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Cytokine Content in the Hypothalamus and Hippocampus of C57Bl/6J Mice with Depressive-Like Behavior

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The content of pro- and anti-inflammatory cytokines in the hypothalamus, hippocampus, and blood serum of C57Bl/6J mice with depressive-like behavior induced by 20-day social stress was analyzed in 4 h after immune stimulation with LPS (250 μg/kg). These animals are characterized by a tendency to an increase in the blood content of IL-6 and a decrease in the level of IL-10. Changes in cytokine content in the brain of mice with depressive-like state developed under these conditions were observed only in the hippocampus: the levels of IL-1β, IL-6, TNFα, and IL-10 increased and the content of IFNγ decreased in comparison the corresponding parameters in the controls (not exposed to social stress) and aggressive animals. No changes in the levels of IL-2, IL-4, and IL-17 were revealed in the hypothalamus and hippocampus.

Key Words: *depressive-like behavior; social stress; hypothalamus; hippocampus; pro- and anti-inflammatory cytokines*

Depressive disorders represent one of the most significant problems of modern psychiatry due to their high prevalence, diversity of their forms, etiological and neurochemical heterogeneity. The important role of inflammation and cytokines, the key regulators of the inflammatory processes, in the pathophysiological mechanisms of depression is beyond doubts [4,7-9,14].

It is known that affective disorders, including depression, are stress-dependent, and social stress plays the most important role in their development. Experiments on the model of chronic social stress showed confrontations with aggressive partners lead to the development of a depressive state in submissive mice. Along with depressive behavior (Porsolt test, open field), depressive state is characterized by activation of the hypothalamic—pituitary—adrenal axis and changes in the content of serotonin and dopamine and their metabolites in brain structures [5]. The analysis of the immune status of these animals revealed reduced immunological reactivity [2,5,6], changes in cytokine content in the peripheral blood [14], and changes in spontaneous and mitogen-stimulated cytokine production in the culture of spleen cells [8]. However, changes in cytokine content in certain brain structures under these conditions are little studied.

At the same time, numerous clinical studies and studies of depressive-like states formed in other experimental models (prenatal and early postnatal stress, social isolation, *etc*.) showed that depressive disorders are characterized by changes in the cytokine profile not only in the periphery, but also in brain structures [4,7-9,14].

It is known that cytokine production in the brain occurs in the hypothalamus, hippocampus, prefrontal cortex, and basal ganglia that are involved in the formation of depression [4,11,12]. The hypothalamus and hippocampus, as was shown earlier [3,5], participate in psychoneuroimmunological modulation and are most often studied in depressions.

Our aim was to study the profile of pro- and anti-inflammatory cytokines in the blood serum and brain structures (hypothalamus and hippocampus) in mice

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with depressive-like state formed as a result of chronic social stress.

MATERIALS AND METHODS

Experiments were carried out on 2.0-2.5-month-old male C57Bl/6J mice weighing 22-30 g (n=65). Animals were obtained from the vivarium of the Research Institute of Physiology and Fundamental Medicine and were kept under standard vivarium conditions at regular 12/12 h light/darkness regime with free access to water and food. The experiments were carried out with strict adherence to Humanity Principles stated in EU Directive for the protection of laboratory animals (86/609/EEC) and approved by local Ethics Committee.

Depressive-like and aggressive types of behavior were formed in mice by repeated experience of social victories and defeats over 20 days (the method of paired distant sensory contact) [10]. The mice demonstrating active (sideways and upright defense postures and running away from the attacking opponent) and passive defense behavior (freezing and supine posture) were considered submissive. Aggressive behavior in mice was assessed by the number of attacks during confrontation testing with a subordinate partner, latency of the first attack, and duration of each attack.

The control group included animals not subjected to social stress and housed in individual cages over 5 days to prevent group influence and antagonistic interaction.

In the next day after the last agonistic confrontation, the animals with depressive-like and aggressive behavior, and control mice received intraperitoneal injection of physiological saline (PS) or endotoxin LPS (*E. coli* serotype 055:B5; Sigma), a component of the bacterial wall of gram-negative bacteria, commonly used as immune activator. LPS was injected in a single dose of 250 μg/kg (in 0.1 ml PS/mouse); PS was also injected in a volume of 0.1 ml.

The animals were divided into 6 groups: control mice (not exposed to social stress) injected with PS (group 1) or LPS (control 2); mice with depressive-like behavior receiving PS (group 3) or LPS (group 4); aggressive mice receiving PS (group 5) or LPS (group 6).

The animals were decapitated 4 h after injection of PS or LPS. The brain was promptly removed and the hippocampus and hypothalamus were isolated in cold (samples of each structure from 2-3 mice were pooled; at least 5 pools were prepared) and immediately transferred in liquid nitrogen. For the analysis of cytokines, detergent-soluble fractions of brain tissues were prepared by adding lysis buffer containing PBS, 0.1% Triton X-100, 1 mM EDTA, and 1 mM PMSF to the tubes with samples. The samples were homogenized with plastic pestils and incubated on ice for 30-40 min. Tissue extracts were centrifuged (4500 rpm, 20 min, 4°C; Centrifuge 5415 R). The supernatants were collected with a pipette and used for cytokine assay. The concentration of cytokines in 1 ml supernatant was normalized to the weight of the tissue sample.

To prepare blood serum, trunk blood was collected after decapitation from each mouse of groups 1, 2, 3, 4 into a separate tube and after 30 min centrifuged at 2500 rpm at room temperature for 10 min. The brain and serum samples were stored at -70°C until analysis.

The concentrations of pro- and anti-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-17, TNFα, and IFNγ) in the brain structures (pg/mg), as well as IL-6 and IL-10 in the blood serum (pg/ml) were measured on Milliplex Luminex 200 analyzer for proteins and nucleic acids (Merk Millipore) by the xMAP technology on magnetic microspheres using standard kits according to manufacturer's instruction.

The results were processed using Statistica 10.0 software. As the data were not normally distributed according to Kolmogorov—Smirnov test, non-nonparametric Kruskal—Wallis test was applied. The results are presented as the median (Me), 25 and 75 percentiles, and minimum and maximum values. The differences were significant at $p<0.05$.

RESULTS

In 4 h after systemic administration of LPS (250 μg/kg), the blood concentrations of proinflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10 significantly increased in both the control (group 2) and mice with depressive-like behavior (group 4) in comparison with the corresponding groups receiving PS.

There was a tendency towards an increase in IL-6 content in animals with depressive-like behavior in comparison with the control, which agrees with previous reports [14]. In mice with depressive-like behavior, this trend was noted after administration of PS: 29.52 (26.8; 32.1) pg/ml *vs*. 16.1 (13.2; 16.8) pg/ml in the control (p=0.05) and LPS: 19,140.5 (16,608.7; 21506.5) pg/ml *vs*. 14,295.5 (12,415.8; 16,053.8) in the control (p=0.05). At the same time, the content of IL-10 in depressive-like mice tended to decrease after LPS injection: 17.43 (11.2, 57.9) pg/ml *vs*. 50.68 (42.47; 74.0) pg/ml in the control (p=0.05), *i.e*., proinflammatory cytokines in the serum of these animals prevailed over the anti-inflammatory cytokines.

The spectrum of proinflammatory (IL-1β, IL-2, IL-6, IL-17, IFNγ, and TNFα) and anti-inflammatory (IL-4 and IL-10) cytokines in brain structures (hypo-

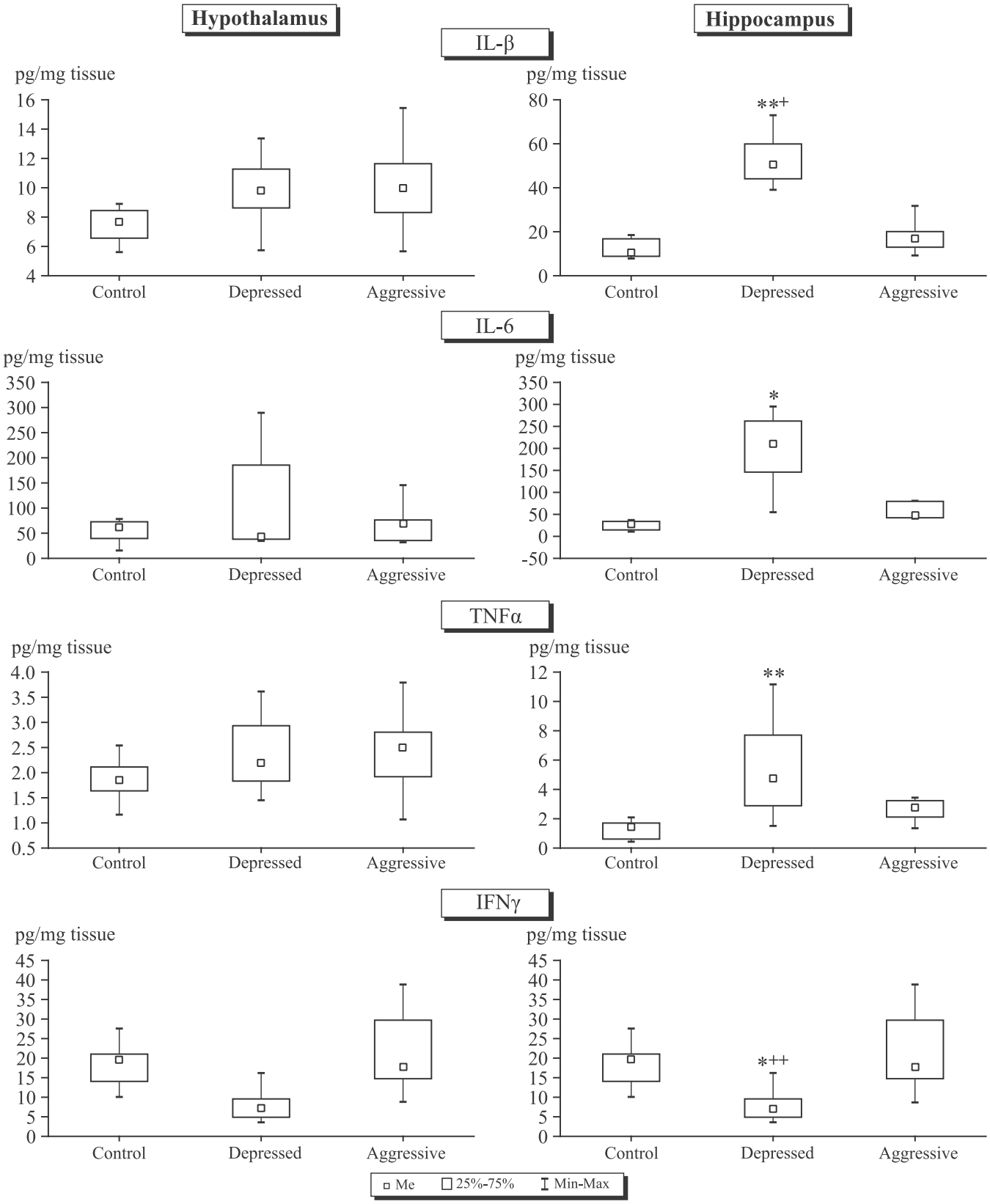

Fig. 1. Contents of IL-β, IL-6, TNFα, and IFNγ in the hypothalamus and hippocampus of C57Bl/6J mice with depressive-like behavior induced by 20-day social stress in 4 h after LPS (250 μg/kg) administration. *p<0.05, **p<0.01 in comparison with the control; ^{+}p<0.01, ^{++}p<0.001 in comparison with aggressive mice.

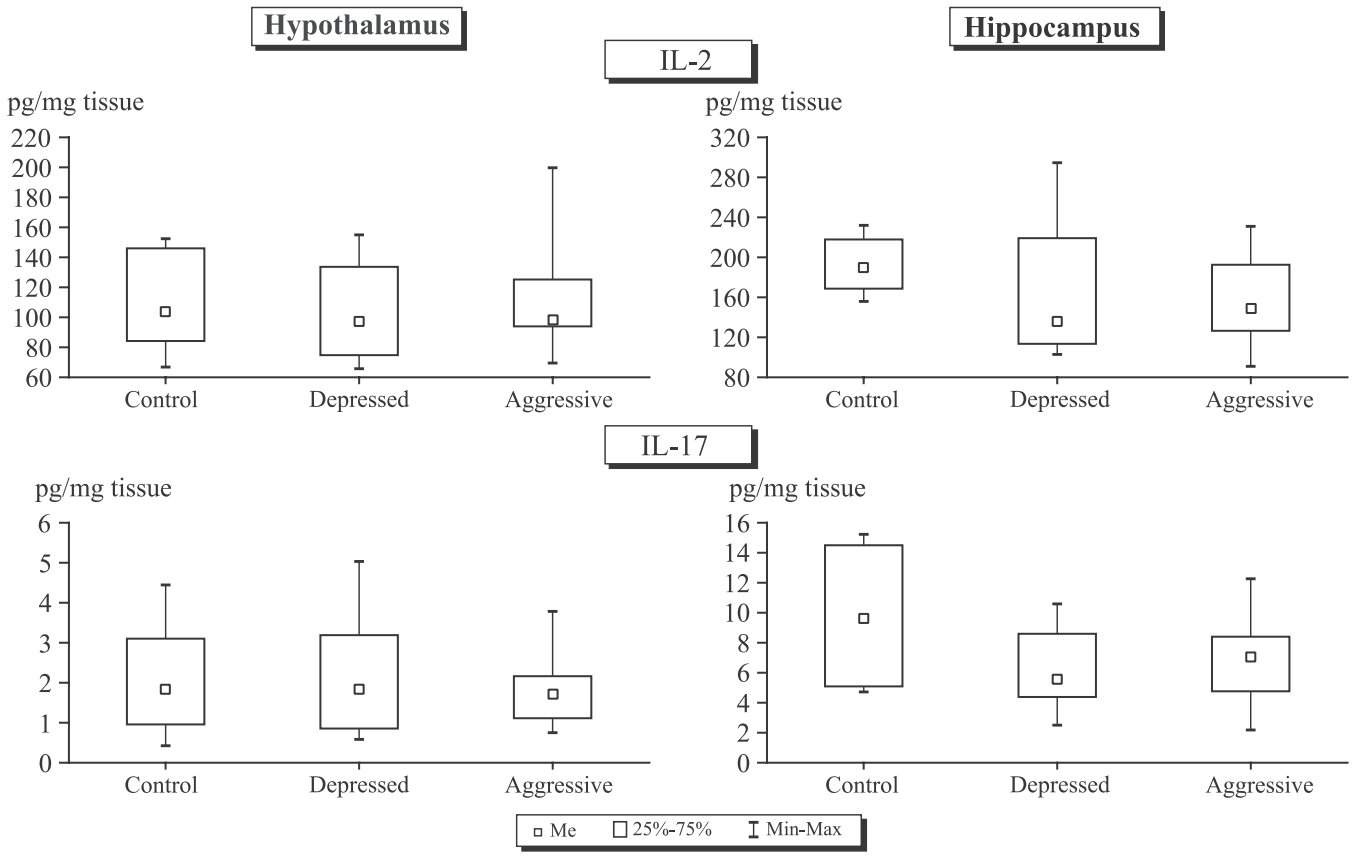

Fig. 2. Contents of IL-2 and IL-17 in the hypothalamus and hippocampus of C57Bl/6J mice with depressive-like behavior induced by 20-day social stress in 4 h after LPS (250 μg/kg) administration.

thalamus and hippocampus) of mice with depressive-like behavior was compared with not only control animals (not exposed to social stress), but also animals demonstrating aggressive behavior induced by chronic social stress.

Before immune stimulation, the levels of cytokines in the hypothalamus and hippocampus were low and practically did not differ between the groups. In 4 h after LPS injection, cytokine levels increased similar to that in the serum, and these changes depended to the type of behavior, brain structure, and the analyzed cytokine.

In the hypothalamus, IL-1β concentration was similar in control and experimental mice receiving LPS (Fig. 1). In the hippocampus, IL-1β content in mice with depressive-like behavior was significantly higher than in controls ($p<0.001$) and aggressive animals ($p<0.02$) (Fig. 1).

Analysis of the content of IL-6 in brain structures revealed similar changes. In the hypothalamus, the levels of IL-6 were similar in all mice irrespective of their behavior (depressive, aggressive or control), whereas in the hippocampus, IL-6 content was significantly higher in depressive LPS-treated mice in comparison with the control ($p<0.05$). In aggressive mice, only a tendency to an increase in IL-6 concentration was noted under these conditions (Fig. 1).

The content of TNFα, similar other proinflammatory cytokines, does not change in the hypothalamus, but increases in the hippocampus only in depressed mice in comparison with that in the control group ($p<0.005$) (Fig. 1). A similar increase in the content of IL-1β, IL-6, and TNFα in the hippocampus and prefrontal cortex was observed in another model of depressive-like behavior induced by chronic mild stress in C57BL/6J mice or ob/ob mice with obesity [15].

It should be noted, that unlike other proinflammatory cytokines, the content of IFNγ was reduced almost 3-fold in the hippocampus of mice with depressive-like behavior in comparison with the control ($p<0.05$) and aggressive mice ($p<0.05$) (Fig. 1). In the hypothalamus, the content of IFNγ was similar in the control and experimental groups. We have previously demonstrated that mitogen-stimulated production of IFNγ in the culture of spleen cell was also reduced in comparison with the control (by 8.3 times) [8].

At the same time, analysis of other proinflammatory cytokines (IL-2 and IL-17) under conditions of immune stimulation with LPS revealed no significant

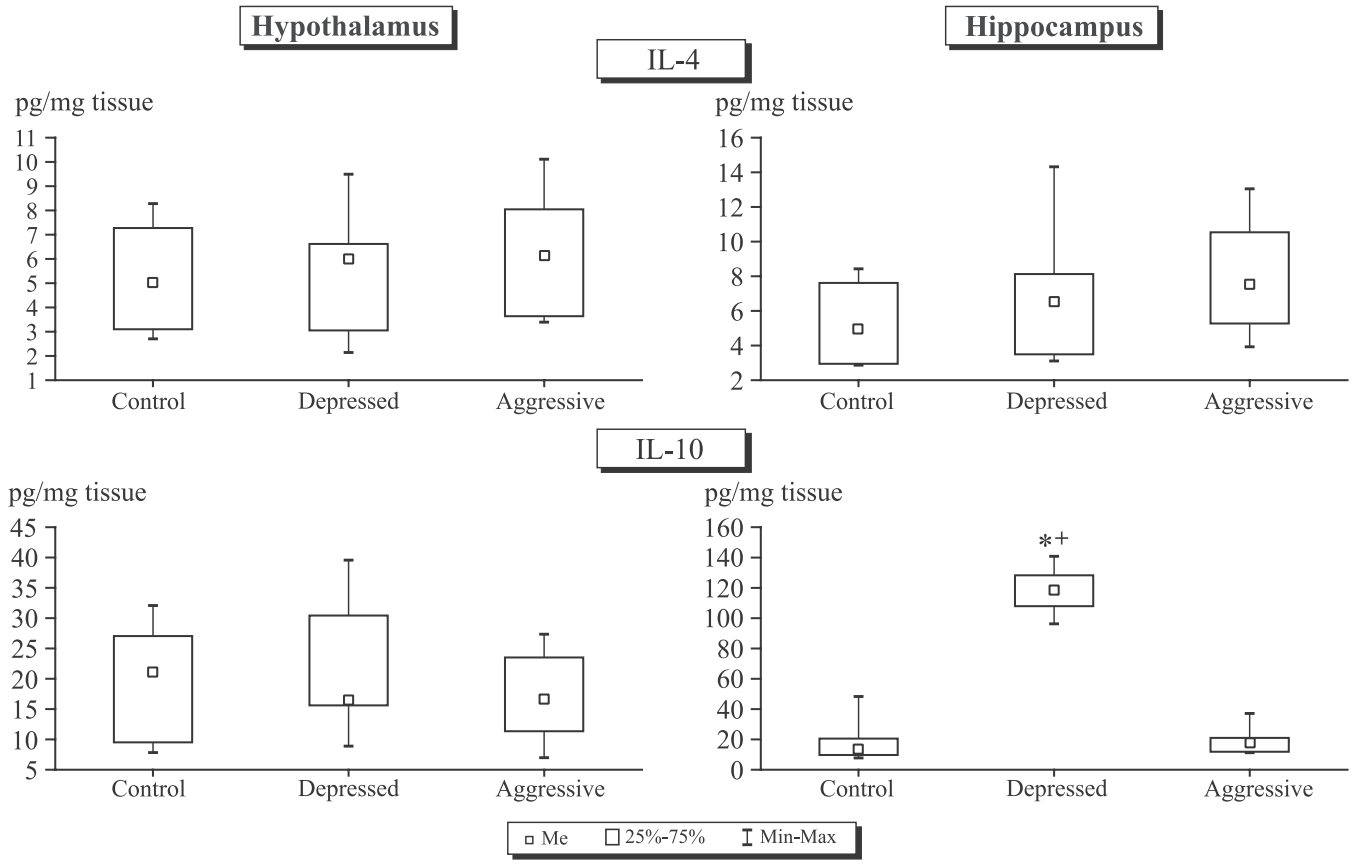

Fig. 3. Contents of IL-4 and IL-10 in the hypothalamus and hippocampus of C57Bl/6J mice with depressive-like behavior induced by 20-day social stress in 4 h after LPS (250 μg/kg) administration. *p<0.01 in comparison with the control; ^{+}p<0.01 in comparison with aggressive mice.

differences in their content in studied brain structures of animals with different types of behavior (Fig. 2).

As for anti-inflammatory cytokines, it has been shown that the level of IL-4 in mice with depressive-like behavior in both brain structures did not differ from that in control mice (p>0.05) and aggressive animals (p>0.05) (Fig. 3). The content of another anti-inflammatory cytokine IL-10 in 4 h after LPS injection considerably increased in the hippocampus of mice with depressive-like behavior in comparison with that in the control (p<0.01) and aggressive animals (p<0.01) and remained unchanged in the hypothalamus (Fig. 3).

Thus, the content of proinflammatory cytokines in 4 h after LPS injection was changes only in the hippocampus of mice with depressive-like behavior, the levels of IL-1β, IL-6, TNFα increased and the concentration of IFNγ decreased in comparison with the control. The levels of IL-2 and IL-17 remained unchanged.

Interestingly, depressive phenotype of mice was associated with increased levels of IL-6, the key cytokine of depression, not only in the brain (the hippocampus), but also at the periphery (blood serum) and as was shown earlier in the spleen [8]. However, the content of IL-10 in the serum and spleen of depressed animals was reduced and in the brain elevated in comparison with the control.

The effects of proinflammatory cytokines are regulated by anti-inflammatory factors, including IL-10 [13]. According to published data, the increase in the level of proinflammatory cytokine is accompanied by a compensatory increase in IL-10 production aimed at suppression of inflammation not only at the periphery, but also in CNS [1,13].

It is known that various clinical forms of depression and depressive-like behavior in animals are associated with morphological (abnormal structure of dendritic spines, smaller volume of the structure, astrocyte atrophy, and microglia activation) and metabolic (activation of the kynurenine pathway of tryptophan degradation) changes in the hippocampus as well as impairment of neuronal plasticity and hippocampal neurogenesis [4,12], and cytokines are involved all these processes [7,9].

The absence of significant changes in the cytokine profile in the hypothalamus can be explained by different dynamics of cytokine content in different brain structures, while in our study, only one time point after LPS administration was analyzed.

Thus, depressive behavior formed under conditions of chronic social stress is characterized by a peculiar pattern of changes in the content of pro- and anti-inflammatory cytokines in the hippocampus. It is known that changes in the cytokine profile important for the development of depression can occur, in addition to the hippocampus and hypothalamus, in other brain structures, *e.g*. in the prefrontal cortex, also involved in the formation of this pathology, which requires further study.

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