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# ИММУНОКОМПЕТЕНТНЫЕ КЛЕТКИ В КАЧЕСТВЕ ПОТЕНЦИАЛЬНОГО ТЕРАПЕВТИЧЕСКОГО АГЕНТА В ЛЕЧЕНИИ ДЕПРЕССИИ

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**Резюме.** Иммунная и нейроэндокринная системы играют решающую роль в поддержании динамического гомеостаза в нормальных условиях и при психической дезадаптации. Психо- и иммунопатология тесно взаимосвязаны: патологические изменения в функционировании обеих систем происходят одновременно и взаимозависимы. Депрессия, как психическое расстройство, – серьезная проблема общественного здравоохранения. Экспертные оценки показывают рост заболеваемости депрессией в будущем. Однако применяемая в настоящее время терапия депрессии не обеспечивает полного излечения. Известно, что нарушение нейроиммунного взаимодействия является существенным звеном в патогенезе заболевания, негативно влияя на его течение, ухудшая клиническую картину, снижая эффективность терапии, поэтому актуален поиск нового подхода к лечению. Имеется достаточное количество данных о ведущей роли иммунных клеток и их биологически активных продуктов в патогенезе депрессии. Однонаправленное действие большинства психоактивных препаратов на центральную нервную систему и иммунную систему подтверждает межсистемную взаимную регуляцию и позволяет рассматривать иммунные клетки в качестве модельных объектов для влияния на межсистемную функциональную взаимосвязь; в силу чего, клеточная иммунотерапия может быть методом выбора при лечении депрессивных расстройств. Мы впервые продемонстрировали возможность направленной регуляции поведения экспериментальных животных путем трансплантации иммунных клеток с определенными функциональными характеристиками, в том числе с функциональной активностью, модулированной экстракорпорально психоактивным веществом. Основываясь на предыдущих результатах, мы исследовали влияние иммунных клеток, модулированных *in vitro* кофеином, на иммунный и поведенческий фенотипы у депрессивно-подобных сингенных реципиентов. Было показано, что трансплантация прекультивированных с кофеином спленоцитов депрессивно-подобных доноров вызвала редактирование депрессивно-подобного поведения у сингенных реципиентов, что проявилось в снижении ангедонии, стимуляции ориентировочно-исследовательского поведения в тесте «открытое поле» и двигательной активности в тесте вынужденного плавания по Порсолту. Поведенческие изменения у депрессивно-подобных реципиентов после клеточной трансплантации регистрировались на фоне снижения уровня провоспалительных цитокинов (TNFα, IL-1β, IL-6, IFNγ) и увеличения уровня IL-10 в патогенетически значимых для депрессивно-подобного состояния структурах головного мозга (гиппокампе, гипоталамусе, фронтальной коре, стриатуме), что свидетельствует о снижении нейровоспаления. Выявлена также модуляция функциональной активности

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иммунной системы реципиентов. Цитокин-опосредованные механизмы редактирования депрессивно-подобного поведения модулированными *in vitro* кофеином иммунными клетками обсуждаются.

*Ключевые слова: нейроиммунное взаимодействие, депрессивно-подобное поведение, иммунокомпетентные клетки, кофеин, мозг, цитокины*

# IMMUNE CELLS AS A POTENTIAL THERAPEUTIC AGENT IN THE TREATMENT OF DEPRESSION

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**Abstract.** The immune and neuroendocrine systems play a critical role in maintaining a dynamic homeostasis in normal conditions and at mental maladaptation. Psycho- and immunopathology closely interrelated: pathological changes in the functioning of both systems occur simultaneously and are interdependent. Depression, as a mental disorder, is a major public health concern. The estimations are showing rise of the depression's incidence in the future. However, currently used therapy of depression doesn't provide a complete cure. It is known that a violation of neuroimmune interaction is an essential link in the pathogenesis of the disease, having a negative impact on its course, making the clinical picture worse, reducing effectiveness of the therapy, therefore, it's urgent to search for a new treatment approaches. There are a sufficient amount data on the immune cells and their biologically active products leading role in the pathogenesis of depression. The unidirectional effect of most psychoactive substances on the central nervous system and the immune system confirms intersystem mutual regulation and allows considering the immune cells as model objects for influencing the intersystem functional relationship; so, cells immunotherapy can be the method of choice in the treatment of depressive disorders. We first demonstrated the possibility of animal's behavior directed regulation by the transplantation of immune cells with definite functional characteristics, including those with functional activity modulated extracorporeally by a psychoactive substance. Based on the previous results we investigated the effect of the *in vitro* caffeine- treated immune cells on the behavior and immune phenotypes in depressive-like singeneic recipients. Transplantation of caffeine-treated splenocytes from depressive-like donors has been shown to induce depressive-like behavior editing in syngeneic recipients, which was manifested in anhedonia decrease, stimulation of exploratory behavior in the Open Field test and motor activity in the Porsolt forced swimming test. Recipient's behavioral changes were registered on the background of decreased brain pro- inflammatory cytokines (TNFα, IL-1β, IL-6, IFNγ) and IL-10 increased in some pathogenetically significant for depressive-like state brain structures (hippocampus, hypothalamus, frontal cortex, striatum), which indicates a decrease in neuroinflammation. It was also detected recipient's immune system functional activity modulation. The cytokines-mediated mechanisms of depressive-like behavior editing by the *in vitro* caffeine- modulated immune cells are discussed.

*Keywords: neuroimmune interaction, depressive-like behavior, immune cells, caffeine, brain, cytokines*

## Introduction

The immune and neuroendocrine systems play a critical role in maintaining a dynamic homeostasis in normal conditions and at mental maladaptation. Psycho- and immunopathology are closely interrelated: pathological changes in the functioning of both systems occur simultaneously and are interdependent. For decades, the consequences of acute and chronic stress caused by information overloads, environmental problems, natural disasters, man-made disasters, military clashes, interethnic conflicts, acts of terrorism, which can lead to painful "breaking" of socio-biological mechanisms of adaptation, decrease in adequacy while assessing the environment and how consequences, the formation of disorders in the psychoemotional sphere, manifested in the development of behavioral disorders, including depression. All this leads to their steady growth in modern world and an increase in the number of related somatic diseases. Depression, as a mental disorder, is a major public health concern. The estimations denostrate a rise of the depression incidence in the future. However, currently used therapy of depression does not provide a complete cure. It is known

that a disturbed neuroimmune interaction is an essential link in the disease pathogenesis, having a negative impact on its course, aggravating clinical picture, reducing effectiveness of the therapy [1, 3, 4, 5, 7, 14]; therefore, it is urgent to search for new treatment approaches. Obviously, this is due to formation of a "vicious circle", which can be broken only by normalizing the neuroimmune regulatory relationship. Cell immunotherapy can be a method of choice in treatment of depressive disorders. There is a whole body of data on the immune cells and their biologically active products exerting a leading role in the pathogenesis of depression [1, 3, 4, 5, 6, 7, 14]. The unidirectional effect of most psychoactive substances on the central nervous system and the immune system confirms intersystem mutual regulation and allows to consider the immune cells as model objects for influencing the intersystem functional relationship. Cell technologies related to critical technologies, based on manipulation of cells outside the body, as a result whereby the cells acquire a higher therapeutic potential. It is assumed that cellular immunotherapy will gain an advantage over pharmacological treatment, since the therapeutic effect will be manifested unnecessarily long-term treatment of patients and the body will be "deprived" of drug side effects. Introduced cells due to the presence of chemotactic signals have a great opportunity to enter the focus of damage. It is also important that injected cells are capable of secreting a wide spectrum factors produced at physiological concentrations and having a regulatory effect on the functional activity of both homeostatic systems, which is absolutely impossible to accomplish by using a complex of individual regulatory molecules. In addition, cellular immunotherapy will allow to overcome the resistance of patients currently manifested in psychiatry to the generally accepted psychopharmacological agents. We first demonstrated the possibility of animal behavior directed regulation by the transplantation of immune cells with defined functional characteristics, including those with functional activity modulated by a psychoactive substance, which was later confirmed by other researchers [2, 8, 9, 10, 12, 13]. It was shown also the ability of splenocytes with *in vitro* caffeine modulated functional activity to stimulate passive behavior in animals in a dose-dependent manner [12, 13].

Based on the previous results we investigated an effect of *in vitro* caffeine-modulated immune cells on the immune and behavior phenotypes in depressive-like syngeneic recipients.

## Materials and methods

The 3.5-4 months old (CBA × C57Bl/6) F1 depressive-like male mice, weighing 25-30 g were used as donors and recipients. Mice were obtained from the husbandry of the Institute of Pharmacology and Regenerative Medicine named after E.D. Goldberg, Tomsk National Research Medical Centre, Siberian Branch of the Russian Academy of Sciences). The animals were housed on a standard diet, with drinking water *ad libitum*, as well as under normal light conditions in cages of 10 mice/cage for at least 2 weeks prior to experiments. The experiment was carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the rules of laboratory practice (Order of the Ministry of Health of the Russian Federation of June 19, 2003, No. 267).

The presence in the population of (CBA × C57Bl/6) F1 mice individuals with active and passive types of behavior, representatives of which have certain structural and functional characteristics of the nervous and immune systems and various psychophysiological forms of reaction to stress factors has been shown [8, 15]. Based on the above noted, homogeneous experimental groups were formed after preliminarily testing of all mice in the "open field" followed by including only individuals with passive behavior as being least resistant to stress influences [15]. Depressive-like phenotype in these mice (n = 129) was developed by modeling repeated social defeat-induced depression using the sensory contact model, as was described earlier [6, 12]. Depression-like behavior was characterized by forced swimming and "open field" tests, and via automatic measurement of sucrose preference in IntelliCage (fully automated system for behavioral and cognitive phenotyping, TSE-systems).

Immune cells for transplantation were obtained under sterile conditions from splenocyte suspension, modulated *in vitro* with caffeine and injected intravenously to syngeneic recipients, as was described earlier [12]. In the control group of animals, immune cell preparation and transplantation were carried out under the similar experimental conditions; the only exception was that the last were cultured without caffeine. Behavioural pattern of depression-like recipients 48 hours after the immune cell transplantation was evaluated in the "open field" test (locomotion, exploration and anxiety) and Porsolt forced swimming test (depression-like behaviour) using a modern hardware and software complex EthoVision XT (Noldus Information Technology, The Netherlands). Anhedonia was assessed in the sucrose preference test based on individual consumption of a 1% sucrose solution and water under *ad libitum* conditions for 10 days in IntelliCage.

Lysates of various brain regions (hippocampus, hypothalamus, striatum, frontal cortex) were obtained from recipient mice by homogenisation in RPMI-1640 medium supplemented with 0.1% Triton X-100,

followed by centrifugation (10,000 rpm, 3 min). Cytokine (IL-1β, IL-2, IL-4, IL-6, IL-10, TNFα, IFNγ) concentrations were measured in supernatants by ELISA using relevant kits (R&D Systems, UK), according to the manufacturer's instructions.

Statistical data analysis was performed by using an analytics software portfolio Statistica 10.0 for Windows (StatSoft, Tulsa, OK, USA). Normally distributed data with low variance were analysed using Student's t-test; in case of no normal distribution, Mann–Whitney U test was applied. Results are presented as mean (M) and standard error of the mean (SE) (M±SE). $P < 0.05$ was considered statistically significant.

## Results and discussion

Anhedonia is considered as one of the main symptoms of major depression in humans, also being the main sign of depression in experimental models and estimated by decreased animal consumption of sucrose solution. Assessing mouse behavior in Intelli Cage allowed to find that depressive-like recipients after the transplantation of caffeine-modulated immune cells in a free choice demonstrated increased consumption and preference of 1% sucrose solution, as compared with the control group [11, 12].

Behavioral despair in mice is a primary screening test for antidepressants. Forced swimming test revealed in depressive-like recipients a significantly increased mobility and high mobility time, decreased periods of passive swimming (drift + complete immobility) with the disappearance of complete immobility periods in water. Differences in these indicators between the control and experimental groups of depressive-like recipients were recorded within 10 days after cell transplantation and were associated with the stimulation of neurogenesis in the hippocampus [11, 12].

**TABLE 1. DEPRESSIVE-LIKE RECIPIENTS (CBA × C57Bl/6) F1 BEHAVIORAL PARAMETERS IN THE OPEN FIELD TEST (M±SD)**

| | Horizontal activity | | | Vertical activity |
|---|---|---|---|---|
| | Number of crossed peripheral square | Number of crossed central square | total | Rearing postures (total) |
| **Control (n = 61)** | 3.2±2.8 | 0 | 3.2±2.8 | 0.2±0.9 |
| **Experimental (n = 68)** | 103.0±49.9* | 0.7±2.4* | 103.7±46.4* | 2.9±3.2* |

**Note. Note. Control, group of mice-recipients after the transplantation of splenocytes pre-cultured without caffeine. Experimental, group of mice-recipients after the transplantation of caffeine-treated splenocytes; testing period – 5 min; * p < 0.01, as compared to control.**

**TABLE 2. CYTOKINES CONTENTS IN VARIOUS BRAIN STRUCTURES IN DEPRESSIVE-LIKE RECIPIENTS (CBA × C57Bl/6) F1 AFTER THE CAFFEINE-TREATED IMMUNE CELLS TRANSPLANTATION, M±SE**

| Brain structures | IL-1β | IL-4 | IL-6 | IFNγ | IL-10 | IL-2 | TNFα |
|---|---|---|---|---|---|---|---|
| ***Hypothalamus*** | | | | | | | |
| **Control** | 233.5±29.8 | 15.5±5.8 | 1261.0±195.1 | 225.3±25.5 | 19.9±6.9 | 16.9±6.6 | 0 |
| **Experimental** | 124.7±18.9* | 23.2±6.6 | 732.1±142.2* | 106.1±13.6* | 26.7±7.6 | 19.8±6.9 | |
| ***Hippocampus*** | | | | | | | |
| **Control** | 377.2±38.1 | 19.1±6.2 | 1863.0±255.3 | 352.9±38.3 | 5.21±1.50 | 39.5±8.9 | 10.6±4.1 |
| **Experimental** | 245.8±31.0* | 33.9±7.7 | 815.7±150.6* | 132.6±16.3* | 26.1±7.6* | 48.8±9.8 | 1.0±1.0* |
| ***Frontal cortex*** | | | | | | | |
| **Control** | 274.5 ±33.8 | 25.7±6.9 | 2012.0±270.2 | 273.8±30.4 | 29.9±7.9 | 24.2±2.4 | 0 |
| **Experimental** | 206.6±27.1 | 24.5±6.7 | 1992.0±268.2 | 98.3±12.8* | 33.3±8.3 | 46.8±3.6 | |
| ***Striatum*** | | | | | | | |
| **Control** | 70.3±13.4 | 14.7±5.8 | 836.7±152.7 | 92.9±12.3 | 7.7±5.8* | 22.7±7.2 | 4.3±5.4 |
| **Experimental** | 57.8±12.2 | 13.7±5.7 | 848.9±153.9 | 83.3±11.3 | 26.0±7.2 | 31.6±8.1 | 0.0±5.0 |

**Note. As for Table 1. n = 12 in each group; * p < 0.05, as compared to control (Mann–Whitney U Test).**

We have shown also that *in vitro* caffeine-modulated immune cell transplantation in depressive-like recipients caused stimulation of motor and exploratory activities in the "open field"test (Table 1). Hence, caffeine-treated immune cell transplantation caused the behavioral phenotype editing, which was manifested in the anhedonia decrease, stimulation of exploratory behavior and motor activity in the forced swimming test.

Scientific community widely debates about phenomenon of "cytokine-induced depression". A number of cytokines, in addition to immunological effects, may cause symptoms of depression. Such effects are typical for signaling molecules, providing conjugation cascade mechanisms of inflammation and immune response. These cytokines affect the nervous system functional activity, being one of depression-related pathogenic factors [1, 4, 5, 6, 7, 14]. Behavioral changes mentioned above after the caffeine-treated immune cell transplantation were accompanied by decreased levels of several pro- inflammatory cytokines (TNFα, IL-1β, IL-6, IFNγ) and increased IL-10 in some brain structures pathogenetically significant for depressive-like state, which indicates a decreased neuroinflammation (Table 2).

Caffeine-treated immune cell transplantation also led to recipient immune system functional activity modulation expressed in stimulating the immune response, the proliferative activity of splenocytes and reducing tryptophan catabolism therein [11]. A decreased production of pro-inflammatory cytokines (IL-1β, IL-6 spontaneous production, IL-6, IFNγ and TNFα mitogen-induced production), increased production of IL-2 and anti-inflammatory cytokines IL-4, IL-10 by depressive-like recipient spleen cells (data not shown) were observed. Multiple studies have reported that baseline levels of certain cytokines or cytokine changes during treatment with specific antidepressants were associated with antidepressant treatment response [4, 7]. Hence, the modulation in the central and peripheral cytokine production may be one of the mechanisms for immune and behavior phenotypes editing after the *in vitro* caffeine-treated immune cells.

## Conclusion

The current data demonstrated that *in vitro* caffeine-modulated immune cells in depressive-like recipients, affecting the main pathogenetic mechanisms of depression, have a positive psychoneuroimmunomodulating effect, which determines the possibility and prospects of immunotherapy of depression by autologous immune cells with modulated *in vitro* functional activity.

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