UDC 612.112:612.176 (045)

LYMPHOCYTIC CHALONES UNDER STRESS CONDITIONS

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It was shown that the production and activity of lymphocytic chalones - inhibitors of lymphocyte proliferation when exposed to stress factors was depended on the nature of stress factors, dose and timing of their actions.

Keywords*: lymphocytic chalones, lymphocytes, stress.*

Stress is a general biological adaptive reaction of the organism. Stress – is a complex phenomenon that is specific for all living organisms, as well as the reaction of the organism to various emergencies of external and internal nature.

Stress – translated from English means "tension" the state of disturbed homeostasis and the factors that cause this condition are stressors. The body meets the stress with stress-reaction, which is aimed at restoring homeostasis and maintaining normal life activity. The founder of the stress theory is a Canadian scientist, physiologist and pharmacologist Hans Selye [18, 19]. He offered to divide the stress on "eustress" (good stress) and "distress" (bad stress), hard, long and exhausting. Through his experiments, H. Selye described stress as a tension reaction that develops gradually and goes through the following stages: 1/ anxiety; 2/ resistance stage (resistance build-up period and resistance decline period); 3/ exhaustion stage. H. Selye defined stress reaction a "general adaptation syndrome", characterized by specificity and non-specificity. Specificity is manifested through the same type reaction of thymus, spleen, lymph nodes, adrenal glands and other organs, and non-specificity is caused by different factors acting on the body [1, 2, 18, 19]. The doctrine of Selye H. on stress is the way to solve the problem of general biological interaction with the environment [3, 12, 15, 16].

Adaptive responses that are aimed at supporting constant internal environment occur in complex of multicomponent and inter-system processes. It is believed that basic systems that support the adaptation of the organism are nervous, endocrine and immune.

There are two major classes of stress responses: physiological and psycho-emotional. Among physiological stress factors there are distinguished mechanical (burns, wounds, trauma, and surgery), physical (cooling, radiation, etc.), chemical (pesticides, toxic substances, heavy metals) and biological (protozoa, viruses, bacteria, fungi, toxins alien cells) [1–3, 5].

Stressful changes affect adaptive (protective) systems of the organism, among which is the immune system. According to H. Selye believe, "the immune system, among others present the somatic expression of mobilization of the organism defenses". Formation and maturation of the immune system and nonspecific resistance factors begin with the early timing of prenatal development and continues throughout the period of pregnancy and after birth. Stress manifestations in terms of the whole organism are very diverse and depend, apparently, on many factors, among which the first place take: the depth and duration of stress loads; initial state of adaptive systems of the body, its backup capabilities, among the latter, to our opinion, not the last role is played by such factors as age, gender, functional status of the nervous system, type of food and habitat place [4, 5, 12, 15, 16].

All data on the effect of stress on the immune competence of the organism can be divided into three major groups: stimulating, depressing and complete absence of them. Most sensitive to the stress load is a T-cell immunity. The reasons for this phenomenon are embedded apparently to a lesser resistance to the T-lymphocytes by glucocorticoid hormones comparison with B-lymphocytes.

Early period of stress – "reaction of anxiey" – characterized by a redistribution of lymphocytes, which is expressed in the devastation of thymus, spleen and lymph nodes [4, 5, 15, 20]. At this stage of the "urgent adaptive reaction" it may be an increase of the functional activity of cells of lymphoid lineage, the intensification of cooperative interactions, increase of the content of stem cells in the bone marrow.

Probably immunostimulatory effect in the early stages of development of stress is caused by suppression of T-regulators (suppressors), that is evidenced by their high sensitivity to the action of elevated concentrations of glucocorticoids. At this stage, there may be an increase of autoimmune pathologies (allergic diseases, rheumatoid arthritis, etc.).

If the stressor by its power is not ultraboundary for the organism, then the "stage of resistance" comes, in which the organism, "induced its backup capabilities," able to withstand to adverse impacts. Often repetitive stress reaction takes it to the stage of exhaustion. Populations of immunocompetent cells differ by no the same resistance to damaging stress factors [4, 5, 11, 20].

Studies of mane authors have shown the reduction in the number of T-lymphocytes, disruption of normal relations between the T-and B-lymphocytes and macrophages, accompanied by a decline of immune potency of the whole organism [4, 5, 15, 16, 20]. Later the changes occur in nonspecific defense reactions, functional activity of macrophages decreases, though the levels of immunoglobulin can remain at normal levels.

For long-term expressed effects of stress the depression covers all major subset of T lymphocytes (T-killer cells, T-suppressor regulators, T-helpers), which under severe trauma, surgical interventions and deep psycho-emotional stress is considered as a poor prognostic sign. Further deepening of stress disorders is accompanied by failures in the activity of B-lymphocytes: reduce of functional activity of B-lymphocytes, reduce of the intensity of proliferative processes, reduces the number of B-cells in the lymphoid organs and, of course, reduce of immunoglobulin levels in the blood until their complete disappearance [15, 20].

Disturbances of cooperative processes of immunocompetent cells under stress may be a result of suppression of the functional activity of these cells, or the effect of suppressing the synthesis and secretion of cytokines (interleukins, interferons, etc. [4, 5, 8, 9, 11, 15, 16, 20].

Lymphocytic chalone and immune homeostasis. In 1970–1980 a new class of biologically active substances – chalones derived from 20 kinds of tissues was

revealed. A number of studies (Bullough W.; Garcia-Giralt E.et al.; Mathe G.; Neustroev G.; Ketlinskaya S. et al.; Y.Romanov et al.) describe chalones properties: they are exempt from differentiated cells and inhibit their proliferation. The action of chalones is tissue-specific and phase-specific but not species-specific. Chalones are not toxic, their action is reversed. All this indicates general biological character of chalones regulation directed to support interstitial homeostasis and shows the universality of chalones regulation of cell proliferation.

Lymphocytic chalones were discovered in 1970 (Mathe G.; Kiger N.et al.; Garcia-Giralt E et al.; Ketlinskaya S.A. et al, Morgunov I. et al; E. Garkava). It was shown that the spleen and thymus chalones inhibit proliferation of PHA-stimulated lymphocytes in vitro, decrease lymphocyte proliferation in the follicles of the spleen, lymph nodes, the number of retarded plaque forming cells prolongs graft transplants leather. Like all chalones, lymphocytic ones have tissue specificity but not species specificity.

Lymphoid tissue is one of the first to react on the stress. Thus, under the experimental conditions it was demonstrated that administration of lymphocytes to healthy animals from animals that are under stress cause changes similar to stress reaction [15, 20]. It was found that immune cells have receptors for many stress mediators: catecholamines, serotonin, endorphins, corticosteroids and others [11, 15, 20]. It was also noted that under stress in the organism there is an appearance of antibodies to its own tissues, after the stressor is terminated. The most active and early immune response to stress – are cellular changes in the lymphoid tissue arising from the depression of proliferation, destruction of cells, mobilization and redistribution of lymphocytes. There is a change in the ratio of T- and B-lymphocytes in the bone marrow, spleen, lymph nodes and peripheral blood. In the early stages of the stress reaction there is reduction of mass of the thymus and spleen, a large number of lymphocytes colonize to the bone marrow and connective tissue. These modifications aim at improving the cell interaction in the immune response [5, 11, 15, 20]. There is an amplification of bone marrow hematopoiesis, significantly increases the number of stem cells in the bone marrow. Immune disorders are under

stress tend to phase character. As per the degree of immune disorders, the outcome of stress situation can indirectly be predicted. During the crush syndrome in the first three days there was a decrease of cellular and humoral immunity, with restoration of disturbed indicators start on the 7th day. Similar changes were typical for uncomplicated disease. If on the 7th day there was of a sharp decrease in cellular immunity without a tendency to restore them under the activation of humoral immunity, the course of the disease was complicated. When there was a lethal outcome, the fall of activation of cellular and humoral immunity occurred since the first days [4, 5, 15, 20].

Heavy and prolonged stress activates the tumors growth. The protective effect of normal killer cells (lymphocytes) takes an important place in the anti-tumor immunity. In the experiment on the model of emotional and painful stress, the following patterns were revealed: after a short stress, exposure for 1–1,5 hours there was observed an increase in the activity of normal killer cells in more than two time. With increase of exposure duration up to three hours the activity of normal killers was remained elevated, but to a lesser extent. With a significant increase in the duration of stress up to 6 hours there was an observe of expressed depression in activity of normal killers. This is possible dynamics of resistance reduction to the tumor growth. Further observations revealed the restore of normal killer lymphocytes to normal level by day 7, followed by twice activation with the final normalization of their activity. Adaptation to short repetitive stress situation largely prevents the depression killer activity of normal lymphocytes [15, 20].

Resistance of organism to stress is largely determined by spare capacity of the immune and antioxidant systems[1–3, 8, 10]. In the adaptation phase, there is an increase of the T-helpers quantity, the raise of sensitivity to acetylcholine receptors and the expression of cell receptors, which stabilizes the membrane and reduces the oxidation therein. During the long and severe stress the activity of the antioxidant system is being reduced, free radicals are gaining damaging properties against immune cells, resulting in reduced content of T-and B-lymphocytes and their functional activity[1–4, 8–10, 20].

Our works done in 2000, 2001 and 2014 have shown the presence of immunoregulatory and antioxidant properties in lymphocytic chalone.

To create a model of stress the antioxidant deficiency antigenic load – immunization was used.

Model of antioxidant deficiency was created by the example of E-vitamin deficiency. First group of rats received casein diet supplemented with vitamins A, D and E during 2 months. Second group of rats did not receive vitamin E (state AON) throughout the experiment. Animals of group 3 to correct AON were administered with α -tocopherol-acetate (50mg/kg) for 4 days prior to slaughter. Research results showed that under these conditions there was an increase in lymphocytic chalone production. When used for correction of vitamin E antioxidant quantity the produce of lymphocytic chalone was also quite high and exceeded the control values by two times. Inhibitory activity of lymphocytic chalones that were removed from the spleen of experimental animals, was also relatively high as to antibody production and in control groups with AON was – 66 %, under correction with vitamin E – 54 % and 43 %, respectively [7, 9]. These experiments point to abnormalities in lymphocyte chalone system with antioxidant deficiency. The level of these disorders depends on the nature of the stress factor and the time of exposure.

Using a model of antigen load – immunization with sheep erythrocytes (T-dependent antigen) and lipopolysaccharide of *E. coli* (T-independent antigens) the following results were obtained during the development of an immune response on the first, third and fifth day. Group of immunized animals was a control group and the research group was immunized animals with administration of exogenous chalone. During the first day, in the inductive phase of the immune response, number of lymphocytic chalone was increased compared to the control by 2 times using a T-dependent antigen and remained at control levels if using *E. coli*. During the proliferative phase, the third day of the immune response the number of lymphocytic in control and experimental groups chalone was decreased, but remained at control values when using sheep erythrocytes and was 2 times higher in the experimental groups with LPS of *E. coli*. On the fifth day, the productive phase of the immune

response, the amount of lymphocytic chalone was decreased as compared to proliferative phase, but while using a different nature of the T-antigen was 2 fold above the control values [9].

After determination of the level of production of lymphocytic chalone, the experiments were conducted to assess its activity. On the first day all received chalones except chalones that seized in the experimental groups with *E. coli* LPS immunization and sheep red blood cells, DNA (synthetic activity of heterologous lymphocytes) was inhibited. On the third day the inhibition activity against heterologous lymphocytes was remained in chalones of control and experimental groups, where there was immunization only with sheep erythrocytes. Chalones of animal groups where *E. coli* LPS and chalones were used, inhibited DNA – synthetic activity of autologous lymphocytes. No action of chalones in all research groups on autologous lymphocytes could be due to change in the physico-chemical structure of the receptors to chalones or their absence on lymphocytes during the induction phase of the immune response. As not all periods of the cell cycle and G 1 - G 2 – phase are sensitive to the action of chalones, then possibly due to different mitotic cycle intact heterologous lymphocytes and transformed autologous cells such action of chalones are recorded. On the fifth day chalones of all groups slowed DNA synthetic activity of endogenous and heterologous lymphocytes. Conducted studies have shown that the activity of endogenous lymphocytic chalones in respect to DNA synthetic activity of lymphocytes and their products are varied depending on the phase of the immune response and T-dependent nature of antigens [8, 9].

Thus, production and activity of lymphocytic chalones was depended on the nature of the stress factor, the dose and timing of its action.

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ЛІМФОЦИТАРНІ КЕЙЛОНИ ЗА УМОВ СТРЕСУ

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Показано, що продукція та активність лімфоцитарних нейлонів – інгібіторів проліферації лімфоцитів за умов впливу стрес-факторів залежить від природи стрес-факторів, дози та часу їх дії.

***Ключові слова**: лімфоцитарні нейлони,лімфоцити, стрес.*

ЛИМФОЦИТАРНЫЕ КЕЙЛОНЫ И СТРЕСС

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Показано, что продукция и активность лимфоцитарных кейлонов – ингибиторов пролиферации лимфоцитов под воздействием стресс-факторов зависит от их природы, дозы и времени действия.

***Ключевые слова:** лимфоцитарные кейлоны, лимфоциты, стресс.*