SOLUBLE LIGANDS OF THE TUMOUR NECROSIS FACTOR SUPERFAMILY sTNF-α, sFas-L, sTRAIL AND sCD40L IN THE PATHOGENESIS OF VILIUISK ENCEPHALOMYELITIS

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Abstract. Viliuisk encephalomyelitis (VE) is a neurodegenerative disorder that afflicts the aboriginal people of Yakutia in Siberia. The disease is characterized by a progressive duration and aseptic inflammatory episodes, with intrathecal synthesis of oligoclonal IgG (OCBs) in some patients. The aim of this study was to evaluate the role of soluble ligands and receptors of the tumour necrosis factor (TNF) superfamily as potential participants in VE pathogenesis. To achieve this goal, we measured the levels of sTNF-α, sFas-L, sTRAIL, sCD40L ligands, and sCD40 receptor by ELISA in the plasma of VE patients compared with healthy individuals of the same population and patients with demyelinating diseases, including multiple sclerosis (MS) and neuromyelitis optica (NMO), as examples of disorders involving immune pathology. In addition, the same markers were analyzed in the CSF of VE patients and patients with demyelinating diseases. The results obtained showed that the increased level of plasma sTNF-α in VE patients was associated with the detection of OCBs ($p = 0.01$; two-tailed Student's t-test). The sCD40L level in plasma was significantly increased in VE patients, regardless of the presence of an inflammatory component ($p = 0.001$; Student's t-test), and their healthy relatives ($p = 0.004$; Student's t-test). Our results suggested that increased blood sCD40L levels are associated with the chronic form of VE and may participate in the predisposition to the disease. Increased blood sCD40L levels may lead to pathology of the vascular endothelium in the brain and the development of VE pathology.

Keywords: Viliuisk encephalomyelitis; TNF-α; CD40 ligand; Fas ligand; TRAIL; neurodegeneration; inflammation; CNS.

List of Abbreviations

VE – Viliuisk encephalomyelitis
MS – multiple sclerosis
NMO – neuromyelitis optica
CNS – central nervous system
BBB – blood–brain barrier
CSF – cerebrospinal fluid
OCBs – oligoclonal IgG
TNF – the tumour necrosis factor
TRAIL – TNF-related apoptosis-inducing ligand
IFN – interferon
MRI – magnetic resonance imaging
SD – standard deviation

Introduction

Viliuisk encephalomyelitis (VE) is a degenerative disease of the central nervous system (CNS) that is found only among the Yakuts (Sakha) aboriginal population in Northeast Russia (Goldfarb & Gajdusek, 1992). The main clinical features of VE are bulbar and motor disorders caused by damage to the pyramidal and extrapyramidal systems and the cerebellum and which are reflected in the Yakut popular name of the disease - bokhoror (translated as stiffness) (Goldfarb & Gajdusek, 1992; Goldfarb *et al.*, 2014). Dementia develops in most VE patients and is expressed in the forgetfulness of recent events and difficulties in reproductive memory. Neuroimaging reveals diffuse atrophy of the brain, mainly the cerebral cortex, cerebellum, and upper spinal cord (Goldfarb et al., 2014). Pathological investigations showed the significant loss of neurons in all parts of the CNS. In patients with an inflammatory episode, scattered necrosis foci with infiltration in the fibrous membrane of small vessels are added (McLean *et al.*, 1997). Only some patients experience an acute phase, which clinically manifests as aseptic encephalomyelitis and can have fatal outcomes (Goldfarb & Gajdusek, 1992; Sivtseva *et al.*, 2018). In the chronic stage, which can take a long time, often more than 20 years, the inflammatory process is significantly weakened and has a self-limiting nature

(McLean *et al*., 1997); however, intrathecal synthesis of oligoclonal IgG (OCBs) persists (Sivtseva *et al*., 2018). At present, the primary chronic form of the disease, which develops gradually with age without severe inflammation, is the most common (Goldfarb *et al*., 2014). As of 01.01.2021, 45 people with a diagnosis of chronic VE were registered in 6 districts of the Vilyui region of the Republic of Sakha (Yakutia).

The chronic form of this disease is characterized by immunosuppression (tolerance to the pathogenic brain). The peripheral blood of VE patients showed inhibition of IFN alpha production, decreased numbers of T-cells and Th2 subpopulations, predominantly CD^{4+} cells (Gankina *et al*., 1992; Fedorov & Osakovsky, 2000). The nitroblue tetrazolium dye test demonstrated low phagocytic activity of leukocytes in VE patients (Zakharova *et al*., 1995). In cerebrospinal fluid (CSF), a decreased level of inflammatory cytokines was detected compared to other groups of neurological disorders (IFN gamma, IL4) (Osakovsky *et al*., 2012). The pathological process in the brain proceeds with weak activity of microglia, as well as functional insufficiency of oligodendrocyte proliferation, which does not respond to severe neuronal lesions and lacks signs of regeneration (McLean *et al*., 1997).

TNF superfamily ligands, such as TNF-α, Fas, TRAIL, CD40L and their receptors, are important regulatory systems in the maintenance of immune homeostasis and the development of a protective immune response (Hehlgans & Pfeffer, 2005; Bremer, 2013). The members of this superfamily expressed on immune and nonimmune cells take part in various biological processes: innate immunity, apoptosis, redox signalling in endothelial cells and other processes (Bremer, 2013; Dostert *et al*., 2019). Membrane-bound ligands or their receptors can be chipped off by metalloproteinases, particularly those proven to be involved in this process ADAM10 and ADAM17 (Black *et al*., 1997; Seidel *et al*., 2021). The soluble forms have altered functions and participate in the reactions of a nonspecific immune response with the development of pathophysiological processes (Ferdinand *et al*., 2018). TNF superfamily members have the ability to influence neuroinflammation through interaction with blood-brain barrier (BBB) endothelial cells (Sonar & Lal, 2015).

The aim of this study was to analyse the participation of ligands sTNF-α, sFas-L, sTRAIL, sCD40L and sCD40 receptor in the pathophysiology of VE disease. To achieve this goal, we estimated the levels of these markers in the blood of VE patients compared with healthy individuals of the same population and patients with demyelinating diseases, including multiple sclerosis (MS) and neuromyelitis optica (NMO), as examples of disorders involving immune pathology. In addition, the same markers were analyzed in the CSF of VE patients and groups of patients with demyelinating diseases.

Materials and Methods

Patients

The study covered a total of 75 patients. The patients were examined in the Scientific Research Center of Medical Institute, M.K. Ammosov North-Eastern Federal University, and Republic's Hospital No. 2 – Center of Emergency Medicine, Yakutsk, Republic of Sakha (Yakutia). All the patients involved in this study were informed about the study and gave their consent. The study was carried out in accordance with the Declaration of Helsinki (2013) and approved by the local ethics committee at Yakut Scientific Centre of Complex Medical Problems, Siberian Branch of Russian Academy of Medical Sciences.

We studied a group of patients with chronic VE consisting of 27 people. Clinical and demographic data are presented in Table 1. All patients were ethnic Yakuts. The diagnosis of VE was based on a long-term and detailed examination of the patients, including evaluation of their neurological and neuropsychological status in the dynamics, routine blood and CSF analysis during the disease course, CSF oligoclonal band (OCB) examination and neuroimaging performed by magnetic resonance imaging (MRI) of the brain. Patients did not take immunomodulatory, antithrombotic drugs or statins at the time of the study. Patients were

Table 1

Clinical and demographic data of VE patients

Demographic data	
Female /male	12/15
Age (years, mean [SD], range)	53.1 [9.1], 39 to 74
Age of onset (years, mean [SD], range)	33.3 [9.6], 18 to 63
Disease duration (years, mean [SD], range)	18.7 [8.9], 3 to 39
Clinical features	
Acute onset	11 of 27 (40.7%)
Spastic paresis	27 of 27 (100%)
Dysarthria	17 of 27 (63.0%)
Dysphagia	3 of 27 (11.1%)
Dementia	15 of 27 (55.6%)
Muscle atrophy	9 of 27 (33.3%)
Pelvic disorders (constipation or urinary incontinence)	16 of 27 (59.3%)
Cerebral atrophy on MRI	13 of 17 (76.5%)
Oligoclonal bands	15 of 25 (55.6%)
CSF protein >450 mg/l at the time of examination	0 of 25 (0%)
CSF cell count >5 per μl at the time of examination at the time of examination	2 of 25 (8%)

Note: SD – standard deviation

Table 2

Clinical and demographic data of MS and NMO patients

Demographic data		
	MS (n = 17)	NMO (n = 6)
Female /male	9/8	6/0
Age (years, mean [SD], range)	40.8 [10.4], 20 to 55	35.2 [14.3], 21 to 61
Age of onset (years, mean [SD], range)	34.0 [9.8], 19 to 53	31.7 [15.6], 18 to 61
Disease duration (years, mean [SD], range)	6.6 [6.9], 1 to 26	3.6 [7.2], 0.04 to 18
Clinical features		
Relapsing-remitting MS	6 of 17 (35.3%)	N/A
Primary progressive MS	2 of 17 (11.8%)	N/A
Secondary progressive MS	9 of 17 (52.9%)	N/A
EDSS	4.2 [2.4], 0 to 9.0	5.8 [2.8], 2.0 to 8.5
Signs of demyelination on MRI	17 of 17 (100%)	6 of 6 (100%)
Oligoclonal bands	6 of 9 (66.7%)	2 of 5 (40%)
Immunomodulating treatment	7 of 17 (41.2%)	0 of 4 (0%)

Note: SD – standard deviation

treated with drugs that improve cerebral blood flow (cavinton, trental) and nootropic drugs (nootropil, piracetam, cerebrolysin, actovegin).

For comparison, the following groups of patients were examined:

1) 20 healthy Yakut people who are not related to neurological patients, with a mean age of 52.9 years (standard deviation (SD) = 11.7 years), ranging from 21 to 72 years;

2) Twenty-three patients with demyelinating diseases, 17 of whom were diagnosed with multiple sclerosis (MS) and 6 with neuromyelitis optica (NMO). Among MS patients, 11 patients were Russian, 1 was Ukrainian and 5 were Ya-

kuts. All patients with ONM were of Yakut nationality. The mean age of this group was 39.3 years (SD = 11.5 years), ranging from 20 to 61 years. Clinical and demographic data are presented in Table 2. Immunomodulatory treatment at the time of the study was received by 30% of patients.

3) Five healthy relatives of VE patients, with a mean age of 28.2 years (SD = 9.4 years), ranging from 19 to 39 years.

Samples

The levels of sTNF-α, sFas-L, sTRAIL, sCD40 and sCD40L were analysed in plasma samples obtained from 20 patients diagnosed with chronic VE, 14 patients with MS and NMO, 20 healthy persons without neurological symptoms and 5 healthy relatives of VE patients. Three plasma samples were obtained repeatedly in three VE patients after several years. Additionally, the levels of these markers were examined in CSF samples obtained by lumbar puncture of 22 VE and 14 with MS and NMO patients.

ELISA

ELISA was performed on an SF-4300-ChroMate-revA STAT FAX 4300 analyser (AWARENESS Technology, USA).

The level of sTNF-α was analysed using the Alpha-TNF-ELISA-BEST kit (A-8756; Vector Best, Novosibirsk, Russia). The level of Fas-L was analysed using the HumanFas-L Platinum ELISA kit (BMS260/2; Bender Med Systems GmbH, Austria). The TRAIL level was analysed using the Human TRAIL Platinum ELISA kit (BMS2004; Bender Med Systems GmbH, Austria). The CD40 and CD40L levels were measured using Human Platinum ELISA kits (BMS239 and BMS265; Affymetrixe Bioscience, Austria).

Detection of oligoclonal IgG

CSF samples and accompanying serum samples were analysed for OCBs in patients with neurological diseases: VE and demyelinating diseases of the CNS. Detection of oligoclonal IgG was carried out by isoelectric focusing on an agarose gel using PharmalyteTM 3–10 and PharmalyteTM 8-10.5 (GE Healthcare, Sweden), followed by immunoblotting with antibodies to human IgG (DAKO, Denmark). The OCB test was considered positive if two or more IgG bands were detected in the CSF but not in the serum.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 22.0. The quantitative data are described as the mean and standard deviation (M (SD)) and median and interquartile range (Me (25–75%)). The normality of the distributions of estimated parameters was determined by the Shapiro–Wilk test. A comparison of the normally distributed parameters between the groups was performed using the two-tailed Student's t-test, and in the case of non-normal distribution, using the nonparametric Mann–Whitney U and Kruskal–Wallis tests. Values of $p < 0.05$ were considered statistically significant.

Results

sTNF-α ligand

Healthy individuals in the Yakut population, according to our analysis, showed an average level of sTNF-α in plasma of 1.7 (SD = 0.7) pg/ml (Table 3). In the general group of VE patients (n = 20) in the chronic stage of disease (with and without intrathecal IgG synthesis), the average level of sTNF-α in plasma was 1.4 pg/ml (SD = 0.8 pg/ml), which statistically did not differ from healthy people and demyelinating disease patients ($p = 0.107$; Kruskal–Wallis test). In the group of VE patients, differences in the level of sTNF-α in plasma are observed depending on the detection of intrathecal IgG synthesis (Fig. 1). In the patients with OCBs, the sTNF-α level was 1.88 (SD = 0.57) pg/ml on average, and in the patients without OCBs, it was 0.98 (SD = 0.68) pg/ml. This difference was statistically significant ($p = 0.01$; two-tailed Student's t-test for equal variances not assumed).

In three VE patients, analyses were performed in plasma samples obtained repeatedly after several years. These patients had a long progressive course of the disease, with a disease

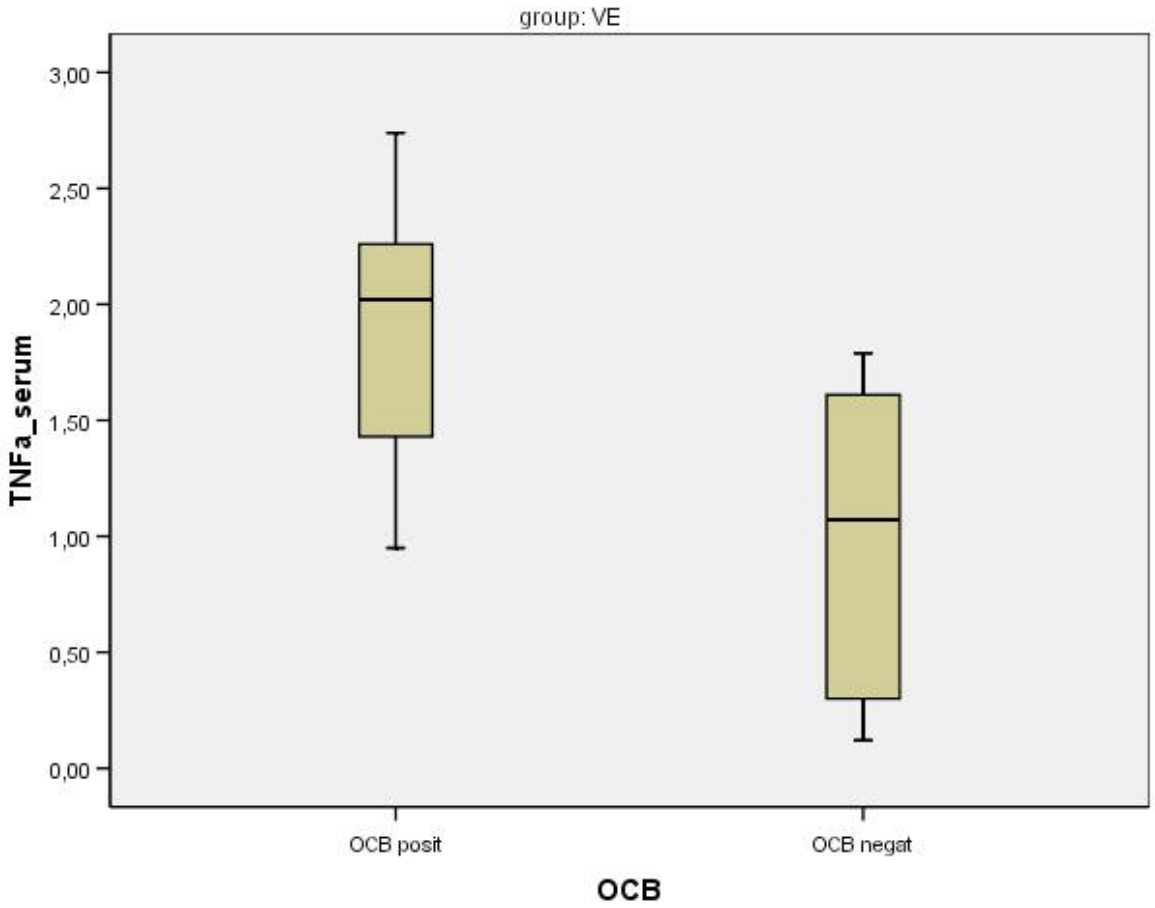

Fig. 1. The sTNF-α level in plasma, pg/ml, depending on OCBs test results in the VE patients (OCB posit – group of VE patients with positive oligoclonal IgG results (n = 10), OCB negat – group of VE patients without oligoclonal IgG (n = 8))

duration of 18, 30 and 28 years and intrathecal IgG synthesis. In all cases, the results showed a decreased level of sTNF-α: from 2.74 to 0.24, from 2.5 to 0.0 and 0.95 to 0.6 pg/ml. These results may indicate that the level of sTNF-α in the blood of VE patients is associated with the development of the inflammatory process, which in the course of the chronic course of the disease tends to attenuate.

In the patients with demyelinating diseases, the level of sTNF-α in plasma was lowest, less than in healthy patients ($p = 0.036$; Mann–Whitney U test).

In the CSF of the VE patients and demyelinating CNS diseases, in most cases sTNF-α was not detected, and there was no difference between groups (Table 4).

sFas-L ligand

In all samples analysed in the three groups, the level of Fas-L did not exceed 128 pg/ml, on average 32.0 (SD 15.0) pg/ml, which does not exceed the level of this indicator expected in healthy people. There was no difference in Fas-L levels between the studied groups ($p = 0.632$; Kruskal–Wallis test) (Table 3). In the CSF samples, sFas-L was not detected in most cases. In the VE patients, the value of this indicator in the CSF was 0.0 in all cases (Table 4).

sTRAIL ligand

The sTRAIL ligand level in the plasma and CSF samples ranged from 11.9 to 176 pg/ml in all analysed groups. In plasma, the sTRAIL levels showed statistically significant differences

Table 3

The sTNF-α, sFas-L, sTRAIL, sCD40 and sCD40L levels in the plasma of patients with VE, demyelinating diseases (MS and ONM) and healthy individuals

Group of patients	n	sTNF-α, pg / ml		sFas-L, pg / ml		sTRAIL, pg / ml		sCD40, pg/ml		sCD40L, pg/ml	
		M(SD)	Me (Q25-Q75)	M(SD)	Me (Q25-Q75)	M(SD)	Me (Q25-Q75)	M(SD)	Me (Q25-Q75)	M(SD)	Me (Q25-Q75)
VE	20	1.4 (0.8)	1.4 (0.9-2.0)	29.6 (8.3)	29.0 (23.3-35.8)	60.1 (23.3)	52.8 (43.5-64.5)	50.0 (76.7)	21.3 (12.7-27.5)	13274.0 (5499.0)	12655.0 (9280.0-17653.0)*
Demyelinating diseases	14	1.1 (1.2)	0.7 (0.1-2.1)*	33.2 (10.2)	30.0 (26.0-44.3)	62.3 (39.4)	48.9 (40.0-62.6)*	27.9 (51.3)	13.3 (8.1-25.5)	5672.1 (5183.5)	3255.0 (2950.0-5995.0)*
Healthy	20	1.7 (0.7)	1.6 (1.2-2.1)*	30.1 (10.5)	28.5 (23.3-33.0)	68.8 (16.6)	70.4 (51.3-80.5)*	23.4 (35.8)	13.5 (11.9-19.1)	7699.5 (3485.1)	6730.0 (4822.5-10287.5)*

Note: M – mean, SD – standard deviation, Me – median, Q25-Q75 – interquartile range, n – number of patients, * – statistically significant differences

Table 4

The sTNF-α, sFas-L, sTRAIL, sCD40 and sCD40L levels in the CSF of patients with VE and demyelinating diseases (MS and ONM)

Group of patients	sTNF-α, pg / ml			sFas-L, pg / ml			sTRAIL, pg / ml			sCD40, pg/ml			sCD40L, pg/ml		
	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n
VE	0.16 (0.37)	0.0 (0.0-0.19)	20	0	0	20	34.9 (13.0)	33.0 (25.7-40.4)	15	6.6 (1.4)	6.7 (5.2-7.5)	18	611.7 (67.1)	620.0 (582.5-660.0)	18
Demyelinating	0.05 (0.10)	0.0 (0.0-0.06)	11	0.9 (3.0)	0.0 (0.0-0.0)	11	31.6 (8.5)	32.2 (24.4-35.7)	9	7.0 (1.9)	6.9 (5.3-8.7)	10	607.8 (96.3)	620.0 (590.0-660.0)	10

Note: M – mean, SD – standard deviation, Me – median, Q25-Q75 – interquartile range, n – number of patients

Table 5

The CSF/plasma ratio of the TNF-α, sFas-L, sTRAIL, sCD40 and sCD40L levels in patients with VE and demyelinating diseases (MS and ONM)

Group of patients	sTNF-α, pg / ml			sFas-L, pg / ml			sTRAIL, pg / ml			sCD40, pg/ml			sCD40L, pg/ml		
	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n	M(SD)	Me (Q25-Q75)	n
VE	0.05 (0.17)	0.0 (0.0-0.0)	12	0	0	12	0.65 (0.27)	0.55 (0.43-0.88)	12	0.34 (0.23)	0.29 (0.18-0.62)	14	0.05 (0.04)	0.04 (0.03-0.05)	14
Demyelinating	0	0	4	0	0	4	0.46 (0.24)	0.36 (0.25-0.71)	5	0.42 (0.22)	0.44 (0.21-0.6)	4	0.19 (0.04)	0.2 (0.15-0.22)	4

Note: M – mean, SD – standard deviation, Me – median, Q25-Q75 – interquartile range, n – number of patients

in the studied groups (p = 0.032; Kruskal–Wallis test) (Table 3). However, the level of plasma sTRAIL in VE patients did not differ from that in other groups. Statistically significant differences were shown in the comparison of demyelinating diseases and healthy controls (p = 0.01; Mann–Whitney U test).

The sTRAIL level in the CSF was approximately two times lower than in plasma. In some patients sTRAIL level in CSF was close to that in plasma. There was no difference in sTRAIL ligand levels in the CSF samples between the two studied groups (p = 0.457; two-tailed Student's t-test for equal variances not assumed (Table 4).

sCD40 receptor and sCD40L (ligand)

The sCD40 receptor level was characterized by a wide range of values from 4.8 to 323.4 pg/ml in plasma in all studied groups. The general group of VE patients showed an increased level of sCD40 receptor (an average of 50.3, a median of 21.3 pg/ml) compared with the healthy and demyelinating patients (Table 3). These differences were not statistically significant (p = 0.066 in comparison with the healthy individuals, and p = 0.077 in comparison with the demyelinating individuals; Manna-Whitney test and p = 0.076; Kruskal–Wallis test).

Unlike the sCD40 receptor, the level of the ligand sCD40L did not have sharp fluctuations. The baseline level of sCD40L in healthy representatives of the Yakut (Sakha) ethnos was 7700 pg/ml on average (Table 3). The highest level of soluble ligand (13274.0 (SD 5499.0) pg/ml) was found in the VE patients (p = 0.000; Kruskal–Wallis test), which was higher than that in the healthy controls (p = 0.001; Student's t-test) and demyelinating diseases (p = 0.000; Manna-Whitney test). In addition, four healthy relatives of the VE patients were examined. They also showed an elevated level of sCD40L of 11850 (SD = 1630) pg/ml on average, which was significantly higher than that in the healthy group unrelated to the VE patients (p = 0.004; Student's t-test).

The sCD40 receptor and ligand sCD40L values in CSF were several times lower than those in peripheral blood (Table 4). The differences between the groups of patients were not revealed (p = 0.576; two-tailed Student's t-test for equal variances not assumed for receptor sCD40; and p = 0.864; Kruskal–Wallis test for ligand sCD40L).

There was some statistically insignificant increase in ligand sCD40L in the group of VE patients with intrathecal IgG synthesis (p = 0.204; two-tailed Student's t-test for equal variances not assumed). Thus, in the patients with positive OCB results, the sCD40L level was on average 630.1 (SD = 52.5) pg/ml, and in the patients without intrathecal IgG synthesis, it was 582.9 (SD = 81.2) pg/ml.

The ratio of the TNF-α, sFas-L, sTRAIL, sCD40 and sCD40L levels in the CSF and plasma

For a better understanding of the role of the studied markers in the pathological process in the CNS, we calculated the ratio of the TNF-α, sFas-L, sTRAIL, sCD40 and sCD40L levels in CSF and plasma in patients who obtained both biological fluids (table 5). Generally, both VE and demyelinating diseases are similar in this indicator. In the chronic form of these diseases the sTRAIL and, to a lesser extent, the soluble forms of the CD40R-CD40L system are most represented in the CSF. These indicators is most pronounced in VE. Comparison of this indicator between groups revealed statistically significant differences in the CSF/plasma ratio of CD40L (p = 0.004; Kruskal–Wallis test). The data obtained confirm the increased level of CD40L in the plasma.

Discussion

We have performed the first analysis of soluble ligands and the receptor of the tumour necrosis factor superfamily in plasma and CSF in patients with VE. Our results showed that the levels of the ligands sTNF-α, sFas-L, and sTRAIL in the plasma of patients with VE did not differ from those in healthy individuals. However, sTNF-α can play a role in the development of the inflammatory component of VE. A difference in the level of this cytokine in the blood was revealed depending on the intrathecal synthesis of IgG. The patients without oli-

goclonal IgG had a low level of sTNF-α in the blood, 48.4% less than in the patients with inflammation. We also found a decrease in the plasma sTNF-α level in patients with chronic VE during the disease course, which confirms the suppression of the innate immune response in this disease.

The obtained data indicate that the soluble CD40L ligand can play a role in the pathogenesis of VE. The VE patients showed increased sCD40L levels in the plasma compared to the healthy individuals and the demyelinating disease patients by approximately 2 times. A significantly elevated level of the ligand sCD40L was also seen in the relatives of the VE patients. The sCD40L ligand level is several times higher than the level of the sCD40 receptor in both plasma and CSF. A high level of sCD40L, unbalanced with soluble CD40 receptor in the circulating blood, is able to induce pathophysiological processes.

Soluble CD40L is known as an inflammatory marker, and its levels are increased in patients with type 2 diabetes, metabolic syndrome, hypertension and atherosclerosis (Michel *et al*., 2017; Linna *et al*., 2016). Among the healthy group, the highest levels of sCD40L (16230 pg/ml) and sCD40 (172.0 pg/ml) were found in the oldest patient at 72 years of age, which is consistent with the data of other authors, who showed an increase in sCD40L levels with age (Linna *et al*., 2016).

CD40 pathway signalling contributes to microglial activation and is associated with Alzheimer's disease and other types of dementia (Giunta *et al*., 2009). It has been shown that sCD40L is associated with demyelinating diseases; it is believed that this marker may be involved in BBB disruption in MS and the development of CNS inflammation in neuromyelitis optica (Zhong *et al*., 2016; Masuda *et al*., 2017; Du *et al*., 2020). However, some studies have not reported an increase in sCD40L in patients with MS (Zahednasab *et al*., 2017). In our study, patients with demyelinating diseases did not have an increased level of sCD40L, which can be explained by the fact that half of these patients received immunomodulating therapy at the time of sampling. This is confirmed by the fact that the studied group of patients with MS and NMO showed reduced levels of sTNF-α and TRAIL in the blood.

Analysing the reasons for the high level of sCD40L in patients with VE, we assume that it may be associated with platelet activity. The peripheral blood of patients with VE is characterized by an increased level of platelets, in some cases almost twice the norm (Sizikova *et al*., 2008). However, as shown by pathomorphological studies, the increased levels of platelets and sCD40L in the blood of VE patients do not have a significant effect on the cardiovascular system. Previous studies of the cardiovascular system of deceased patients with VE showed only moderate atherosclerotic changes in the aorta, coronary and cerebral arteries (lipoidosis and single fibrous plaques) and cardiac dystrophy of varying severity (Sukhov, 1990). Other authors have noted the absence of signs of atherosclerosis of the cerebral vessels in the pathological examination of deceased VE patients, even those aged 75 years (Avtsyn *et al*., 1994).

The selectivity of the pathology of the brain in VE patients may be related to congenital high sensitivity of the microcirculatory system of the brain parenchyma to elevated levels of sCD40L in circulating blood. Sensitivity to sCD40L in the brains of patients with VE may be related to the constant expression and/or higher density of receptors in the endothelial cells of the microvascular system of the brain parenchyma. The participation of heredity in the development of the disease has been confirmed in a number of studies, but genetic factors and the molecular nature of the sensitivity of the brain parenchyma in VE remain unclear (Goldfarb *et al*., 2014).

Increased plasma CD40L levels may provoke microvascular pathology and be involved in the induction of increased permeability of the BBB. The small size of the sCD40L molecule allows it to penetrate into the brain parenchyma and induce astrogliosis, which leads to compaction of the extracellular matrix, which worsens the availability of oxygen to brain tissues. In addition, sCD40L can be involved in the immunosuppression characteristic of the VE chronic form (Schlom *et al*., 2013). Developing microvascular pathology may be the cause of chronic

hypoxia, inducing degenerative atrophic processes of the brain and one of the major factors in VE pathogenesis.

In the CSF in the most studied VE and other neurological patients, the production of ligands sTNF-α and sFas-L was not observed, and the CD40 receptor and ligand values were several times lower than those in peripheral blood. There were no differences between the studied groups for these markers. In contrast, the level of ligand sTRAIL in the CSF was only two times lower than in plasma. Some VE patients had sTRAIL CSF levels comparable to serum level. The involvement of this ligand in the pathogenesis of the disease requires further consideration because it was the only marker in this study, which showed CSF levels close to plasma levels. Whereas in other neurodegenerative diseases, such as Alzheimer's disease, this marker is not detected in the CSF (Genc *et al*., 2009). The TRAIL ligand has various and complex functions and is involved in multiple pathways depending upon the specific cell and tissue type and may be a potential participant in pathological processes in the CNS (Tisato *et al*., 2016).

The obtained results suggest the participation of soluble forms of TNF superfamily CD40L and TNF-α ligands in the pathogenesis of ethnospecific neurodegenerative disease. These data will be useful for further research on the interaction of the immune system and the central nervous system.

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