

Migration of the Immune System Cells Induced by Moderate Cold Stress

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Abstract

The article is devoted to the study of the migration of cells of the immune system under moderate cold stress, which has the theoretical and practical significance. The aim of a study was to investigate the effect of the once-only reconstitution of moderate cold stress for exploring cell migration and redistribution within the immune system. The experiments were performed on male C57BL mice aged 6 - 8 weeks and of 18 - 20g weight. Moderate cold stress was induced by keeping the mice at +4°C for 15 min. After 4h and 24 h, bone marrow, thymus and spleen parameters, cell cycle phase and apoptotic activity, as well as hematological parameters, including leukocyte formula and reticulocyte content in the blood, were examined.

It is shown that moderate cold stress is characterized by the development within 24h of leukocytosis, reticulocytopenia, a noticeable decrease in cell number in the bone marrow, thymus and spleen, a significant decrease of thymocytes in S and G2-M+S phases and a significant increase of thymocytes and splenocytes which can be regarded as an intense cellular stress redistribution.

Bone marrow cell mobilization, migration of lymphocytes and granulocytes from the thymus and spleen, inhibition of proliferative activity and increased apoptotic rate of thymic and splenic cells, are involved in the development of moderate cold stress. It indicates a combination of catabolic and regulatory processes under cold stress. The obtained data on the peculiarities of the cold stress response can be used for developing new anti-stress care strategies.

Keywords: *Cold Stress; Immune System; Hematopoietic Stem Cells; Lymphocytes*

Introduction

Cold stress is a general adaptive response of the body. It means as its base an excitation by the axis of the hypothalamus-pituitary-adrenal cortex and activation of the autonomic nervous system, mainly its sympathetic division. ACTH and the stimulated production of adrenal steroid hormones and catecholamines are key factors affecting the immune system. There is evidence that the immunosuppressive effect of stress is more not glucocorticoids, but catecholamines and prostaglandins [1]. It is known that stimulation of α - and β -receptors promotes the release of lymphocytes and granulocytes from the spleen [2] and provides neutrophilia under stress [3].

Strong classical stresses cause catabolic processes with apoptosis of sensitive cells of the immune system and the formation of immunodeficiency [4]. The effects of moderate intensity have been studied less [5]. Exposure of rats at +4°C significantly increased the level

of ACTH, adrenaline, angiotensin-II and IL-10. A decrease in the number of CD4+CD25+Foxp3+ T_{reg} lymphocytes was also observed [6]. Meanwhile, from clinical observations it is known that the cold stress uncontrolled by strength, duration and frequency can contribute to opportunistic infections' development and aggravate the course of chronic diseases [7].

Aim of the Study

The goal of the study is to explore the effect of moderate cold stress on cell migration within the immune system.

Materials and Methods

The experiments were performed with use of male mice of the C57BL line aged 6 - 8 weeks and of 18 - 20g weight from the vivarium of Kavetsky Institute of Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, maintained under standard vivarium conditions. All experiments were performed in compliance with the requirements of Article 26 of the Law of Ukraine "On Protection of Animals from Cruelty" (February 21, 2006) and the "The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986). During the experiment, all animals received a balanced diet and had free access to water.

Moderate cold stress was induced by keeping the mice at +4°C for 15 min. Three experimental groups of animals were formed: control (n = 28), mice 4 h after 15 min cold stress (n = 7), mice 24h after 15 min cold stress (n = 7).

After 4h and 24h, bone marrow, thymus and spleen parameters, cell cycle phase and apoptosis activity, as well as hematological parameters, including leukocyte formula and reticulocyte content in the blood, were examined. To determine the phases of the cell cycle and apoptosis, the cell suspension was stained with a solution of propidium iodide with the addition of sodium citrate. Cells were analyzed by flow cytometry analysis. To establish the distribution of cells by phases of the cell cycle, histograms were analyzed, which evaluated the proportion of cells in the region corresponding to the hyperdiploid set of chromosomes. On the histogram of cells stained with propidium iodide, these cells correspond to the peak to the right of the main diploid peak. When using a specially designed program (ModFit LT™) it is possible to analyze this area in more detail and calculate the proportion of cells in different stages of the cycle.

To assess apoptosis on the cytogram by direct and lateral light scattering, the localization of lymphocytes was determined and the red fluorescence of propidium iodide was evaluated for 10,000 cells, among which the percentage of hypodiploid cells was calculated [8].

The obtained results were processed by methods of variation statistics using Excel (MS Office XP). For quantitative characteristics, the mean value (M) and the standard error of the mean value ($\pm m$) were calculated. The nonparametric Mann-Whitney test (U) was used to determine the significance of the differences. In the case of statistical evaluation, $p < 0.05$ was considered plausible.

Results and Discussion

As a result of the development of a moderate stress response after 24h, mice had a high leukocytosis, which was formed, apparently, together with a simultaneous, albeit unreliable, increase in the number of granulocytes and lymphocytes in the blood (Figure 1). At the same time there was a significant decrease in bone marrow cell number (Figure 2a), which suggests a partial bone marrow origin of leukocytosis. It is also possible that a decrease in the number of cells in the bone marrow may be the result of toxic metabolic effects. The bone marrow contains progenitor cells that migrate to the thymus and develop within its microenvironment, but the part of the mature thymocytes returns to the bone marrow after differentiation [9]. The administration of glucocorticoids promotes the migration of T-cells into the bone marrow, and the number of these cells is reduced in the spleen. Neutrophils are a key component of the innate immune system and a key source of inflammation [10]. But the most important regulator of the release of neutrophils from the bone marrow under normal and stress conditions is CXCR4 [11].

Meanwhile, the level of erythrocytes in this period changed little. But the number of reticulocytes decreased significantly (Figure 2c). It means the suppression of erythrocyte hematopoiesis, and it can be considered as confirmation of the presence of toxic effects of metabolites on the bone marrow.

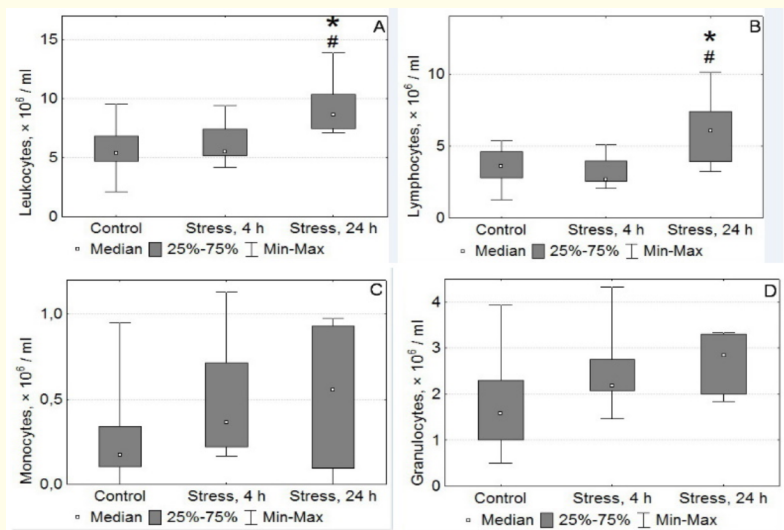


Figure 1: The number of leukocytes (A), lymphocytes (B), monocytes (C) and granulocytes (D) in the peripheral blood of stressed mice. **p* < 0.05 compared with the group of control mice; # *p* < 0.05 compared with a group of 4h mice after 15 min of cold stress.

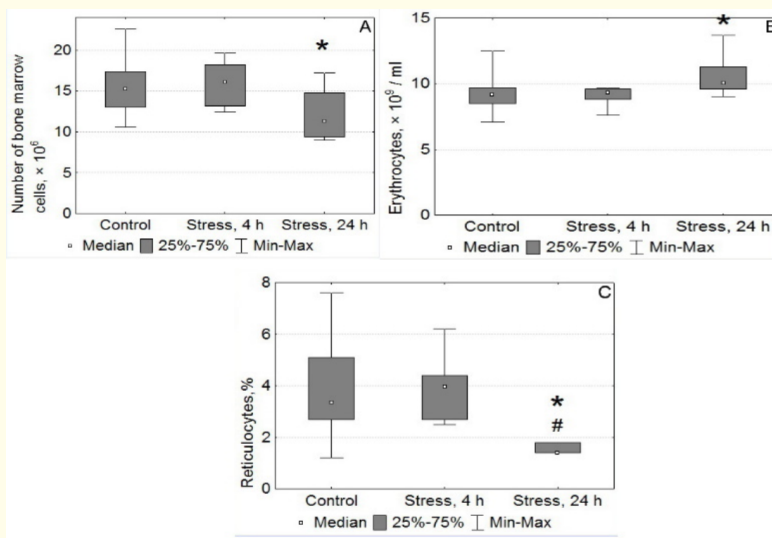


Figure 2: Bone marrow cell content (A), number of erythrocytes (B) and reticulocytes (C) of stressed mice. **p* < 0.05 compared with the group of control mice; # *p* < 0.05 compared with a group of 4h mice after 15 min of cold stress.

Replenishment of the number of lymphocytes and granulocytes in the blood can be carried out not only as a result of bone marrow mobilization of cells, but also due to their migration from the thymus and spleen, as evidenced by a significant decrease in cell number of these organs after 4 and 24 hours (Figure 3 and 4).

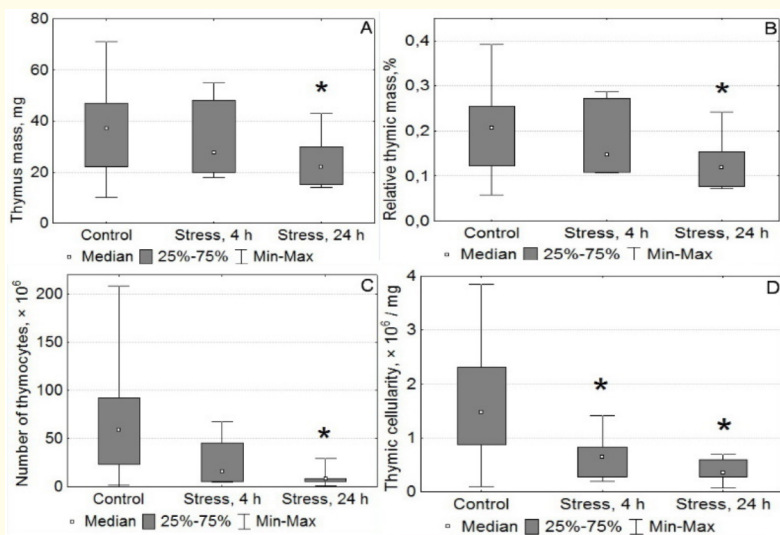


Figure 3: Thymus parameters of stressed mice: A - mass of the thymus; B - relative thymus mass; C - the number of thymocytes per organ; D - thymus cellularity. * $p < 0.05$ compared with the group of control mice; # $p < 0.05$ compared with a group of 4h mice after 15 min of cold stress.

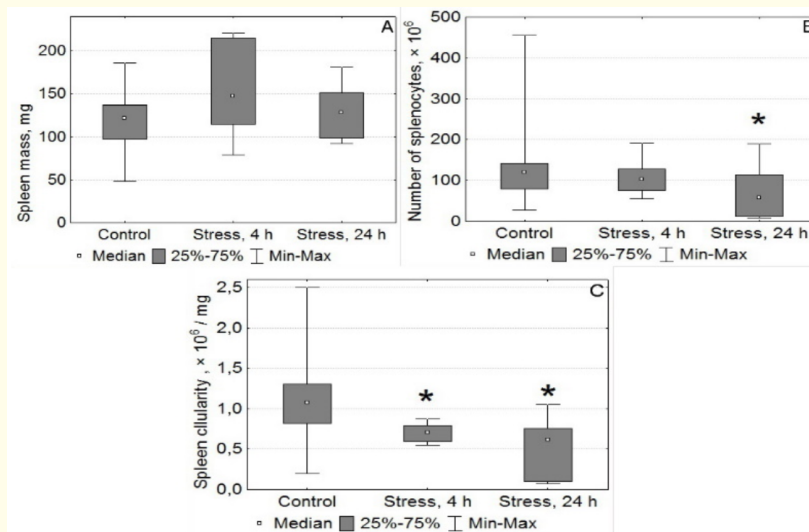


Figure 4: Spleen parameters of stressed mice: A - mass of the spleen; B - the number of splenocytes; C - cell content of the spleen. * $p < 0.05$ compared with the group of control mice.

After 24h from the beginning of the reaction, the number of thymocytes in the G0/G1 cell cycle phase increased significantly and the number of thymocytes in the S and G2-M+S cell phases decreased significantly, which indicates a significant rate of thymic cell proliferation (Figure 5).

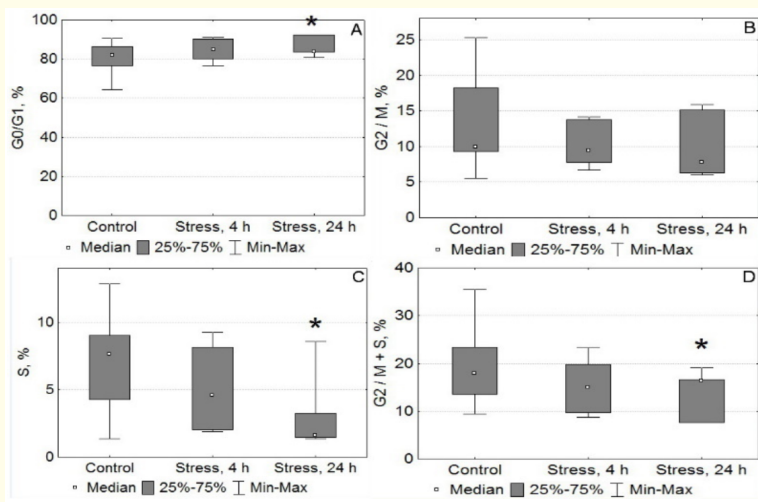


Figure 5: Distribution by phases of the cell cycle of thymocytes of stressed mice: A - G0/G1; B - G2/M; C - S; D - G2-M+S. *p < 0.05 compared with the group of control mice; # p < 0.05 compared with a group of 4h mice after 15 min of cold stress.

In the spleen, the number of cells in the G2/M phase decreased significantly but only after 4h (Figure 6), which apparently reflects the smaller anti-proliferative effect of hormonal stress mechanisms on splenocytes.

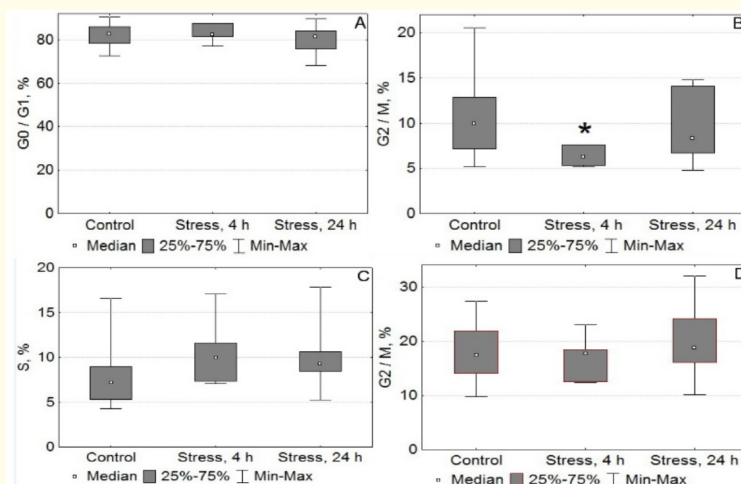


Figure 6: Distribution by phases of the cell cycle of splenocytes of stressed mice: A - G0/G1; B - G2/M; C - S; D - G2-M+S. # p < 0.05 compared with a group of 4h mice after 15 min of cold stress.

The number of bone marrow cells in different phases of the cell cycle at the considered time points did not change significantly (Figure 7), which indicates that the decrease in the number of bone marrow cells by 24h may not depend on changes of proliferative activity (See figure 2).

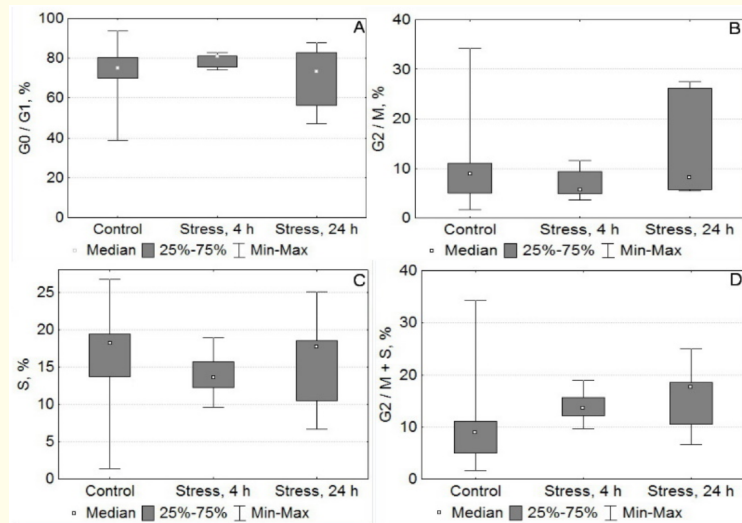


Figure 7: Distribution by phases of the cell cycle of the bone marrow cells of stressed mice: A - G0/G1; B - G2/M; C - S; D - G2-M+S.

Apoptotic processes can be of great importance for cell migration. The greatest increase of apoptotic processes run was observed after 24h in the thymus (Figure 8a), which coincides with data on a significant decrease in the number of thymocytes at this time point in the proliferative phase (Figure 5). Indicators of spontaneous apoptosis of bone marrow cells were approximately at the same level, but with a pronounced tendency to increase after 24 hours. In the spleen, cell apoptosis rate 24h after the onset of stress was significantly higher than normal rate (Figure 8). Lymphocyte apoptosis is caused by glucocorticoids; it is blocked by glucocorticoid receptor antagonists [12]. Increased levels of lymphocyte apoptosis also occur under short-term cold stress in humans.

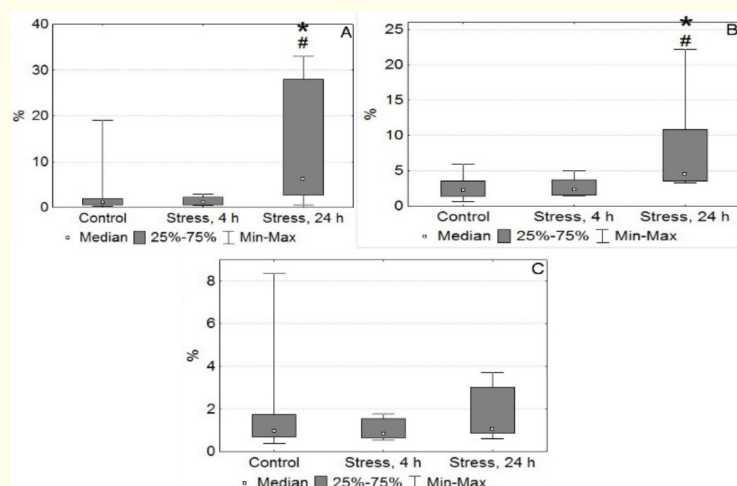


Figure 8: Spontaneous apoptosis level of thymocytes (A), splenocytes (B) and bone marrow cells (C) of stressed mice. * $p < 0.05$ compared with the group of control mice; # $p < 0.05$ compared with a group of 4h mice after 15 min of cold stress.

Thus, it is shown that as a result of reconstitution of moderate cold stress in mice there is a migration of cells within the immune system with an increase of their number in the peripheral blood, their decreased content in bone marrow, thymus and spleen, inhibition of proliferative activity of cells, especially thymocytes, and significant increase of apoptotic cells in the thymus and spleen. The pronounced reticulocytopenia also develops.

Conclusion

1. After 24h moderate cold stress is characterized by the development of leukocytosis, reticulocytopenia, a noticeable decrease in cell number in the bone marrow, thymus and spleen, a significant decrease in the number of thymocytes in S and G2-M+S phases and a significant increase of thymocytes and splenocytes content; it can be regarded as intense cell redistribution processes under stress.
2. In the development of cold stress were involved the processes of the bone marrow cell mobilization, migration of lymphocytes and granulocytes from the thymus and spleen, inhibition of proliferative activity and increased apoptosis of thymic and splenic cells; it indicates a coupling of cold stress catabolic and regulatory processes within the immune system.
3. The obtained data on the peculiarities of the cold stress response can be used for development of new anti-stress care strategies.

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