MICROBIOLOGY AND IMMUNOLOGY

Functional Activity of Spleen and Peripheral Blood Lymphocytes in Stress-Induced Immunodepression

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Study of lymphoid organs and T and B cells in white rats shows that long-term stress causes progressive suppression of the immune response. Blockade of steroid hormone synthesis in the adrenal cortex prevents the development of immunodepression, implying a protective effect of such a blockade against stress-related secondary immunodeficiency.

Key Words: *stress; lymphocytes; immunodepression; metapyrone*

Stress-related secondary immunodepression is a consequence of increased production of steroid hormones by the adrenal cortex under the influence of adrenocorticoid hormone [2,3,5]. High concentrations of glucocorticoids suppress the immune responses [14]. There is evidence suggesting that glucocorticoids and other biologically active substances produce a direct effect on the lymphoid tissue and peripheral blood lymphocytes [6-8,11-13]. It was shown that the immune response is associated with proliferation of the effector cells [1,6,9]. This suggests that diminished function of the immune system in stress-related imbalance of hormones results from changes in the population of lymphocytes and their activity. In this connection, physiological stabilization of stress-related hyperactivity of the adrenal cortex is of interest [4,10].

Our aim was to study the populations of and functional state of spleen and peripheral blood lymphocytes in stress-induced immunodepression and during physiological stabilization of changes in the balance of hormones.

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MATERIALS AND METHODS

Experiments were performed on outbred male albino rats weighing 180-220 g (n=45). Stress was modelled by hyperkinesia achieved by forced swimming for a long time. Three series of experiments were performed. In the first series, rats (n=20) were exposed to stress. In the second series (n=20), stress was applied after treatment with metapyrone (SU-4885, CIBA-Geygy). This agent blocks hyperfunction of adrenal cortex and in a dose of 11 mg/100 g body weight maintains blood glucocorticoids at the baseline level [4,10]. Metapyrone was injected one day before the first stressful situation. Five rats which were given normal saline and were not exposed to stress served as a control. The experiment was carried out for 4 weeks, and material was collected at the end of each week (on day 7). The rats were killed by decapitation on days 7, 14, 21, and 28. Histological investigation of the lymphoid tissue (spleen and Peyer's patches of the small intestine) and quantitative analysis of immunocompetent cells in the T and B zones were performed on paraffin sections by the method of Avtandilov. The sections were stained with hematoxylin-eosin and picrofuchsin after Van Gieson. The enzyme activity of specific cells and endothelial cells of splenic sinu-

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soids and Peyer's patches of the small intestine) was evaluated cytophotometrically (staining for NADH-dehydrogenase, 5-nucleotidase, nucleic acids, etc.).

Proliferative and suppressive activities of spleen and peripheral blood lymphocytes were assessed. T lymphocytes were divided into theophylline-sensitive (T suppressors) and theophylline-resistant (T helpers). Lymphocyte proliferation in response to non-specific activators was evaluated in the blast transformation reaction. Spontaneous and phytohemagglutinin-stimulated (PHA, 10 μg/ml) proliferative activities were evaluated by measuring the incorporation of ^{3}H-thymidine. Proliferative activity of B cells was evaluated with the use of *E. coli* lipopolysaccharide (LPS). Spontaneous and concanavalin A-induced (ConA, 25 μg/ml) suppressor activity of lymphocytes were evaluated by suppression of ^{3}H-incorporation in PHA-stimulated test culture.

RESULTS

All rats developed classical stress reaction: hyperactivity at the initial state stage of the experiment, aggressiveness at the intermediate stage, and asthenia at the end stage, which was accompanied by acute ulcerous-necrotic alterations of the alimentary canal mucosa. At the end of the experiment, histological studies revealed a considerable decrease in the number of stromal cells and the development of "fibroadenias" in splenic follicles and Peyer's patches with a

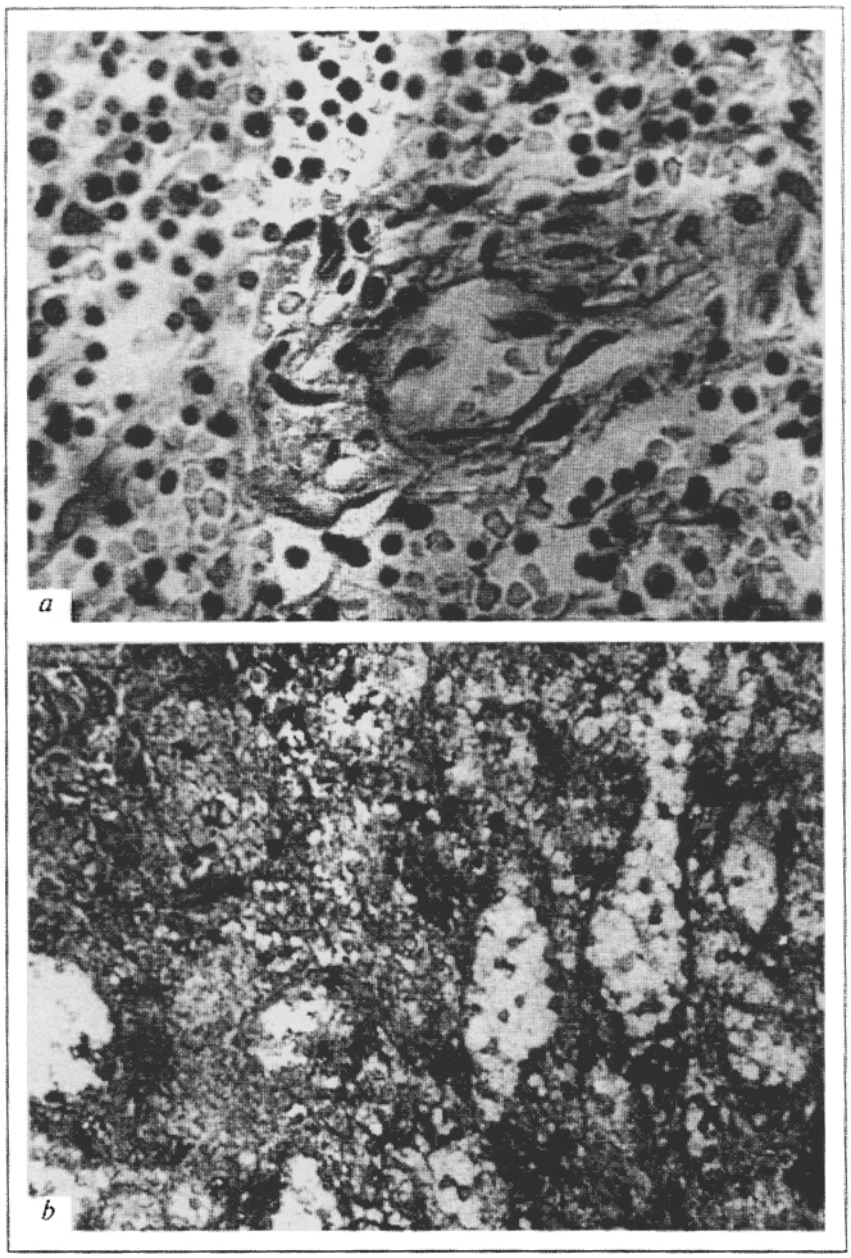

Fig. 1. Stress-induced changes in rat spleen. Light microscopy. *a*) "depletion" of the T-dependent zones of white pulp by lymphoid cells. Hematoxylin and eosin staining, × 400. *b*) 5-nucleotidase staining of the cytoplasm of endothelial cells of splenic sinusoids. Berston's method of nitrogen coupling (pH 8.3). × 400.

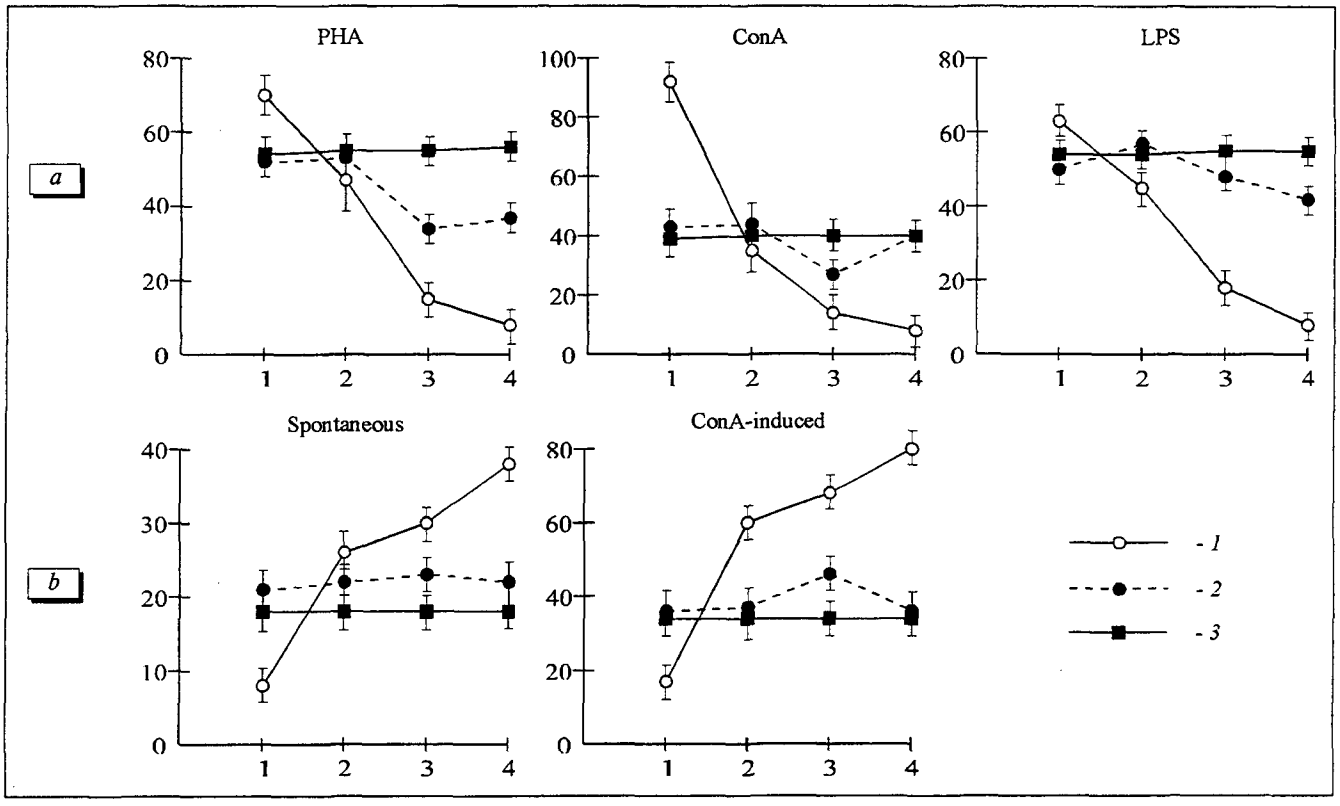

Fig. 2. Proliferative (*a*) and suppressor (*b*) activity of rat spleen lymphocytes under conditions of stress. Here and in Fig. 3: ordinate: ^{3}H-thymidine incorporation, cpm (*a*), suppression index, % (*b*); abscissa: experimental period, weeks. *1*) stressed rats; *2*) stressed rats treated with metapyrone; *3*) control animals.

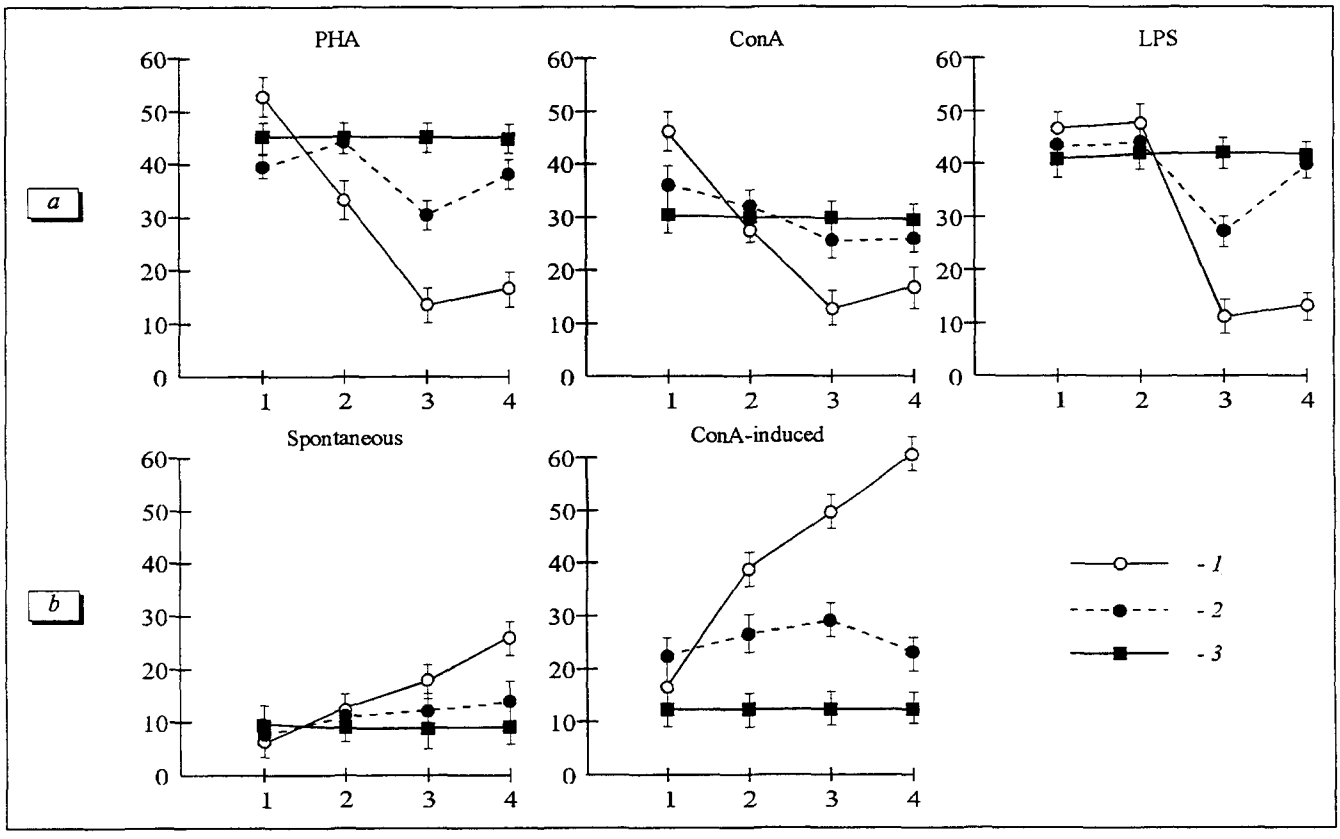

Fig. 3. Proliferative (*a*) and suppressor (*b*) activity of rat peripheral blood lymphocytes under conditions of stress.

simultaneous atrophy of specific parenchyma. The specific volume of splenic white pulp and the number of intestinal Peyer's patches decreased with the time of stress.

Marked changes in the vascular bed and cellular composition of these organs were attended by delymphatization of T- and B-dependent zones (Fig. 1). By the end of stress, the number of lymphocytes and plasma cells in the spleen decreased, while the number of macrophages in T-dependent zone increased.

There were no stress-related changes in the immune system of metapyrone-treated rats. Histochemical studies showed that in stressed rats the enzyme activity of endothelial cells of splenic sinusoids and specific parenchyma cells of the spleen and Peyer's patches of the small intestine was markedly changed, protein synthesis was suppressed (as evidenced by decreased incorporation of labeled precursors in nucleic acids), while the activity of the enzymes involved in energy metabolism (NADPH) and transendothelial transport (5-nucleotidase) was increased. These histological and histochemical modifications correlated with changes in the lymphocyte population and lymphocyte activity.

Proliferative and suppressor activities of blood and spleen lymphocytes was evaluated by changes in the lymphocyte population. After one week of stress, functional activity and populations of blood and spleen T and B cells were changed. The suppressor activity of spleen and peripheral blood lymphocytes was slightly decreased on day 7, as evidenced by enhanced incorporation of ^{3}H thymidine and slightly increased proliferation in response not only to ConA and PHA, but also to LPS, a B cell mitogen (Fig. 2). Metapyrone normalized functional activity of peripheral blood and spleen lymphocytes. On the 2nd and 3rd weeks of stress, both spontaneous and ConA-induced suppressor activity of blood and spleen lymphocytes markedly increased. Spontaneous incorporation of ^{3}H-thymidine by blood and spleen lymphocytes declined, remaining still higher compared with the control. The proliferative activity of blood lymphocytes in response to PHA, a T cell mitogen, also decreased considerably, which may be indicative of the effect of the stress-induced hormonal imbalance on the mature population of T cells. Both PHA-, ConA-, and LPS-induced proliferation of blood and spleen lymphocytes markedly decreased on the 3rd week of stress. Thus, functional activity of mature and immature precursors of T and B cells was suppressed. Administration of metapyrone on the 2nd and 3rd weeks of stress prevented the increase in the suppressor activity of spleen and blood lymphocytes, thus preserving the proliferative response to T and B cell mitogens (Fig. 3). On the 4th week of stress, the suppressor activity of blood and spleen lymphocytes increased, while the proliferative activity decreased to a greater extent. At the end of the experiment, the suppressor activity of T cells was high, while the proliferative activity of both blood and spleen lymphocytes dramatically decreased. There was no activation of T suppressor cells in the spleen and peripheral blood in the metapyrone-treated rats.

Thus, stress-induced changes in the population of lymphocytes and their functional activity in the spleen and peripheral blood is not associated only with the direct effect of glucocorticoids and other biologically active substances on the histohematological barrier of lymphoid organs, but may be due to increased suppressor activity of immunoregulating cells on other lymphocyte populations.

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