on the brain vasculature through second messengers such as NO and prostanoids[39]. We describe several lines of evidence suggesting the participation of plasma and CSF kinins and cytokines in the pathogenesis of active NPL manifestations.

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