Effects of Short-Term Hypothermal and Contrast Exposure on Immunophysiological Parameters of Laboratory Animals

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Experiments on inbred animals showed that short-term exposure in cold water significantly modified structural and functional parameters of the immune system at different levels of its organization, from bone marrow hemopoiesis to effector stage of the immune response to antigen. The thermal factor caused changes in nonspecific and specific mechanisms of the immune system. Hypothermal exposure (7-9°C, 5 sec) increased the thymic index and bone marrow lymphocyte count, reduced absorption capacity and stimulated metabolic activity of phagocytes, stimulated cell-mediated and suppressed humoral immunity. Contrast exposure in cold and hot water (7-9°C, 5 sec/ 40-42°C, 30 sec) increased monocyte count in bone marrow and reduced it in the their peripheral blood, reduced metabolic activity of phagocytes, stimulated cell-mediated and suppressed humoral immunity. These data demonstrate physiological mechanisms of interactions between the thermoregulatory and immune systems.

Key Words: *hypothermal and contrast exposure; myelogram; peripheral blood; macrophages; cell-mediated and humoral immunity*

The effects of cold conditioning on functional activity of the immune system attracted great attention of scientists in recent years. Natural factors, *e.g.* water and air, usually serve as thermal factors for this conitioning. Water conitioning is most effective, because thermal exposure is potentiated by mechanical and physicochemical factors [3,7]. The efficiency of this type of conitioning depends on physical characteristics, intensity, and duration of exposure in water. The findings indicate that extreme conitioning can produce suppressive effects on the immune system. The phagocyte function is reduced, while the expression of activation and apoptosis markers on immunocompetent cells elevated in individuals practicing ice-hole swimming for more than 1 year. Phagocytic activity, cell-mediated and humoral immunity are suppressed in individuals practicing ice water shower twice weekly for more than 5 years [1]. Experimental studies demonstrated that hypothermal exposure led to changes in the spectrum and concentration of interleukins in systemic circulation [9], reduced the intensity of the formation of specific immune response to antigen [2], phagocyte system activity, and intensity of cell-mediated and humoral immunity reactions. On the other hand, thermal exposure according to some protocols can improve organism's resistance. For example, 30-second exposure of rats in cold (4°C) water 3 times weekly for 5.5 weeks limited the negative effects of X-ray exposure [6].

It is assumed that conitioning phenomenon is based on the nonspecific regularities of adaptation.

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The relationship between thermoregulation and immunogenesis systems through the neuroendocrine [1] and cytokine [9] regulation is discussed. Despite numerous studies, the effects of conitioning measures of moderate intensity on the physiological mechanisms of the immune system remain little studied.

We studied the effects of short-term hypothermal and contrast exposure on immunophysiological parameters in experimental animals.

MATERIALS AND METHODS

Experiment was carried out on models modulating activities of different components of the immune system by hydrothermal exposure [4,5]. These models were based on evaluation of the length of exposure of laboratory mice in cold or hot water, during which the changes in the functional activities of cell-mediated and humoral immunity became statistically significant.

The experiment was carried out on 144 F_1(CBA/Bl6) mice weighing 18-20 g. Group 1 animals (hypothermia) were exposed in cold (7-9°C) water for 5 sec. Group 2 animals were subjected to contrast exposure by transferring them from cold (7-9°C) water (5 sec) into hot water (40-42°C; 30 sec). Group 3 animals served as the control and were exposed in water of indifferent temperature (33-35°C) for 15 sec. Due to this control, the impact of "water stress" on laboratory animals is excluded and the effect of the thermal factor can be evaluated. Manipulations on animals were carried out individually once a day for 5 days. The animals were sacrificed by cervical dislocation. The blood was collected after decapitation.

The thymic and adrenal indexes (organ weight to body weight ratio, %) were evaluated. Cell composition of the peripheral blood and bone marrow was evaluated by impression smears (myelogram) per 1000 cells (in %). The capacity of splenic macrophages adhering to glass to absorb (PI) inactivated yeast cells and their metabolic activity in spontaneous NBT test were evaluated. Cell-mediated immunity was evaluated in delayed-type hypersensitivity (DTH) test after Crowle (1975). Humoral immunity was evaluated by Cunningham's method (1968). The counts of nucleated cells (NC) in the spleen, antibody-producing cells per 10^6 NC (APC/10^6) and in the spleen (APC/spl) in response to intraperitoneal immunization by thymus-dependent antigen (sheep erythrocytes; SE) were evaluated. The significance of differences between the groups was evaluated by Student's *t* test using SPSS 11.5 for Windows software.

RESULTS

The first question was whether short-term thermal exposure is a stress factor for warm-blooded animals. It is known that stress exposure can lead to a decrease in the thymic index and elevation of adrenocortical activity [8].

Short-term thermal exposure caused no stress: contrast alteration of water temperature did not lead to changes in the morphology and function of the thymus and adrenals (Table 1). Short-term hypothermia led to an increase of the thymic index and reduction of the adrenal index in comparison with the control.

The next step of the study was to evaluate the effect of the thermal factor on structural and functional parameters of the immune system (from bone marrow hemopoiesis to effector stage of the immune response to antigen).

Short-term thermal exposure led to significant changes in cell composition of the bone marrow (Fig. 1).

Hypothermia reduced the level of undifferentiated blasts ($p<0.01$), eosinophils ($p<0.01$), basophils ($p<0.01$), and increased the percent of lymphocytes ($p<0.01$) in comparison with the control. Contrast exposure reduced eosinophil count ($p<0.01$) and increased the counts of monocytes ($p<0.05$) and basophils ($p<0.01$) in the bone marrow. These changes can be regarded as an evidence of physiological mechanisms of thermoregulation control over bone marrow hemopoiesis (including lymphopoiesis) intensity.

Cellular composition of the peripheral blood after short-term thermal exposure is presented in Figure 2. Short-term hypothermia virtually did not change the proportion of cells in the peripheral blood. Contrast exposure in cold/hot water reduced

TABLE 1. Visceral Indexes (%; *n*=12)

Parameter	Control	Hypothermia	Contrast exposure
Adrenal index	0.074±0.005	0.047±0.008**	0.069±0.005
Thymic index	0.290±0.018	0.356±0.015*	0.275±0.011

Note. *$p<0.05$, **$p<0.01$ compared to the control.

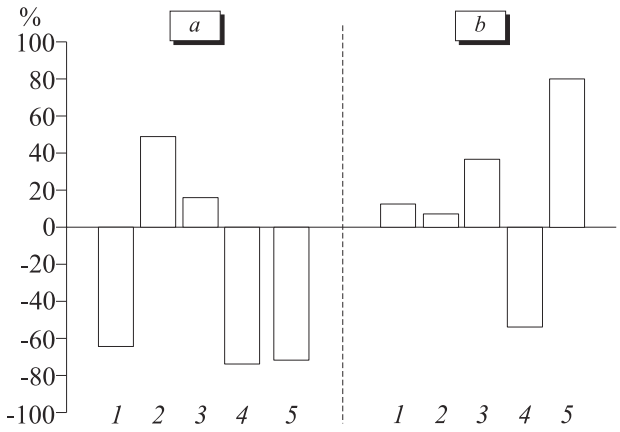

Fig. 1. Effect of thermal factor on cell composition of mouse bone marrow. *a*) hypothermia; *b*) contrast exposure. *1*) undifferentiated blasts; *2*) lymphocytes; *3*) monocytes; *4*) eosinophils; *5*) basophils. Here and in Figs. 2, 3: 0: control group. Ordinate: deviations from the values in the control.

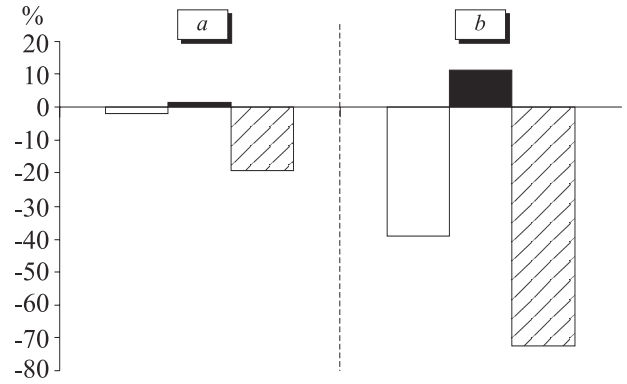

Fig. 2. Impact of thermal factor for cellular composition of mouse peripheral blood. *a*) hypothermia; *b*) contrast exposure. Light bars: neutrophils; dark bars: lymphocytes; cross-hatched bars: monocytes.

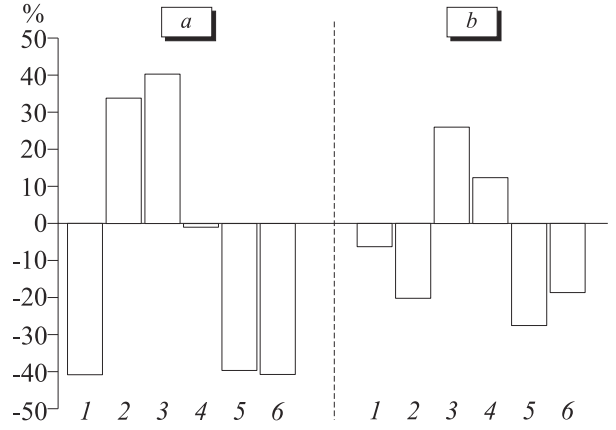

Fig. 3. Impact of thermal factor for functional activity of the mouse immune system. *a*) hypothermia; *b*) contrast exposure. *1*) absorption activity of macrophages (PI); *2*) metabolic activity of macrophages (NBT); *3*) DTH intensity; *4*) APC count/10^6 NC; *5*) NC count in the spleen; *6*) APC count in the spleen.

the level of neutrophils ($p<0.05$) and monocytes ($p<0.01$) in comparison with the control. Shifts in the counts of peripheral blood neutrophils and monocytes can be explained by their greater need in organs and tissues under the effect of the thermal factor.

A relationship between activity of nonspecific immune resistance and specific immunity reactions, on the one hand, and thermal exposure protocol, on the other, was detected (Fig. 3).

Hypothermia increased metabolic (NBT, $p<0.05$) and reduced absorption activity of splenic macrophages (PI, $p<0.01$), while contrast exposure reduced their metabolic activity (NBT, $p<0.05$). Hypothermia and contrast exposure similarly stimulated cell-mediated (DTH; $p<0.01$, $p<0.05$), but not humoral immunity (APC/10^6; $p>0.05$). However, thermal exposure reduced NC count ($p<0.01$ in both cases), which eventually caused a significant suppression of the humoral immunity at the level of the entire spleen (APC/spl; $p<0.01$, $p<0.05$, respectively). The results indicate that hypothermia and contrast exposure stimulate cell-mediated and reduce the intensity of humoral immunity; in other words, they promote polarization of the immune response to thymus-dependent antigen (SE) in favor of Th1-dependent response.

Hence, simulation experiments demonstrated heretofore unknown mechanisms of the effects of hardening measures on immunophysiological parameters. Moderate hydrothermal exposure causes changes in the nonspecific and specific mechanisms of the immune system. Thermal factor modulates physiological mechanisms of the immune system at different levels of its organization, including the specific immune response to antigen. These data indicate the presence of physiological (presumably specific for thermoregulatory system) mechanisms of interactions with the immune system. However, additional studies are needed for using these results for improvement of disease resistance, particularly during seasonal changes.

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