Neuroimmune interactions represent a highly dynamic mechanism for the regulation of cognitive function in chronic cerebral ischemia (CCI). The aim of this study was to investigate changes in salivary proinflammatory cytokines IL1β and IL6 and anti-inflammatory IL10 in patients with CCI (mean age 65.4 ± 9.1 years) before and after cognitive tests. After cognitive tests, the levels of salivary IL1β and IL6 were significantly elevated by 101.6 ± 19.1 pg/ml (n = 74) and 32.8 ± 6.1 pg/ml (n = 74), respectively. Using one-way ANOVA and non-parametric statistical methods, we were able to demonstrate associations between changes in salivary interleukins and cognitive performance. In the group of patients with a significant increase in IL1β, some cognitive parameters were lower than in the group with negative or zero dynamics of this cytokine: the patients made more mistakes in the subtraction test (F = 11.5; n = 63; p = 0.001) and performed worse in the Luria test (F = 6.8; n = 65; p = 0.01). For IL6, Spearman’s rank correlation coefficient for the number of mistakes in the subtraction test was positive and differed significantly from 0 (r = 0.26; n = 62; p = 0.042). The group with positive IL10 dynamics performed better in N-back test (F = 5.2; n = 67; p = 0.03) and made fewer mistakes in the subtraction test (F = 6.8; n = 63; p = 0.01) in comparison with patients who demonstrated negative IL10 dynamics. Good performance in other cognitive tests was not correlated with interleukin dynamics. The article also discusses possible mechanisms underlying interleukin effects on cognitive function in patients with CCI and applications of the obtained data.

Keywords: neuroimmune interactions, vascular encephalopathy, interleukins, IL1β, IL6, IL10, cognitive function

Author contribution: Fokin VF performed data analysis and wrote the manuscript; Shabalina AA performed biochemical analysis of cytokines and participated in writing the manuscript; Ponomareva NV collected and analyzed psychometric data; Medvedev RB performed clinical examinations, analyzed the literature and proposed the study design; Lagoda CV analyzed clinical data and proposed the study design; Tanashyan MM proposed the study design, summarized clinical data and participated in the context of the obtained results.

Compliance with ethical standards: The study was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 11/14 dated November 19, 2014); all study participants signed informed consent to participate.

Correspondence should be addressed: Vitaly F. Fokin
Volokolamskoe shosse, 80, Moscow, 125367; mfmail.ru

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immune system. Neuroimmune interactions are the crucial component of the underlying pathogenetic mechanism as they largely determine the course of the disease and the level of cognitive functioning [2–8].

Understanding the pattern of changes in the salivary concentrations of pro-inflammatory interleukins (IL1β, IL6 and IL10) in response to cognitive stress is an important avenue of contemporary neuroimmunology research. Chronic cerebral ischemia (CCI) is a pathological form of vascular aging, i.e. profigredent chronic vascular insufficiency accompanied by non-focal neurological symptoms and cognitive decline caused by cortical or subcortical lacunar infarcts. Ischemia triggers inflammation, production of reactive oxygen species and activation of microglia and other glial cells, thereby stimulating secretion of cytokines including proinflammatory interleukins [9, 10].

Proinflammatory interleukin IL1β plays a role in inflammatory response and other immune processes; its modulatory effects span the nervous, immune and endocrine systems. IL1β and its receptors are present in the brain and especially abundant in the hippocampus. There are reasons to believe that IL1β is involved in the modulation of hippocampal plasticity and memory formation [4]. According to the majority of IL1β studies, elevated IL1β has a negative impact on cognitive function. However, some studies report that IL1β either does not affect or has a beneficial effect on learning and memory. Physiological concentrations of IL1β promote post-tetanic potentiation but its abnormally high levels can be inhibitory and interfere with learning and memory. Furthermore, IL1β, IL6 and a few other cytokines are activated in the brain upon induction of long-term potentiation [5]. It is reported that central administration of IL1β to rats affects inflammatory response and enhances conditioned memory; this cognitive effect is correlated with glucocorticoid levels [6]. In addition, IL1β can interact with the autonomic nervous system [7], which, in turn, may mediate its effects on cognition.

Interleukin 6 (IL6) is one of the major mediators of acute inflammation. The sources of IL6 in the central nervous system are represented by neurons, astrocytes, microglia and endothelial cells. IL6 plays a definitive role in the pathogenesis of inflammatory diseases and the normal homeostasis of nervous tissue [8].

Recently, IL6 has been hypothesized as having an impact on cognitive function. Some researchers applied the analysis of variance to identify factors affecting the levels of IL6. Age, hypertension, diabetes, smoking, moderate consumption of alcohol, total homocysteine, carotid intima-media thickness and body mass index were reported to be positively correlated with IL6 concentrations. In a multidimensional linear regression model, IL6 was negatively correlated with Mini mental state examination scores adjusted for social, economic and vascular risk factors. It is known that IL6 can be expressed by brain cells, including neurons during their depolarization [11]. Perhaps, this process can be activated by cognitive stress.

Elevated IL6 is observed in patients with declining cognitive function, which may be associated with the regulation of post-tetanic potentiation [12, 13]. Similar to IL1β, IL6 interacts with the autonomic nervous system [14].

IL10 is an anti-inflammatory cytokine that inhibits secretion of proinflammatory IL1β, IL6 and tumor necrosis factor alpha (TNFα) and stimulates release of other anti-inflammatory cytokines, including the IL1β receptor antagonist, which exerts anti-inflammatory effects. IL10 reduces IL1β and TNFα levels after traumatic brain injury in rats and improves neurologic recovery [15]. Hyperactive microglial response observed in persistent inflammation is often associated with increased expression of inflammatory IL1β and anti-inflammatory IL10 [16], which often occurs in the setting of sympathetic nervous system activation [17]. IL10 blocks the inhibitory effect of IL1β on post-tetanic potentiation [18].

We hypothesize that interleukin effects on cognitive function are characterized by certain selectivity and mostly target long-term memory.

The aim of this study was to assess associations between cognitive processes and the dynamics of pro- and anti-inflammatory interleukins in patients with CCI.

METHODS

The study recruited 31 male and 63 female patients with CCI aged 42 to 85 years (the mean age was 65.4 ± 9.1 years). Pathomorphologically, cognitive decline in patients with CCI is characterized by the presence of diffuse and multiple lacunar lesions in the subcortical white matter and the cortex; subcortical defects are often associated with either cerebral atherosclerosis or lipohyalinosis of small penetrating arteries supplying deeper brain regions. Etiologically, CCI has a variety of causes, including atherosclerosis, high blood pressure and hypertensive heart disease, venous insufficiency, diabetic angiopathy, vasculitis of various etiology, hematologic disorders, etc. Our patients with CCI and cognitive decline differed in the extent of memory impairment, ability to work, irritability, brainstem symptoms, etc. The following inclusion criteria were applied: stage I–II dyscirculatory encephalopathy (early or subcompensation stage according to the classification by Levin OS [19–20]); right-handedness; MoCa scores of ≥ 26 (patients who scored < 26 were included in the study if they were not demented and did not need daily care). Exclusion criteria: pronounced dementia (> 1 points on the Clinical Dementia Rating Scale); a history of acute cerebrovascular accidents; traumatic brain injury; severe cardiac or metabolic (type 2 diabetes mellitus) pathology; renal insufficiency; uncompensated thyroid dysfunction.

The patients underwent a battery of tests to assess their cognitive function. The tests were performed in strict sequence. The first task was an alphabetic version of the N-back test: the patients were asked to find all occurrences of a specified two-letter combination in a text without space characters in 3 minutes. This test is based on the Kirchner n-back task with n = 1. As a rule, healthy subjects are able to complete this task without or with only one mistake.

The N-back test was followed by a verbal fluency test: the patients were asked to name as many words as possible starting with each of the specified Russian letters (C, K and A). The number of words was summarized and averaged.

The next test initially proposed by Luria aimed to assess verbal memory. First, the patients were asked to memorize and recall 10 words (each series of words was repeated 5 times). Then, the patients counted backwards from 100 by sevens. Finally, the patients were asked again to reproduce the memorized words. Immediate and delayed scores were counted. Healthy subjects were able to remember 9–10 words in the immediate recall test, made no mistakes in the subtraction test, and reproduced 8–10 words in the delayed recall test.

Overall cognitive function and patient eligibility for the study were assessed using the Montreal cognitive assessment scale (MoCa). Salivary interleukins were measured before and after cognitive tests by means of a sandwich ELISA. IL10 was measured using eBioscience reagents (Bender MedSystems; Austria); IL1β and IL6 concentrations were determined using reagents by Vector-Best (Russia). The detection range was from 1 to 2,000 pg/mL. Assay calibrators were purchased from the
Table 1. Statistical characteristics of interleukin levels before and after cognitive tests

| Variable          | n  | Mean, pg/ml | Standard deviation, pg/ml | Standard error, pg/ml | ρ  |
|-------------------|----|-------------|---------------------------|-----------------------|----|
| IL1β, before      | 94 | 584.9468    | 275.1796                  | 28.38263              | < 0.000001 |
| IL1β, after       | 74 | 678.9865    | 272.2785                  | 31.65174              | < 0.000001 |
| IL1β, shift       | 74 | 101.8216    | 170.5874                  | 19.83039              | 0.000002  |
| IL6, before       | 94 | 152.5844    | 66.2574                   | 6.83393               | < 0.000001 |
| IL6, after        | 74 | 32.8459     | 52.4934                   | 6.10223               | 0.000001  |
| IL10, before      | 92 | 0.9592      | 0.1949                    | 0.02032               | < 0.000001 |
| IL10, after       | 74 | 0.9159      | 0.2505                    | 0.02912               | < 0.000001 |
| IL10, shift       | 74 | -0.0177     | 0.2047                    | 0.02379               | 0.459261  |

Note: ρ — level of statistical significance.

Table 2. Shifts in IL1β and IL10 salivary concentrations following cognitive stress in 2 groups of patients

| Group                  | Mean IL1β, pg/ml | Standard error IL1β, pg/ml | Mean IL10, pg/ml | Standard error IL10, pg/ml |
|------------------------|------------------|----------------------------|------------------|---------------------------|
| Group 1 (below average)| -0.34 (n = 44)   | 14.7 (ρ = 0.98)            | -0.10 (n = 40)   | 0.02 (ρ < 0.000001)       |
| Group 2 (above average)| 251.2 (n = 30)   | 26.8 (ρ < 0.000001)        | 0.13 (n = 33)    | 0.02 (ρ < 0.000001)       |

Note: ρ — level of statistical significance.

Table 3. Associations between shifts in IL1β concentrations and cognitive function

|                     | n  | F    | ρ   |
|---------------------|----|------|-----|
| Subtraction (100–7)  | 63 | 11.49| 0.001|
| Delayed recall (based on Luria tests) | 65 | 6.84 | 0.01 |

Note: F — Fisher's coefficient; ρ — level of significance.
Comparison of figures 1 and 2 reveals that cognitive function is negatively correlated with pro- and anti-inflammatory interleukin response.

The Kolmogorov-Smirnov test demonstrated that IL6 shifts did not conform with normal distribution; therefore, associations between IL6 shifts and cognitive function were measured by Spearman’s rank correlation used for nonparametric samples. Spearman’s correlation coefficient for the subtraction test and IL6 shifts significantly differed from zero ($\rho = 0.26; n = 62$; $p = 0.042$). Similar to IL1β, the higher number of mistakes was associated with a higher increase in IL6 levels.

Some cognitive indicators were insensitive to changes in interleukin concentrations, including MoCa, verbal fluency and immediate recall (Luria) scores.

**DISCUSSION**

Reported physiological salivary concentrations of interleukins in general and IL10 in particular vary across studies [22–23]. In people of advancing age, salivary interleukin levels are affected by a multitude of factors, from the past history of diseases to the quality and number of dentures. So, it may be reasonable to analyze and compare relative indicators within one group of patients; some authors rely on the ratios of different proinflammatory cytokines, for example, IL1β to IL10, or measure interleukin response to stress. Proinflammatory interleukins, including IL10, are elevated in the setting of stress [23]. Cognitive tests cause an elevation of IL1β and IL6 levels, but induce no changes in IL10 concentrations. The absence of IL10 dynamics in response to cognitive tests can be explained by weak stress limited to sympathoadrenal activation.

Many proteins originally detected in the immune system are also found in neuronal synapses participating in cognitive processes [24]. Our work demonstrates an increase in salivary interleukins during cognitive tests in patients with CCI. Higher expression of proinflammatory cytokines was correlated with worse performance in cognitive tests. Patients with CCI had more pronounced inflammation in comparison with healthy individuals [25]. In such patients, neuronal activation enhances secretion of proinflammatory interleukins, but the intensity of this enhancement depends on the severity of the disease. It is known that sympathetic neurons secrete IL6 and produce paracrine or autocrine signals in response to the presence of the soluble IL6 receptor [26]. So, hypothetically neuronal activation in patients with CCI will be accompanied by an increase in the levels of proinflammatory interleukins. It is known that proinflammatory cytokines disrupt normal neuronal function in an adult brain by exerting a direct effect on neurons or by triggering mechanisms mediated by non-neuronal cells (like microglia or astrocytes) [27]. Patients with CCI will inevitably experience mild stress during cognitive testing because they perceive cognitive tasks as psychoemotional strain. Stress might trigger cytokine secretion (IL1β, IL6, etc.) On the one hand, physiological levels of IL1β are indispensable to learning and memorizing; on the other hand, elevated IL1β is detrimental to cognitive performance. Increased production of IL6 in the setting of stress exacerbates inflammation by stimulating IL1β secretion in the brain and thus promotes anxiety [28]. Effects of IL1β on memory and learning are often associated with its effects on synaptic mechanisms of long-term potentiation in the hippocampus. IL1β induces hyperpolarization and modulates synaptic inhibition of preoptic and frontal hypothalamic neurons; it also neutralizes long-term depression of synaptic transmission in the hippocampus. The cytokine network consisting of IL1β, IL18, IL6 and TNFa interacts with neurons during long-term potentiation and learning. Blockade of endogenous IL1β is beneficial to memory formation [29].

Effects exerted by IL10 are opposite to those of proinflammatory cytokines. It is known that IL10 is involved in cytokine regulation (feedback loop) and inhibits effects of proinflammatory cytokines. This interleukin demonstrated no significant dynamics during cognitive stress, which might be explained by its low involvement in cognitive processes as such. The impact of IL10 on cognitive function might be mediated by its effects on proinflammatory IL1β and IL6 [30].

Cognitive performance correlated with changes in interleukin concentrations was reflective of memory retention, i.e. plasticity processes. Notably, immediate word recall in the Luria test was not associated with changes in the levels of proinflammatory interleukins, whereas delayed word recall was associated with

**Table 4.** Associations between IL10 response and cognitive function

| IL10            | n  | F      | $\rho$ |
|-----------------|----|--------|--------|
| Subtraction (100–7) | 63 | 6.83   | 0.01   |
| Detected patterns | 67 | 5.16   | 0.026  |

Note: $F$ — Fisher’s coefficient; $\rho$ — level of significance.

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Fig. 1. Performance during cognitive tests in groups 1 and 2 of IL1β shifts. A — group 1, B — group 2. A. Number of subtraction mistakes (100–7). B. Delayed words recall in the Luria test

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changes in IL1\(\beta\). This confirms the potential role of IL1\(\beta\) in inhibiting post-tetanic potentiation. Perhaps, this mechanism underlies many effects of proinflammatory interleukins.

Our findings may be clinically useful. Current recommendations for people of advancing age struggling with chronic vascular diseases point to the benefits of intense cognitive load (doing crossword puzzles, studying foreign languages, etc.) Our findings suggest that in some cases cognitive load can lead to elevated proinflammatory cytokines and contribute to oxidative stress, which raises questions about the benefits of such recommendations. Further research could be aimed at exploring biological markers of proinflammatory interleukins that could be conveniently used for controlling cognitive load and monitoring the response of proinflammatory cytokines.

CONCLUSION

The dynamics of pro- and anti-inflammatory interleukins IL1\(\beta\), IL6 and IL10 were associated with performing cognitive tasks in patients with CCI. Cognitive stress was accompanied by the reliable increase in IL1\(\beta\) and IL6 in the mixed sample of men and women. Changes in the levels of all studied cytokines reflected performance scores. Elevated salivary IL1\(\beta\) and IL6 were associated with worse performance in the subtraction test (100–7); heightened IL1\(\beta\) was associated with poor scores in the delayed recall test. Patients with positive dynamics of salivary IL10 made fewer mistakes in the subtraction test and did better in the N-back test than patients with negative IL10 dynamics. Thus, the significant increase in the levels of salivary proinflammatory cytokines induced by cognitive stress was accompanied by a decline in cognitive function in delayed memory tests, while similar changes in anti-inflammatory IL10 were associated with better cognitive performance. MoCa, verbal fluency and some other scores were not associated with changes in interleukin levels. Further discussion is needed to understand the mechanisms underlying interleukin effects on cognitive function. There is a tight link between the levels of proinflammatory cytokines and some types of mental activity, suggesting that patients with CCI should be monitored for the levels of proinflammatory cytokines during cognitive tasks.

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