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INCREASED LEVELS OF TUMOR NECROSIS ALPHA AND SOLUBLE VASCULAR ENDOTHEL ADHESION MOLECULE-1 IN THE CEREBROSPINAL FLUID OF PATIENTS WITH CONNECTIVE TISSUE DISEASES AND MULTIPLE SCLEROSIS*

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The aim of the present study was to investigate the serum and cerebrospinal fluid (CSF) concentrations of tumor necrosis factor alpha (TNF-alpha) and soluble vascular cell adhesion molecule-1 (sVCAM-1) in patients with primary progressive form of multiple sclerosis (MS) and in patients with connective tissue diseases (CTDs) complicated with central nervous system (CNS) involvement. Stimulation of sVCAM-1 release by TNF-alpha was demonstrated on endothelial cells of brain vessels. We intended to present the TNF-alpha stimulated elevation of sVCAM-1 in the serum and CSF in any cases of CNS lesion. Fifty patients with several CTDs complicated with neuropsychiatric symptoms and 25 MS patients with primary chronic progressive form of the disease were selected. Determinations of TNF-alpha and sVCAM-1 were performed using ELISA methods. TNF-alpha and sVCAM-1 concentrations were elevated in the CSF of all patients, intrathecal synthesis of sVCAM-1 was demonstrated in MS patients. The changes in the TNF-alpha and sVCAM-1 concentrations were independent from the clinical manifestations, immunoserological changes and quality of neuropsychiatric symptoms of the CTDs. The stimulatory effect of TNF-alpha was more pronounced in the CSF of MS patients.

Keywords: TNF-alpha, sVCAM-1, CSF, MS, CTDs

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Introduction

Connective tissue diseases (CTDs) are frequently complicated with involvement of the central nervous system (CNS). Several symptoms of CNS lesions are present in as many as 70% of systemic lupus erythematosus (SLE) [1], 20% of primary Sjogrens syndrome (PSS) [2], and 10% of rheumatoid arthritis (RA) [3] patients. Coexistence of systemic autoimmune diseases and immune/autoimmune diseases of the nervous system was described [4, 5, 6, 7]. In several cases a common genetic background was predisposed [4, 6, 8]. Immunoserological tests are frequently positive in autoimmune diseases of nervous system [9]. Concentrations in body fluids of immunoglobulines [10], cytokines [11] and soluble adhesion molecules [12] showed some similarities in systemic and organ specific diseases. The pathomechanism of the involvement of CNS is not clarified. In any case, blood–brain barrier damage is necessary early in the development of lesions in the CNS because it permits entry of cellular and soluble mediators with pathogenic potential into the CNS. TNF-alpha seems to stimulate the release of sVCAM-1 from endothelial cell surface [13]. Positive correlation between the serum and cerebrospinal fluid (CSF) concentrations of TNF-alpha and adhesion molecules was described in multiple sclerosis (MS) [14]. SLE patients with cerebrovascular complications were characterized with elevated levels of serum TNF-alpha and sVCAM-1 [15]. The aim of the present study was to investigate the serum and CSF concentrations of TNF-alpha and sVCAM-1 in patients with primary progressive form of the MS and in patients with CTDs complicated with CNS involvement. We demonstrated the presence of the TNF-alpha stimulated elevation of sVCAM-1 in the serum and CSF in any cases of CNS lesion.

Materials and methods

Twenty five female patients with primary chronic progressive form of clinically defined MS, according to Poser criteria [16] and 50 female patients with several forms of CTDs were selected. Diagnostic criteria of SLE [17], primary Sjogrens syndrome [18] or rheumatoid arthritis (RA) were not completely present. 25 patients were listed to SLE, 15 patients showed several symptoms characteristic for primary Sjogren syndrome and in 10 patients symptoms of joint lesions dominated. Patients presented several clinical symptoms, and immunoserological abnormalities. Clear-cut nosological classification was, however, impossible during a two-year observation period. Different symptoms of CNS lesions were presented

[1]. Twenty five female patients with degenerative disease of spinal column served as a control group. The study was conducted in compliance with the guidelines set out in the 1966 Declaration of Helsinki. Basic clinical and laboratory characteristics are presented in Table I. Patients with serious side effects of corticosteroide treatment, hypertony, nephrosis syndrome, infections or presence of antiphospholipide antibodies were excluded.

Magnetic resonance imaging (MRI) investigations were performed several days before the lumbar puncture. MRI scans of MS patients showed multifocal white matter lesions and some newly formed lesions with Gadolinium enhancement. Ten patients with autoimmune diseases had no pathology on the brain MRI scans. Lesions of the spinal cord were presented. Nine of the CTD patients had symptoms of slight brain edema. One patient was characterized by a large ischemic lesion, without perifocal edema. All other patients had small white matter lesion with or without Gadolinium enhancement.

Intrathecal synthesis of IgG was presented in 92% of MS and 96% of CTD patients. Oligoclonal IgG bands were presented in all MS and in 30% of CTD patients. Neither intrathecal synthesis of immunoglobulines, nor oligoclonal IgG bands were demonstrated in the control patients (Table I).

Table I

Clinical and laboratory characteristics of the patients groups

		Control (25)	MS (25)	CTD (50)
Age [years]		38±4.5	39±1.5	43±3.5
Duration of illness [years]			4.52±0.7	6.8±4.3
Duration of neuropsychiatric symptoms [years]				1.8±0.5
Therapy			azathioprine	low doses of steroid 20, cytostatics 10, combined 20
Intrathecal IgG synthesis			23/25	48/50
ESR			0/25	50/50
Anti-dsDNA			1/25	40/50
Anti-SSA/Ro			1/25	5/50
Anti-SSB/La			0/25	8/50
Rheuma factor			0/50	14/50
Neuropsychiatric symptoms				
	cranial nerves		25/25	8/50
	brainstem		20/25	12/50
	pyramidal		10/25	25/50
	cerebellar		5/25	3/50
	spinal cord		15/25	10/50
	psychiatric disturbances		15/25	40/50

MS = multiple sclerosis, CTD = connective tissue disease

Concentrations of anti-dsDNA, -SSA/Ro, -SSB/La, -Sm, -Sm/RNP and anticardiolipin antibodies were determined using commercial ELISA assays.

The investigations of the CSF were performed at the time of exacerbation of the neuropsychiatric symptoms. CSF and serum samples were taken simultaneously. Lumbar puncture was performed after 24-hour bed rest in sitting position. The first 2 mls of the CSF were used for the determinations of cell count, proteins and immunoglobulines. The 3rd–5th mls of the CSF and the sera were frozen at –70 °C. Concentrations of TNF-alpha and sVCAM-1 were determined using an ELISA assay of R&D systems for quantitative determination of TNF-alpha and of sVCAM-1 in cell culture, supernate, serum and plasma. CSF samples were examined without dilution.

The significance of differences was tested using the t-test (2 samples assuming unequal variances) and the Wilcoxon's test. Correlation between variables was analyzed using Spearman rank coefficient, and the Pearson correlation. Intrathecal synthesis of the TNF-alpha and sVCAM-1 was calculated using the formula serum albumin/CSF albumin × CSF TNF-alpha (sVCAM-1)/serum TNF-alpha (sVCAM-1) [19].

Results

The age and the duration of illness did not differ between the patient groups investigated. In 23% of CTD patients the neuropsychiatric symptoms developed relatively early (within less than 1 year) after the beginning of the autoimmune disease. Intrathecal IgG synthesis was presented in the majority of the MS and CTD patients. CTD patients showed elevated erythrocyte sedimentation rates (ESR). Elevated anti-dsDNA and anti-SSA/Ro antibody concentrations were present in two different MS patients and in several CTD patients. Rheuma factor was detectable only in CTD patient group (Table I). Anti-Sm, -Sm/RNP and NCA antibodies were present in 1–10% of CTD patients (data not shown).

An increased level of serum TNF-alpha relative to control and MS patients was present in patients with CTD. TNF-alpha concentrations in the CSF were elevated in both patient groups. No differences were found between the MS ad CTD groups. Intrathecal synthesis of TNF-alpha was not detectable (Table II). However, most of the patients showed elevated indices (Figure 1). Differences between the groups of CTD patients closed to several diagnostic categories were not presented. TNF-alpha concentrations in CSF and serum in patients with several neuropsychiatric symptoms were not different (data not shown). Weak correlations

Table II

TNF-alpha concentrations

TNF-alpha	CSF [pg/ml]	Se [pg/ml]	Index [<200]
Control (25)	0.37±0.03	1.68±0.19	142± 42
MS (25)	1.88±0.62*	1.54±0.33	306±118
CTD (50)	2.56±1.05*	3.48±1.22*	328±108

MS = multiple sclerosis, CTD = connective tissue disease, CSF = cerebrospinal fluid, Se = serum
Elevated serum levels of TNF-alpha were observed only in the CTD patient group. Concentrations of TNF-alpha in the CSF were elevated in patients with MS and CTD. Difference between the MS and CTD patients was observed only in serum concentrations. *$p < 0.01$

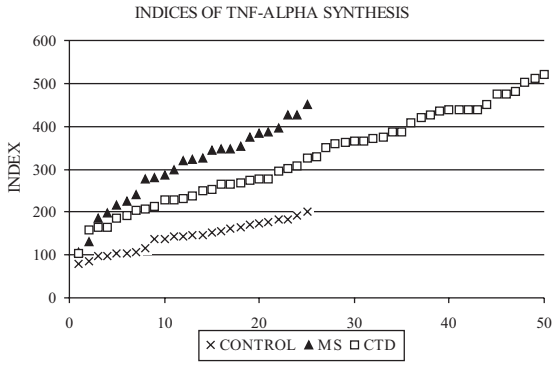

Figure 1. The number of patients is figured on the *x* axis. The values of the calculated indices are presented on the *y* axis. 76% of the MS and 84% of the CTD patients showed elevated indices – intrathecal TNF-alpha synthesis

between the CSF and serum concentrations of TNF-alpha were demonstrated in control ($r = 0.30$, $p < 0.1$), MS ($r = 0.27$, $p < 0.1$) and AI ($r = 0.25$, $p < 0.1$) patients.

Significant elevation of sVCAM-1 concentrations in serum and CSF was present in MS patients. Concentrations of sVCAM-1 were elevated in serum and CSF in CTD patients. This difference was not significant relative to the control values. Differences between the MS and CTD patients were not demonstrated. Intrathecal synthesis of sVCAM-1 was demonstrated in MS patients (Table III). Several patients of both groups showed elevated indices (Figure 2). sVCAM-1 concentrations did not differ in CTD patients closed to several nosological categories or with different neuropsychiatric symptoms (data not shown). CTD group of patients ($r = 0.28$, $p < 0.1$) and control patients ($r = 0.25$, $p < 0.1$) showed weak correlations between the CSF and serum concentrations of sVCAM-1. Correlation between the CSF and serum concentrations of sVCAM-1 in MS patient groups was not detected ($r = 0.06$, NS.).

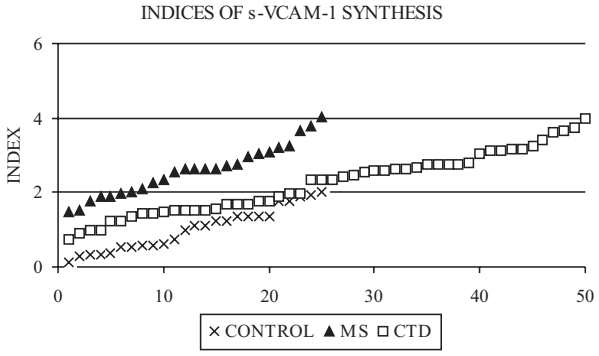

Figure 2. The number of patients is figured on the *x* axis. The values of the calculated indices are presented on the *y* axis. 72% of the MS and 50% of the CTD patients showed elevated indices – intrathecal sVCAM-1 synthesis

Table III

sVCAM-1 concentrations

sVCAM-1	CSF [ng/ml]	Se [ng/ml]	Index [<2]
Control (25)	2.26±0.26	250±84	1.02±0.30
MS (25)	5.80±1.63*	784±56*	2.59±0.10*
CTD (50)	3.81±1.10	783±234	2.26±0.83

MS = multiple sclerosis, CTD = connective tissue disease, CSF = cerebrospinal fluid, Se = serum
Statistically significant elevation of serum and CSF levels and intrathecal synthesis of sVCAM-1 were demonstrated in MS patients. Elevation in CSF and serum concentrations and intrathecal synthesis of sVCAM-1 were demonstrated in CTD patients. Statistical significant differences between the MS and CTD patients were not present. *$p < 0.01$

Concentrations of TNF-alpha and sVCAM-1 in the serum and in the CSF did not correlate in control patients (data not shown). Significant correlation was found between TNF-alpha and sVCAM-1 concentrations in the serum of MS ($r = 0.58$, $p < 0.01$) and CTD ($r = 0.68$, $p < 0.01$) patients. Concentrations of TNF-alpha and sVCAM-1 in the CSF did not correlate (MS: $r = 0.27$, $p < 0.1$, CTD: $r = 0.35$, $p < 0.1$).

Discussion

The aim of the study was to investigate the TNF-alpha stimulated elevation of sVCAM-1 in the CSF and serum in CTDs in cases with CNS involvement. We compared patients with autoimmune disease of the CNS (MS) and patients with a

systemic disorder. Diagnostic criteria for each nosological entities in CTDs were not taken into account, however previous studies described several differences concerning the TNF-alpha and sVCAM-1 serum concentrations [15]. Selection of patients with primary chronic form of MS was based on the immunological differences between the several clinical forms of the disease [20].

Increased levels of TNF-alpha [14, 21] and sVCAM-1 [12, 20, 22] in MS patients, at the time of exacerbation of clinical symptoms were described previously. Data confirming our findings on increased TNF-alpha and sVCAM-1 in the CSF of connective tissue diseases were not available. Increases of TNF-alpha [15, 23] and sVCAM-1 [15, 23, 24] concentrations in serum were described.

Lack of correlation between the TNF-alpha and sVCAM-1 concentrations in serum and CSF and presence of intrathecal synthesis in majority of patients suggest the possibility of an immunological process occurring in the CNS, in addition to the blood-brain barrier damage. The more pronounced elevation of TNF-alpha concentrations in sera of patients with connective tissue diseases can be explained as an ongoing immunological process first on the periphery. The moderate elevation of sVCAM-1 concentrations in the CSF of patients with systemic diseases can be a consequence of the time-dependent stimulation of the excretion of sVCAM-1 by TNF-alpha [13]. At the time of the investigation the stimulatory effect was in a progressive phase.

Correlation between the symptoms of clinical exacerbation and inflammatory markers in MS patients is widely discussed [25, 26]. The data presented suggest simultaneous elevation of TNF-alpha and sVCAM-1 concentrations in the CSF at the time of clinical impairment. An elevation of TNF-alpha concentrations in serum was not demonstrated, in contrast to previous reports [27]. The discrepancy can be explained as a consequence of chronic cytostatic treatment, suppressing the immunological processes mainly on the periphery. Another possible explanation is, that the immunological process started before the examination was performed.

The stimulation of sVCAM-1 excretion by TNF-alpha was more pronounced in the CSF of MS patients. Stimulatory role of CSF cytokines on myeloid dendritic cells was described [28]. The data presented can be explained by the dominance of T cell dependent immunological processes in the CNS. Nonetheless the direct effect of TNF-alpha on the brain endothelial cells [29] cannot be excluded.

The data presented suggest that the TNF-alpha stimulated sVCAM-1 excretion is present in the CSF in connective tissue diseases complicated with CNS involvement. The changes in the TNF-alpha and sVCAM-1 levels were independent

from the clinical symptoms and immunoserological changes in the patients with connective tissue disease. They were independent from the character of the neuropsychiatric symptoms. The stimulatory effect of TNF-alpha was more pronounced in the CSF of MS patients. Following the blood–brain barrier damage (TNF-alpha stimulated excretion of sVCAM-1) the same type of immunological processes seems to be generated in the CNS. Differences can be suspected concerning the type of predominantly activated cells and in the order of activation.

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References

1. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. ACR ad hoc committee of neuropsychiatric lupus nomenclature. Arthritis and Rheumatism **42,** 599–608 (1999).
2. Hietaharju, A., Yli Kerttula, U., Hakkinen, V., Frey, H.: Nervous system manifestations in Sjogrens syndrome. Acta Neurol Scand **81,** 144–152 (1990).
3. Nadeau, S. E.: Neurologic manifestations of connective tissue disease. Neurol Clin **20,** 151–178 (2002).
4. Lindvall, B., Bengtsson, A., Ernerudh, J., Eriksson, P.: Subclinical myositis is common in primary Sjogrens Syndrome and is not related to muscle pain. J Rheumatol **29,** 717–725 (2002).
5. Kinnunen, E., Muller, K., Keto, P., Ketonen, L., Helve, T., Sepponen, R.: Cerebrospinal fluid and MRI findings in three patients with multiple sclerosis and systemic lupus erythematosus. Acta Neurol Scand **87,** 356–360 (1993).
6. Hietaharju, A., Peltola, J., Seppa, J., Luukkainen, R., Dastidar, P.: The coexistence of systemic lupus erythematosus and multiple sclerosis in a mother and daughter. Scand J Rheumatol **30,** 120–122 (2001).
7. Iniguez, C., Mauri, J., Medrano, M., Larrode, P., Santos, S., Pina, J., Morales, F.: Sjogren's syndrome and multiple sclerosis. Neurologia **16,** 232–235 (2001) (Spain).
8. Kanellopoulos, P., Baltoyiannis, C., Tzioufas, A. G.: Primary Sjogrens syndrome associated with inclusion body myositis. Rheumatology **41,** 440–444 (2002).
9. Baraczka, K., Lakos, G., Sipka, S.: Immunoserological changes in the cerebrospinal fluid and serum in systemic lupus erythematosus patients with demyelinating syndrome and multiple sclerosis. Acta Neurol Scand **105,** 378–383 (2002).
10. Christenson, R. H., Behlmer, P., Howard, J. F. Jr., Winfield, J. B., Silverman, L. M.: Interpretation of cerebrospinal fluid protein assays in various neurologic diseases. Clin Chem **29,** 1028–1030 (1983).
11. Beebe, A. M., Cua, D. J., de Waal Malefyt, R.: The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). Cytokine Growth Factor Rev **13,** 403–412 (2002).

12. Baraczka, K., Nekam, K., Pozsonyi, T., Jakab, L., Szongoth, M., Sesztak, M.: Concentrations of soluble adhesion molecules (sVCAM-1, sICAM-1 and sLselectin) in the cerebrospinal fluid and serum of patients with multiple sclerosis and systemic lupus erythematosus with central nervous system involvement. Neuroimmunomodulation **9,** 49–54 (2001).
13. Hummel, V., Kalmann, B. A., Wagner, S., Fuller, T., Bayas, A., Tonn, J. C., Benveniste, E. N., Toyka, K. V., Rieckmann, P.: Production of MMPs in human cerebral endothelial cells and their role in shedding adhesion molecules. J Neuropathol Exp Neurol **60,** 320–327 (2001).
14. Sharief, M. K., Noori, M. A., Ciardi, M., Cirelli, A., Thompson, E. J.: Increased levels of circulating ICAM-1 in serum and cerebrospinal fluid of patients with active multiple sclerosis. Correlation with TNF-alpha and blood-brain barrier damage. J Neuroimmunol **434,** 15–21 (1993).
15. Nagahama, M., Nomura, S., Ozaki, Y., Yoshimura, C., Kagawa, H., Fukuhara, S.: Platelet activation markers and soluble adhesion molecule in patients with systemic lupus erythematosus. Autoimmunity **33,** 85–94 (2001).
16. Poser, C. M., Brinar, V. V.: Diagnostic criteria for multiple sclerosis. Clin Neur Neurosurg **103,** 1–11 (2001).
17. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N., Winchester, R. J.: The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis and Rheumatism **25,** 1271–1277 (1982).
18. Vitali, C., Bombardieri, S., Jonsson, R., Moutsopoulos, H. M., Alexander, E. L., Carsons, S. E., Daniels, T. E., Fox, P. C., Fox, R. I., Kassan, S. S., Pillmer, S. R., Talal, N., Weisman, M. H., and the European Study Group on classification criteria for Sjogrens Syndrome. Classification criteria for Sjogrens syndrome: a revised version of the European criteria proposed by American–European Consensus group. Ann Rheumat Dis **61,** 554–558 (2002).
19. Link, H., Tibling, G.: Principles of albumin and IgG analysis in neurological disorders. Scand J Lab Invest **37,** 385–396 (1977).
20. McDonnell, G. V., McMillan, S. A., Douglas, J. P., Droogan, A. G., Hawkins, S. A.: Serum soluble adhesion molecules in multiple sclerosis: raised sVCAM-1, sICAM-1 and sE-selectin in primary progressive disease. J Neurol **246,** 87–92 (1999).
21. Sharief, M. K., Thompson, E. J.: In vivo relationship of tumor necrosis factor-alpha to blood–brain barrier damage in patients with active multiple sclerosis. J Neuroimmunol **38,** 27–33 (1992).
22. Dore-Duffy, P., Newman, W., Balabanov, R., Lisak, R. P., Mainolfi, E., Rothlein, R., Peterson, M.: Circulating, soluble adhesion proteins in cerebrospinal fluid and serum of patients with multiple sclerosis: correlation with clinical activity. Ann Neurol **37,** 55–62 (1995).
23. Gattorno, M., Vignola, S., Barbano, G., Sormani, M. P., Sabatini, F., Biocompagni, A., Picco, P., Pistoia, V.: Tumor necrosis factor induced adhesion molecule serum concentrations in Henoch–Schönlein purpura and pediatric systemic lupus erythematosus. J Rheumatol **27,** 2251–2255 (2000).
24. Neidhart, M., Pataki, F., Fehr, K.: Increased soluble endothel adhesion molecules in rheumatoid arthritis correlate with circulating cytokines and depletion of CD45Ro+ T-lymphocytes from blood stream. Schweiz Med Wochenschrift **125,** 424–428 (1995).
25. Giovannoni, G., Miller, D. H., Losseff, N. A., Sailer, M., Lewellyn-Smith, N., Thompson, A. J., Thompson, E. J.: Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. J Neurol **248,** 487–495 (2001).

26. Vladic, A., Horvat, G., Vukadin, S., Sucic, Z., Simaga, S.: Cerebrospinal fluid and serum protein levels of tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and soluble interleukin-6 receptor (sIL-6R gp80) in multiple sclerosis patients. Cytokine **20,** 86–89 (2002).
27. Sharief, M. K.: Heightened intrathecal release of soluble CD137 in patients with multiple sclerosis. Eur J Neurol **9,** 49–54 (2002).
28. Pashenkov, M., Soderstrom, M., Huang, Z. M., Link, H.: Cerebrospinal fluid affects phenotype and functions of myeloid dendritic cells. Clin Exp Immunol **128,** 379–387 (2002).
29. Frigerio, S., Gelati, M., Ciusani, E., Corsini, E., Dufour, A., Massa, G., Salmaggi, A.: Immunocompetence of human microvascular brain endothelial cells: cytokine regulation of IL-1beta, MCP-1, IL-10, sICAM-1, sVCAM-1. J Neurol **245,** 727–730 (1998).