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 поиска 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 нейромедуцинского взаимодействия в настоящее время является чрезвычайно перспективным. Имеется большое количество клинических и экспериментальных данных, указывающих на взаимосвязь 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, действием которых опосредуется клеточными элементами иммунной системы. Существенная роль иммунокомпетентных клеток в патогенезе агрессии, равно как и однородное 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 иммунокомпетентных клеток на поведенческий фенотип и содержание цитокинов в головном мозге агрессивных сингенных реципиентов. Агрессивное поведение было сформировано у активных мышей-самцов (CBA × C57Bl/6) F1 в результате опыта 20-кратных побед в межцовых конфронтациях (метод парного дистанционного сенсорного контакта). Спленоциты агрессивных мышей обрабатывали in vitro хлорпромазином и внутривенно вводили сингенным агрессивным реципиентам. Агрессивное поведение было сформировано у активных мышей-самцов (CBA × C57Bl/6) F1 в результате опыта 20-кратных побед в межцовых конфронтациях (метод парного дистанционного сенсорного контакта). Спленоциты агрессивных мышей обрабатывали in vitro хлорпромазином и внутривенно вводили сингенным агрессивным реципиентам. Было продемонстрировано, что модулируемые in vitro хлорпрома-
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 charac-
terized 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 un-
der 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 mi-
ces 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
(Version 2008, The Hague, 1951), 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 therapeu-
tic 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 chlo-
ropromazine 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 dis-
tributed 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 ≤ 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 stereotypes [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

| 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 | 228.40±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 intermale 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 psychoneuromodulating 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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