ТУЧНЫЕ КЛЕТКИ ТИМУСА КАК КОМПОНЕНТ НЕЙРО-ЭНДОКРИННО-ИММУННЫХ ВЗАИМОДЕЙСТВИЙ ПРИ СТРЕССЕ

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Резюме. Тучные клетки являются обязательным компонентом микроокружения тимуса, за счет выработки ряда цитокинов они влияют на межклеточные взаимодействия и проницаемость гемато-тимического барьера. Предполагают, что тимус является местом образования и депонирования тучных клеток. Тучные клетки, находясь под сложным комплексным нейроэндокринным контролем, при формировании стресс-реакции могут играть важную роль в процессе временной трансформации тимуса, влияя в том числе на экстратимическую миграцию клеток. Цель данного исследования – оценить функциональную вовлеченность тучных клеток в процесс временной трансформации тимуса при различных гипер- и гиподинамических воздействиях на фоне формирования стресс реакции и без нее.

Эксперимент проведен на самцах крыс линии Wistar. В качестве стресс-факторов применяли физическую нагрузку (плавание) разной интенсивности и иммобилизацию (у животных с сохраненными и удаленными надпочениками), как два полярных состояния динамического стресса. На гистологических препаратах тучные клетки типировали и рассчитывали коэффициент дегрануляции и средний гистохимический коэффициент (синтетическую активность).

В группах с сохраненными надпочечниками после воздействий отмечается достоверное снижение коэффициента массы тимуса, что свидетельствует об ослаблении его функциональной активности в ответ на развитие стресса. При этом тучные клетки тимуса довольно быстро реагируют на нейроэндокринные факторы, выделяемые при стрессе, и вовлекаются в общую реакцию: их активность проявляется в синхронном снижении синтеза гранул в цитоплазме и усиленном выбросе активных веществ, накопленных ранее. В группах с удаленными надпочечниками, напротив, после иммобилизации масса и структура тимуса остаются неизменными, не выявляются и изменения морфофункциональных показателей тучных клеток. Эксперименты с гипо- и гипердинамической нагрузкой животных с сохраненными и удаленными надпочечниками свидетельствуют, что реакция тучных клеток во многом определяется гипоталамо-гипофизарно-надпочечниковой осью эндокринной системы. Удаление надпочечников (невозможность выброса глюкокортикоидов) приводит к отсутствию функционального ответа со стороны тучных клеток тимуса. Стимулирующее влияние глюкокортикоидов надпочечников на тучные клетки при стрессе осуществляется в комплексе с другими нейроэндокринными факторами (катехоламинами, кортикотропин-релизинг-гормоном, адренокортикотропным гормо-

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ном) и при формировании полноценной стресс-реакции организмом, они активно вовлекаются в процесс временной трансформации тимуса через выделение ряда цитокинов, что является важным условием для выработки адаптационных механизмов со стороны иммунной системы.

Ключевые слова: тимус, тучные клетки, стресс, иммунные клетки, адаптация, надпочечники

THYMUS MAST CELLS AS A COMPONENT OF NEURO-ENDOCRINE-IMMUNE INTERACTIONS UNDER STRESS

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Abstract. Mast cells (MCs) are a required component of the thymus microenvironment. They affect intercellular interactions and permeability of the hematothymic barrier through cytokine production. There is speculation that the thymus is the site of MCs formation and deposition. MCs are under complex neuro-endocrine control and they can play an important role in the process of acute transformation of the thymus in the formation of a stress reaction, affecting the extrathymic migration of cells. The purpose of this study is to assess the functional involvement of MCs in the process of the thymus acute transformation at various hyper- and hypodynamic effects during the formation of stress response and without it.

The study was conducted on male Wistar rats. The stress factors were physical activity (swimming) of different intensities and immobilization, which represent two opposite states of dynamic stress. MCs were classified on histological preparations; a degranulation coefficient and a mean histochemical coefficient (synthetic activity) were calculated.

In groups with preserved adrenal glands after exposure a significant decrease in the thymus mass coefficient is noted, which indicates a weakening of its functional activity in response to the development of stress. At the same time, MCs of the thymus quickly respond to neuro-endocrine factors under stress. These cells are involved in a general reaction: their activity consists in a synchronous decrease of the synthesis of granules in the cytoplasm and an increased release of active substances accumulated earlier. The mass and structure of the thymus remain unchanged in groups with removed adrenal glands after immobilization. No changes in morphofunctional indicators of mast cells were detected either. Experiments with hypo- and hyperdinamic loading of animals with preserved and removed adrenal glands indicate that the MCs response is largely determined by the hypothalamic-pituitary-adrenal axis of the endocrine system. Removal of the adrenal glands (inability to release glucocorticoids) leads to a lack of functional response from the thymus MCs. The stimulating effect of adrenal glucocorticoids on MCs under stress is carried out in combination with other neuro-endocrine factors (catecholamines, corticotropin-releasing hormone, adrenocorticotropic hormone). When this axis is activated and a full-fledged stress reaction is formed by the body, MCs are actively involved in the process of acute transformation of the thymus through cytokine secretion. These is an important condition for the development of adaptation mechanisms by the immune system.

Keywords: thymus, mast cells, stress, immune cells, adaptation, adrenal glands

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Introduction

Today, cells of the thymic microenvironment are being actively studied as they play an important role in the differentiation, selection, and migration of T-lymphocytes, macrophages, epithelial, dendritic, and mast cells (MCs). The question of MCs' functions in the thymus continues to be open. There is evidence that MCs are normally involved in the processes of updating the extracellular matrix of the thymus in organogenesis, thymopoiesis, T-lymphocyte selection and angiogenesis [2, 5, 8, 9, 11]. Despite the intensive study of MCs, studies on the features of thymus mast cell population under the action of stress factors on the body and in the development of induced organ involution are rare. Acute thymus involution is known to be a manifestation of adaptation syndrome in response to stress. Under stress, the level of thymus polypeptides increases, which limits the stress-damaging effect of hypothalamic-pituitary-adrenal axis excessive activity [12]. In this case, the content of lymphoid cells in the thymus is reduced, and the number of epithelioreticulocytes and degenerating cells is increased. The researchers noted such a reaction of this organ under completely different extreme influences. It is known that MCs affect intercellular

interactions, permeability of the hematothymic barrier, and migration of thymus lymphocytes through the production of IL-1, IL-2, IL-3, IL-4, IL-6, TNFα, GM-CSF, NGF [4, 6, 10]. Additionally, it is assumed that thymus is the place of formation and deposit of MCs [8, 12]. In this regard, MCs can play an important role in the process of acute transformation of the thymus, affecting the extratimic migration of cells during the development of the stress reaction.

The purpose of this study is to assess the functional involvement of MCs in the process of acute transformation of the thymus at various hyper- and hypodynamic effects during the formation of stress response and without it.

Materials and methods

The study was conducted on male Wistar rats weighing 150-200 g. The conditions of animal housing and treatment of animals used in the experiment were in accordance with the directive of the European Community of 80/609/EEC and were approved by the local ethics committee of the IIF UrO RAS (protocol No. 10 of 03.06.2016).

The stress factors were physical activity (swimming) of different intensities and immobilization, which represent two opposite states of dynamic stress. The animals were divided into seven experimental groups, and each group consisting of five animals (n = 5).

Physical activity (PA) consisted of rats being forced to swim in a special pool under different regimens for 4 weeks: 1) moderate physical activity (MPA) – daily swimming for 2 hours, and 2) maximum physical activity (MaxPA) – 6-day swimming with a load of 20% of their body weight, according to the following scheme: 5 sets of 60 seconds with 3 minutes of rest in between. The rats' weight was measured weekly, and the weight of the load was adjusted accordingly. After the completion of all swimming batches, the animals were removed from the experiment, and their thymi were extracted.

Immobilization stress (IS) was simulated by inducing neuromuscular tension, achieved by immobilizing the animals on their back for 6 hours once on an operating table. Two series of experiments were performed. In the first series, intact animals were subjected to neuromuscular stress, while in the second series, both adrenal glands were removed (AE) in animals 48 hours prior to immobilization stress. The animals were then removed from the experiment and their thymi were extracted. The thymi were extracted in two groups – one with preserved adrenal glands and one with removed adrenal glands – within two periods: immediately after the end of immobilization (groups IS 6 h and IS + AE 6 h), and 48 hours after immobilization (groups IS 48 h and IS + AE 48 h).

Histological preparations of the thymus were stained with hematoxylin-eosin to assess structural changes in the gland. Preparations were also stained with toluidine blue (pH = 2.0) to detect MCs by highlighting acidic sulfated glycosaminoglycans in a blue-violet color, a characteristic feature of metachromasia. MCs were then stained with alcian blue – safranin to assess maturity. Safranin binds to highly sulfated glycosaminoglycans, such as heparin, staining them red or pink. The presence of such granules is an indication of mature MCs. Alcian blue, on the other hand, binds to slightly sulfated glycosaminoglycans, mainly heparin precursors, staining them blue. The presence of such granules is an indication of immature MCs or MCs at an intermediate stage of maturation.

MCs were classified based on the content of granules in the cytoplasm to assess their functional activity. "0" – MCs with diffuse arrangement of granules in the cytoplasm and a clearly visible nucleus, "+1" – cells with a denser arrangement of granules in the cytoplasm and a contoured nucleus, "+2" – cells with a high degree of granularity in the cytoplasm, the nucleus is not clearly visible, the granules are tightly adjacent to each other, "+3" – cells with a very dense content of granules in the cytoplasm, the nucleus and individual granules are not detected. Based on these data, the mean histochemical coefficient was calculated: MHCC = (0 × "0" + 1 × "+1" + 2 × "+2" + 3 × "+3") / Σ all cell types. Cells were divided into three groups based on signs of degranulation: "DG" – actively degranulating MCs with a violation of membrane integrity and observation of granules outside the cell; "PDG" – partially degranulating MCs with a large number of granules outside; "NDG" – non-degranulating MCs with integral membrane and no granules outside the cell. Based on these data, the degranulation coefficient was calculated: DC = ((DG + PDG) / Σ all cell types) × 100%.

Statistical analysis was performed using the Statistica 6.1 program. Group comparisons were made using non-parametric methods, specifically the Mann-Whitney test. Differences were considered significant at $p < 0.05$.

Results and discussion

MCs are obligatory cells of the thymus gland and are more often located in the peripheral part of the lobes around blood vessels, among the premedullary row of cells and accompany nerve fibers. The production of biogenic amines is a constant function of MCs of the thymus both normally and in pathological processes. MCs facilitate contact and transfer of stem cells and lymphocytes into tissues during circulation and promote migration of the formed lymphocytes

by secreting mediators for vascular permeability (histamine, serotonin, catecholamines, etc.).

Normally, the MCs of the thymus is quite large, of different shapes, more often rounded or oval, located in the capsule and connective branch walls (septa) of the gland, the content in 1 mm^2 is 117.9±3.62. Typing thymus MCs according to the degree of maturity, based on sulfation of glycosaminoglycans in granules, showed that 62% were mature cells and 38% were immature. Thus, more cells that have completed the synthesis, accumulation, and maturation of granules with biologically active substances are ready to show their activity by degranulating in response to endo- or exogenous stimuli. TC degranulation is regulatory because granule isolation is generally directed towards target cells (Figure 1, see 3rd page of cover).

In experimental groups with preserved adrenal glands under different PA regimens and 48 hours after IS, a significant decrease in the thymus mass coefficient is observed, on average by 1.5 times. This indicates a weakening of its functional activity (acute transformation) in response to the development of a stress reaction (from 1.394±0.088 mg/g in intact animals to 0.952±0.097 × mg/g in the MaxPA group, and to 1.006±0.156 × mg/g in the IS group 48 hours).

The thymus capsule is part of the fascial skeleton of the mediastinum, directly involved in the blood supply of the organ. There were no significant changes after different types of PA and IS in the capsule thickness and in the ratio of the cortex and the medula of the thymus of rats.

The results of the morphometric analysis of thymus mast cells showed that there were no significant differences in the amount of mast cells present in the gland stroma after exposure to all types of hyperdynamic and hypodynamic stress. It is expected that MCs functional activity will change in groups with preserved adrenal glands. In this case, MCs probably play a role in the temporal involution of the thymus, as they are involved in the formation of the adaptation syndrome during the development of stress.

The assessment of synthetic activity and degranulation activity revealed a decrease in the mean histochemical coefficient and, on the contrary, an increase in the coefficient of MC degranulation in the thymus in all experimental groups with preserved adrenal glands (Figure 2).

In accordance with the data presented in the figures, it can be stated that thymus MCs quickly respond to neuro-endocrine factors and are involved in a stress response, where their activity is manifested in a synchronous decrease in the synthesis of granules in the cytoplasm and an increased release of active substances accumulated earlier. The short-term early response of MCs is confirmed by experiments with immobilization of rats. Furthermore, experiments on continuous PA of different intensities demonstrate the preservation of this trend until the end of the stressor action.

In experimental groups with removed adrenal glands after IE, the thymus mass coefficient and gland structure remain unchanged. No changes in morphofunctional MC parameters were detected in the thymus of adrenalectomized animals after IS. Their amount in the organ, synthetic and degranulatory activity, remained at the level of the intact group (Figure 2).

A generalizing scheme can be presented to illustrate the neuro-endocrine-immune interactions involved in the development of a stress reaction, as well as the role of MCs in this process (Figure 3).

In response to extreme effects in the formation of stress reactions, MCs are influenced by various factors. The main pathway of MC activation under stress remains unclear. First, catecholamines produced by activation of the sympathoadrenal system directly affect MCs through adrenergic receptors [12], and thymus MCs are known to establish connections with catecholaminergic nerve terminals. Second, corticotropin-releasing hormone produced by the hypothalamus affects MC by binding to the KRG-Rl receptor, resulting in the release of histamine, which leads to increased permeability and vasodilation [1]. Third, adrenocorticotropic hormone activates MC secretion via melanocortin receptors [7]. Thus, degranulation of the thymus MCs during the development of stress under the influence of these factors can lead to gland atrophy. Conflicting questions remain about the effects of glucocorticoids, which are known to be cell membrane stabilizers and anti-inflammatory hormones that suppress the activity of MCs in allergies. However, the mechanism of their action on MCs during stress is not fully understood. Our experiments with hypo- and hyperdynamic stress on animals with preserved and removed adrenal glands indicate that the MC response is largely determined by the hypothalamic-pituitary-adrenal axis of the endocrine system. Thus, the removal of adrenal glands, and therefore the inability to release glucocorticoids, leads to the absence of a functional response from the thymus MCs. The stimulating effect of adrenal glucocorticoids on MCs under stress is likely mediated by their interaction with the factors listed above.

Conclusion

Thus, the response of MCs thymus to extreme factors is unidirectional, expressed by the activation of degranulation and the suppression of their synthetic activity. This indicates that the MC response in impaired homeostasis caused by various factors is nonspecific and serves as an integral component in the development of a stress response during the body's adaptation to changing conditions. The peculiarities of

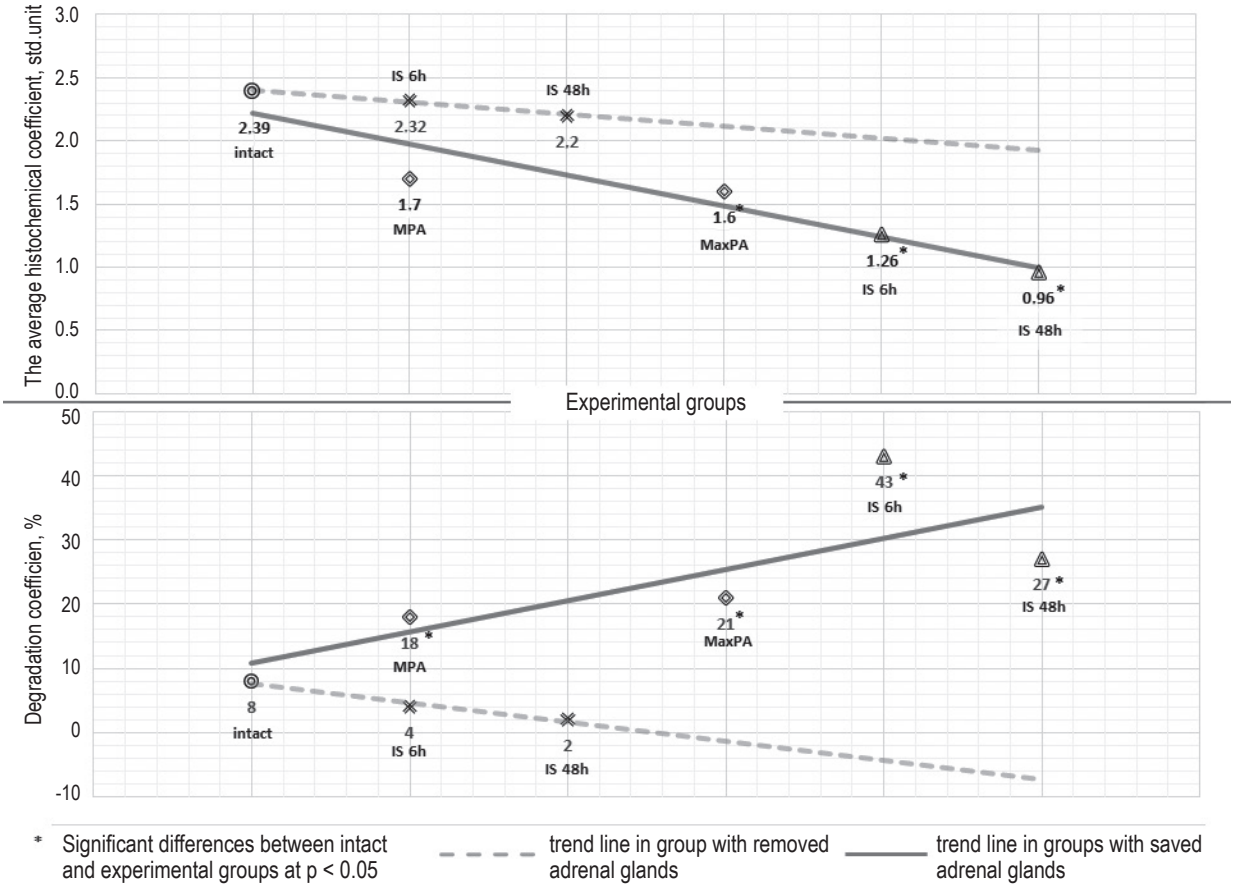

Figure 2. Change in the synthetic and degranulatory activity of thymus mast cells under stress

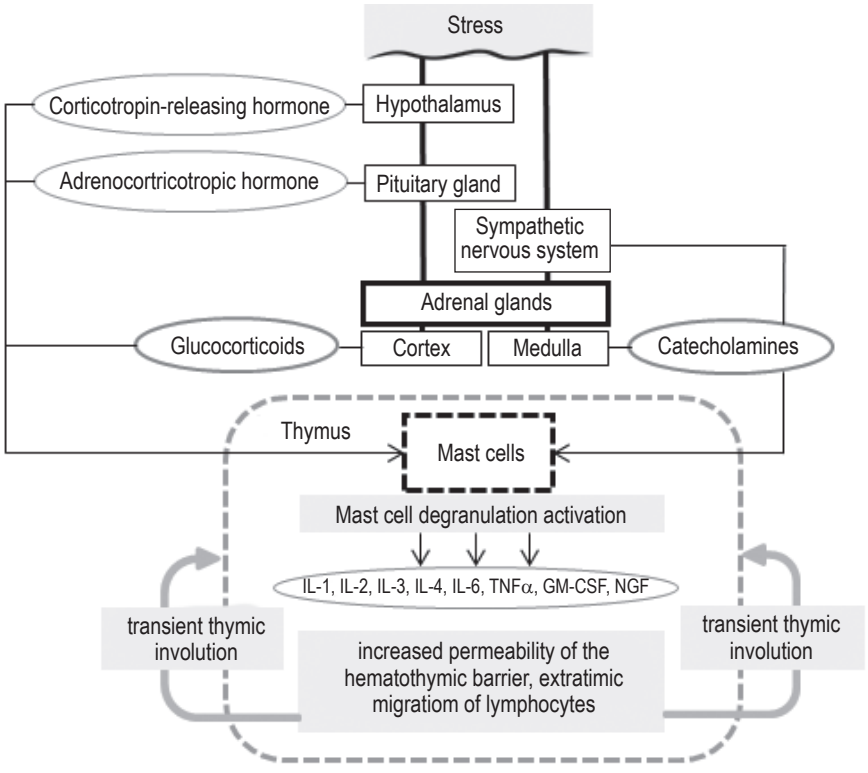

Figure 3. Thymus mast cells as a component of neuro-endocrine-immune interactions under stress (scheme)

the MCs thymus response at hypo-and hyperdinamic load confirm that their reaction is largely determined by the hypothalamic-pituitary-adrenal axis of the endocrine system. When this axis is activated and a full-fledged stress reaction is formed by the body, MCs are actively involved in the process of acute transformation of the thymus through cytokine secretion. This is an important condition for the development of adaptation mechanisms by the immune system, including the thymic-lymphatic apparatus. Under the influence of stress factors, the immune system triggers a variety of regulatory effects aimed at changing the functions of immune cells and redistributing them through the compartments of the body. This process is crucial for maintaining homeostasis and promoting resilience in the face of stress [3].

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References

1. Ayyadurai S., Gibson A.J., D'Costa S., Overman E.L., Sommerville L.J., Poopal A.C., Mackey E., Li Y., Moeser A.J. Frontline Science: Corticotropin-releasing factor receptor subtype 1 is a critical modulator of mast cell degranulation and stress-induced pathophysiology. *J. Leukoc. Biol., 2017, Vol. 102, no. 6, pp. 1299-1312.*
2. da Silva E.Z., Jamur M.C., Oliver C. Mast cell function: a new vision of an old cell. *J. Histochem. Cytochem., 2014, Vol. 62, no. 10, pp. 698-738.*
3. Dhabhar F.S., Malarkey W.B., Neri E., McEwen B.S. Stress-induced redistribution of immune cells-from barracks to boulevards to battlefields: a tale of three hormones-Curt Richter Award winner. *Psychoneuroendocrinology, 2012, Vol. 37, no. 9, pp. 1345-1368.*
4. Durkin H.G., Waksman B.H. Thymus and tolerance. Is regulation the major function of the thymus? *Immunol. Rev., 2001, Vol. 182, no. 1, pp. 33-57.*
5. Komi D.E.A., Wöhrl S., Bielory L. Mast cell biology at molecular level: a comprehensive review. *Clin. Rev. Allergy Immunol., 2020, Vol. 58, no. 3, pp. 342-365.*
6. Mukai K., Tsai M., Saito H., Galli S.J. Mast cells as sources of cytokines, chemokines, and growth factors. *Immunol. Rew., 2018, Vol. 282, no. 1, pp. 121-150.*
7. Naumova E.M., Sergeeva V.E. Histochemical study of mast cells from the thymus of mice receiving ACTH1-24. *Bulletin of Experimental Biology and Medicine, 2004, Vol. 138, pp. 93-96.*
8. Polevshchikov A.V., Guselnikova V.V. Thymic mast cells: From morphology to physiology. I*ntegrative Physiology, 2021, Vol. 2, no. 1, pp. 15-20.*
9. Ribatti D., Crivellato E. The role of mast cell in tissue morphogenesis. Thymus, duodenum, and mammary gland as examples. *Exp. Cell Res., 2016, Vol. 341, no. 1, pp. 105-109.*
10. Soumelis V., Liu Y.J. Human thymic stromal lymphopoietin: a novel epithelial cell-derived cytokine and a potential key player in the induction of allergic inflammation. Springer *Semin. Immunopathol., 2004, Vol. 25, no. 3, pp. 325-333.*
11. Varricchi G., de Paulis A., Marone G., Galli S.J. Future needs in mast cell biology. *Int. J. Mol. Sci., 2019, Vol. 20, no. 18, 4397*. doi: 10.3390/ijms20184397.
12. Yushkov B.G., Chereshnev V.A., Klimin V.G., Artashyan O.S. Mast cells: physiology and pathophysiology. Moscow: Meditsina, 2011. 240 p.

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