Activation of Apoptosis as a Mechanism of Thymus Involution during Repeated Immobilization

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Lymphopenia preceded involution of the thymus during daily immobilization for 1 h. Hypoplasia of the thymus was associated with induction of apoptosis.

Key Words: *stress; apoptosis; thymus; proliferation; hypoplasia*

Repeated 1-h immobilization is characterized by the predominance of stress-limiting systems [3]. On the other hand, involution of the thymus can occur under these conditions [5]. These changes can seem surprising during adaptation to stress. The mechanism of this phenomenon is unknown. It remains unclear whether the observed changed are related to migration of T lymphocytes in other compartment of the blood system [2] or induction of apoptosis [6]. The present work was designed to study this problem.

MATERIALS AND METHODS

Experiments were performed on 45 albino outbred rats. The animals were subjected to daily 1-h immobilization for 3 days. The control group included intact rats. Control and stressed animals were killed under ether anesthesia 24 h after the last immobilization. The thymus was minced in a glass homogenizer. The cells were washed, resuspended in 0.15 N sodium phosphate buffer (pH 7.4, 1×10^6 cells/ml), and stained with a hypotonic solution of propidium iodide (50 μg/ml, 0.1% Triton X-100). The relative number of apoptotic (M_1 peak), G_1-phase (G_1 peak, M_2), and mitotically active cells (M_3 peak) was estimated 3 h after treatment using a FACS CALIBUR flow cytofluorometer (Becton Dickinson) and CELL QUEST software. The significance of differences was evaluated by means of nonparametric Wilcoxon—Mann—Whitney test.

RESULTS

When comparing changes in the number of circulating lymphocytes and count of thymocytes after threefold 1-h immobilization (IM_3) we found that blood lymphocytes are more sensitive to the cytolytic effect of repeated stress (lymphopenia preceded involution of the thymus). The number of circulating lymphocytes decreased by 2.5 times 60 min after the 1st immobilization (Fig. 1, *a*). The number of thymocytes remained practically unchanged (Fig. 1, *b*). Similar changes were revealed after 2-fold immobilization. Twenty-four hours after IM_3 thymocyte number was $100.44\pm7.48\times10^6$ (*vs.* $232.57\pm22.55\times10^6$ cells in control animals, $p<0.01$). Control and stressed rats had the following parameters: M_1 peak (apoptosis), 12.07±2.28 and 41.67±7.05%, respectively ($p<0.01$); M_2 peak (G_1-phase), 81.63±2.36 and 55.91±6.72%, respectively ($p<0.01$); and M_3 peak (mitotically active cells), 16.82±0.34 and 2.300±0.082%, respectively ($p<0.05$). The number of karyocytes in control and stressed animals was $96.31\pm7.85\times10^6$ and $131.48\pm3.46\times10^6$ cells, respectively ($p<0.05$).

Stress-induced lymphopenia disappeared only after the 3rd immobilization. These changes were accompanied by atrophy of the thymus and increase in

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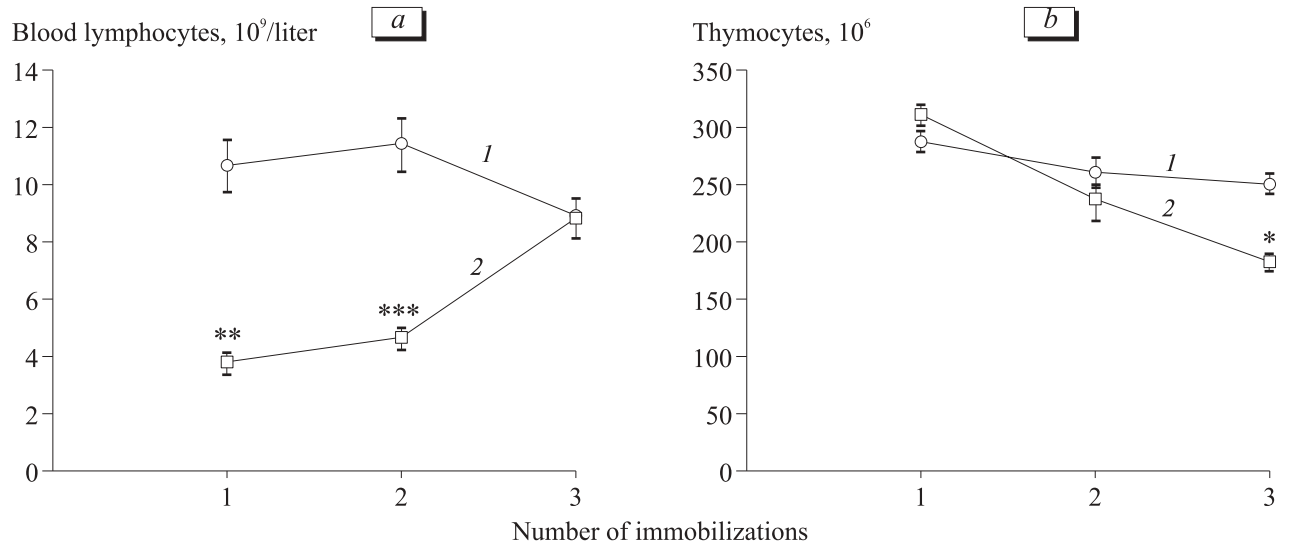

Fig. 1. Effects of repeated immobilization on the number of blood lymphocytes (*a*) and count of karyocytes in the thymus (*b*). Control (*1*) and stress (*2*). **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to the control.

the number of bone marrow karyocytes. Since these processes occurred simultaneously, it could be hypothesized that hyperplasia of the bone marrow and normalization of blood count of lymphoid cells were associated with migration of cells from the thymus. However, our assumption was not confirmed by further studies. For example, the number of apoptotic cells increased by 3 times after IM_3. We revealed a decrease in the number of G_1-phase thymocytes and mitotically active T lymphocytes. Therefore, atrophy of the thymus during IM_3 is associated with cell cycle arrest and induction of apoptosis in T cells [4]. This is a surprising result, because the proapoptotic effect of IM_3 is realized via the steroid-independent mechanisms. Published data show that blood glucocorticoid concentration does not increase during IM_3 [1,5]. Previous studies demonstrated an increase in the concentration of tumor necrosis factor-α [1], which serves as s potent inductor of apoptosis [4]. Involution of the thymus during repeated immobilization is probably related to the cytokine-dependent induction of apoptosis.

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