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КОРРЕКЦИЯ АГРЕССИВНОГО ПОВЕДЕНИЯ ТРАНСПЛАНТАЦИЕЙ МОДУЛИРОВАННЫХ *IN VITRO* ИММУНОКОМПЕТЕНТНЫХ КЛЕТОК

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Резюме. Агрессия – серьезная биомедицинская проблема, связанная с большим процентом пациентов и отсутствием селективных корректирующих средств. Наиболее часто повышенная агрессивность наблюдается у пациентов с депрессивными расстройствами, шизофренией, реактивными психозами и расстройствами адаптации, которые, как известно, характеризуются иммунологической дисфункцией. Нейролептики достаточно широко используются в клинической практике для коррекции психомоторного возбуждения: антипсихотическое действие указанных препаратов проявляется в достижении седативного эффекта. Однако, как и другие психоактивные вещества, они обладают рядом побочных эффектов, ограничивающих их длительное использование, что ограничивает их длительное применение и определяет необходимость поиск новых подходов к коррекции аффективных расстройств. Экспериментальное моделирование агрессии – один из основных подходов к изучению ее патогенетических механизмов и поиску новых эффективных средств для терапии. Изучение патогенетических механизмов агрессии и поиск подходов к ее терапии в рамках нейроиммунного взаимодействия в настоящее время является чрезвычайно перспективными. Имеется большое количество клинических и экспериментальных данных, указывающих на взаимосвязанные изменения функциональной активности нервной и иммунной систем при агрессии. Ведущим звеном патогенетического механизма агрессии является нарушение выработки и взаимной регуляции цитокинов, нейротрансмиттеров, нейропептидов, факторов роста, гормонов, действие которых опосредуется клеточными элементами иммунной системы. Существенная роль иммунокомпетентных клеток в патогенезе агрессии, равно как и однонаправленное действие большинства психоактивных препаратов на клеточные элементы иммунной и нервной систем, позволяет рассматривать иммунокомпетентные клетки в качестве модельного объекта для воздействия на межсистемные функциональные связи для редактирования агрессивного фенотипа. Целью настоящего исследования было изучение влияния трансплантации модулированных *in vitro* нейролептиком иммунокомпетентых клеток на поведенческий фенотип и содержание цитокинов в головном мозге агрессивных сингенных реципиентов. Агрессивное поведение было сформировано у активных мышей-самцов (СВА × С57Bl/6) F1 в результате опыта 20-кратных побед в межцовых конфронтациях (метод парного дистантного сенсорного контакта). Спленоциты агрессивных мышей обрабатывали *in vitro* хлорпромазином и внутривенно вводили сингенным агрессивным реципиентам. Было продемонстрировано, что модулируемые *in vitro* хлорпрома-

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зином спленоциты агрессивных мышей после трансплантации редактируют поведение агрессивных сингенных реципиентов на фоне снижения в патогенетически значимых для агрессии структурах головного мозга цитокинов IL-1β, IL-2, IL-6, IFNγ и повышения уровня IL-4. Механизмы коррекции агрессивной стратегии поведения модулированными *in vitro* нейролептиком иммунокомпетентными клетками обсуждаются.

Ключевые слова: агрессивное поведение, иммунокомпетентные клетки, хлорпромазин, структуры головного мозга, цитокины

AGGRESSIVE BEHAVIOR CORRECTION BY THE TRANSPLANTATION OF *IN VITRO* MODULATED IMMUNE CELLS

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Abstract. Aggression is a serious biomedical problem associated with a high percentage of patients and a lack of selective corrective agents. The most frequent increase in aggressiveness occurs in patients with depressive disorders, schizophrenia, reactive psychoses and adjustment disorders, which are known to be characterized by immunological dysfunction. Antipsychotics are widely used in the correction of psychomotor agitation; the antipsychotic effect of these drugs is manifested in the achievement of a sedative effect. However, like other psychoactive substances, they have a number of side effects that limit their long-term use and determines the need to search for new approaches to the correction of affective disorders. Experimental modeling of aggression is one of the main approaches for studying its pathogenetic mechanisms and searching for new effective therapeutic agents for the treatment. The study of the aggression pathogenetic mechanisms and the search for approaches to therapy within the framework of neuroimmune interaction is currently extremely promising. Currently, there is a large number of clinical and experimental data indicating interrelated changes in the functional activity of the nervous and immune systems during aggression. The leading links in the pathogenetic mechanism of aggression is the violation of the production and mutual regulation of cytokines, neurotransmitters, neuropeptides, growth factors, hormones, the effects of which are mediated by the cellular elements of the immune system. Given the immune cells essential role in the pathogenesis of aggression and the psychoactive substances unidirectional effect on the immune and nervous cells, make it possible to consider immune cells as model objects for influencing the intersystem functional relationship in order to edit the aggressive phenotype. The aim of the study was to investigate the effect of *in vitro* neuroleptic-modulated immune cells transplantation on behavioral phenotype and brain cytokines in aggressive syngeneic recipients. Aggressive behavior was formed in active male mice (CBA × C57Bl/6) F1 as a result of the experience of 20-fold victories in inter-male confrontations (distant sensory contact model). Aggressive mice splenocytes were treated *in vitro* with chlorpromazine and intravenously injected to syngeneic aggressive recipients. It has been demonstrated that modulated *in vitro* by chlorpromazine splenocytes of aggressive mice after transplantation edit the syngeneic aggressive recipient's behavior against the background of a decrease in cytokines IL-1β, IL-2, IL-6, IFNγ and an increase in IL-4 in pathogenetically significant for aggression brain structures. The mechanisms of the aggressive behavior correcting effect of modulated immune cells are discussed.

Keywords: aggressive behavior, immune cells, chlorpromazine, brain structures, cytokines

Introduction

In human society, increased aggressiveness is one of the main social and health problems. More than a million people around the world die each year as a result of violent clashes. Aggression is one of the leading causes of death among people aged 15 to 44 years. Moreover, according to the World Health Organization experts, 20 hospitalizations associated with physical aggression per every such death [15].

Psychoactive drugs used for its correction are quite efficient, the activity of these drugs is manifested

particularly in achieving a sedative effect. At the same time, the positive sedative action of neuroleptics is accompanied by a number of side effects, such as the occurrence of addiction, dependence formation, endocrine disorders, as well as the possibility of inducing late psychoses (psychoses of dopamine hypersensitivity) that occur due to their prolonged use and aggravated course of the underlying disorder, which limits a potential to apply antipsychotics and determines the search for new approaches to correct affective disorders. The most frequent increase in aggressiveness occurs in patients with depressive disorders, schizophrenia, reactive psychoses and adjustment disorders, which are known to be characterized by immunological dysfunction [13, 14, 15]. While using experimental models of various forms of aggression, it was also revealed that an aggressive state is characterized by altered functional activity of immune system. When mice and rats were selected to model high and low aggressiveness, it was found that highly vs. low aggressive animals exhibit higher immunological reactivity [5]. Aggression formed under conditions of prolonged social stress in rats and mice of different strains is accompanied by increased primary immune response to T-dependent antigens, T-cell proliferation, redistribution of T-lymphocyte subpopulations in the bone marrow, blood and spleen. Moreover, it was found that immune dysfunction associated with impaired cytokine production by the immune and brain cells can be involved in the mechanisms of aggressive behavior development [1, 3, 5, 6, 13, 14]. It is known that the immune system cells exert regulatory effect on functions of the central nervous system. It has been established that immune cells are capable of regulating behavioral reactions both in normal conditions and in neuroimmune pathology, and their products display psycho- and neurotropic effects [2, 3, 7, 8, 9, 13, 14]. The unidirectional effect of most psychoactive substances on the nervous and immune system cells suggest the possibility of using immune cells in psychiatry to correct disorders with a pronounced neuroimmune pathogenesis component.

Considering the issues noted above, **the aim of this work** was to study effect of *in vitro* neuroleptic drug-modulated immune cell transplantation on behavioral phenotype and brain cytokines level in aggressive syngeneic recipients.

Materials and methods

Four-month-old male (CBA × C57Bl/6) F1 mice were used in the study; the average weight of the animals was 20-25 grams. The animals were housed in laboratory vivarium, 10 animals per cage, for at least 2 weeks before experiments, reared on a standard diet, with drinking water ad libitum, normal light regime. 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).

Considering the presence of individuals with active and passive types of behavior in the male population (CBA×C57Bl/6) F1 characterized by certain structural and functional characteristics of the nervous and immune systems and different psychophysiological responses to stressful influences [7, 10 12], in order to form homogeneous experimental groups of animals, all mice were pre-tested in the "open field" and only those showing an active type of behavior were included in the study. Aggressive behavior in active mice was formed under conditions of prolonged social stress: experience of victories in inter-male confrontations for 20 days (distant sensory contact model) as described earlier [6].

Splenocytes from aggressive mice were isolated under sterile conditions and treated *in vitro* with chlorpromazine (15×10^6 cells/150 μg drug) for 25 minutes. The concentration of the drug used for cell culture was determined by recalculating the therapeutic dose, taking into account the body weight and metabolic characteristics of animals, as well as preliminary testing [8, 11]. Next, following 3 washouts, splenocytes pre-cultured with chlorpromazine were intravenously inoculated to syngeneic aggressive recipients at a concentration of 15×10^6 cells dissolved in 0.3 ml of saline per animal. In the control group, the preparation and transplantation of splenocytes were carried out under similar experimental conditions, without adding chlorpromazine.

Recipients behavioral phenotyping consisted of assessing exploratory behavior (EB) in the Open Field test, as described earlier [7] and behavior in Porsolt test using a modern hardware and software complex EthoVision XT (Noldus Information Technology, The Netherlands).

Cell proliferative activity after the *in vitro* chlorpromazine treatment was assessed by a standard radioactive label (H3-thymidine) incorporation in the nucleoprotein fractions of cells.

The cytokine profile was assessed by ELISA for mouse cytokines manufactured by R&D Systems (Great Britain), according to the manufacturer's instructions.

Statistical data analysis was performed using an analytics software portfolio Statistica 10.0 for Windows (StatSoft, Tulsa, OK, USA). Normally distributed data with low variance were analyzed by using Student's t-test; in case the data were not normally

distributed, Mann–Whitney U test was applied. Results are presented as mean (M) and standard error (SE) (M±SE). $P \leq 0.05$ was considered statistically significant.

Results and discussion

Previously, the features of the aggressive mice immune cells functional activity were described [6]. Splenocyte pre-exposure to chlorpromazine modulated their functional activity, revealed as significantly decreased spontaneous proliferative activity (628.9±204 cmp/min and 144.0±47.6 cmp/min in the control and experimental groups respectively; $p < 0.05$).

It has been shown that repeated experience of aggression, accompanied by victories, leads to changed behavior in male mice with increased motor activity, irritability, severe anxiety, and the appearance of stereotypies [4, 11, 12, 14]. Transplantation of chlorpromazine-modulated splenocytes in aggressive mice-recipient was accompanied by changed EB parameters, manifested as decreased horizontal motor activity parameters, reflecting the behavioral motor component (peripheral: 144.0±13.9 and 70.7±9.9 in the control and experimental groups, respectively; central: 8.0±4.0 and 0.4±0.1 in the control and experimental groups, respectively; total: 152.0±17.9 and 71.1±10.1 in the control and experimental groups, respectively; $p < 0.01$), and vertical motor activity, reflecting the exploratory component of the behavior (free postures: 0.8±0.2 and 0.2±0.5 in the control and experimental groups, respectively; sideways: 2.2±1.1 and 0.7±0.1 in the control and experimental groups, respectively; total vertical activity: 2.9±1.3 and 1.0±0.6 in the control and experimental groups, respectively. $p < 0.01$). At the same time, aggressive recipients also showed decreased latency period for entering the central squares of the field with a decreased emotional reactivity, recorded by the number of fecal boluses (3.7±1.2 and 1.4±1.1 in the control and experimental groups, respectively; $p < 0.05$), which indirectly indicates the anxiolytic effect of the immune cell transplantation in this group of recipients.

Assessment of the aggressive recipient's behavior in the forced swimming test showed a pronouncedly reduced periods of mobility and increased periods of passive water swimming (drift + complete immobility) [11]. The data obtained indicate aggressive behavior editing in mice-recipients.

As mentioned above, cytokines are involved in the central mechanisms of various behavioral reactions regulation and significantly contribute to the development of mental disorders. The aggressive behavior strategy formation correlates with changes in cytokine profile in some brain structures, such as

TABLE 1. CYTOKINES CONTENTS IN VARIOUS BRAIN STRUCTURES IN AGGRESSIVE MICE-RECIPIENTS (CBA × C57Bl/6) F1 AFTER THE CHLORPROMAZINE-MODULATED IMMUNE CELLS TRANSPLANTATION, M±SE

Brain structures	Cytokines (pg/ml)				
	IL-1β	IL-4	IL-6	IFNγ	IL-2
Hypothalamus					
Control	220.05±28.40	15.65±5.90	1350.4±203.8	217.4±14.7	24.01±7.41
Experimental	189.79±25.40	22.41±5.60*	1900.1±259.3	172.94±18.03*	27.71±7.77
Hippocampus					
Control	208.83±27.30	21.07±6.40	1760.5±245.4	234.16±26.40	59.17±10.90
Experimental	102.9±16.7*	20.08±6.30	1190.4±117.2*	158.27±18.80*	28.93±7.89*
Frontal cortex					
Control	212.36±27.60	25.78±6.90	1830.1±251.5	231.74±26.2	27.22±7.72
Experimental	132.66±29.70*	25.55±6.60	1940.8±262.5	226.40±25.6	19.84±6.98
Striatum					
Control	163.35±22.70	23.18±6.60	1740.0±242.7	226.13±25.60	49.34±9.93
Experimental	175.03±23.90	24.11±6.70	2004.0±272.8	205.67±23.60	41.47±9.14

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

the hypothalamus, hippocampus, striatum and frontal cortex; particularly increased levels of IL-1β, IL-2, and IL-6 has been shown in mice dominating in inter-male collisions [1, 4].

We have shown that behavioral changes in aggressive recipients mentioned above were accompanied by changes in the cytokines content in some brain structures: IL-1β, IL-2, IL-6, IFNγ levels in the hippocampus were decreased; IL-4 level in the hypothalamus was increased and IFNγ – decreased; decreased IL-1β level in the frontal cortex was recorded (Table 1).

Opposite changes in the brain cytokines during formation of behavioral aggressive strategy and its arrest by chlorpromazine-modulated splenocytes testify in favor of the cytokine-mediated psycho-neuromodulating effect bound to transplanted immune cells. Changes in the activity of the 5-HT and DA-systems playing an important role in the psychoneuroimmunomodulation are observed in the brain of animals with long-term experience of aggression [4, 12, 13]. Taking into account the well-known cytokine effect on the 5-HT and DA-systems activities [3, 4, 13], it can be assumed that the key cytokine modulation after the immune cell transplantation changing the brain neurochemical system reduces aggressive manifestations.

So, chlorpromazine-modulated immune cells have a positive aggressive behavior editing effect being involved in the mechanisms underlying the development of aggressive reactions.

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