late the immune response but acquires a phagocytosis-stimulating property which is not exhibited by low doses (6.5×10^{-2}-6.5×10^{-4} mg/kg). A similar characteristic is inherent in cerebrolysin as well. Aviamine tested in a dose of 65 mg/kg (used in poultry raising and producing a trophic effect) [3] inhibits the immune response but, like levamine-70 and cerebrolysin, boosts PAN. A decrease of the dose to 6.5×10^{-4} mg/kg results simultaneously in a loss of the phagocytosis-stimulatory effect and a boost of the immune-stimulatory action.

Thus, the abolition of the immune-stimulatory property for the use of a high (trophic) dose attests to a reverse dependence of the immune-stimulatory and trophic functions. The effect of high doses of preparations on phagocytes, unlike their influence on lymphocytes, probably stems from phylogenetically determined trophic properties of neutrophils.

The differences in the action of various doses of amino-acid preparations as well as in the effects of certain amino acids and peptides [4] on the immune response and phagocytosis attest to the relative autonomy of the specific and of nonspecific components of defense.

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Immunological Responses during the Organism's Adaptation to a Dosed Thermal Factor

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UDC 612.591:616-097

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 5, pp. 502-504, May, 1994
Original article submitted November 7, 1993

Overheating of mice over the course of 10 and 20 days suppresses the proliferative activity of splenic cells in response to stimulation with phytohemagglutinin, concanavalin A, lipopolysaccharide, pokeweed mitogen, and alloantigens. The number of antibody-producing cells in the spleen drops on day 5 of overheating and is still low on days 10-20. Forty days after the start of overheating the functional activity of lymphocytes is restored. Overheating of animals does not change the colony-forming activity of hemopoietic stem cells.

Key Words: *overheating; lymphocyte; immunity; adaptation; immune system*

A high temperature of the external environment is an unfavorable physical factor frequently acting on the human organism under natural conditions and in a specific industrial setting. It has been shown that exposure of the organism to exogenous hyperthermia leads to disturbances in the state of different organs and systems [2,4]. This provides an incentive to study the functional systems of the organism during hyperthermia and adaptation to heat and to seek ways of raising the organism's resistance under given conditions. Published data on the functions of the immune system during adaptation of the organism to thermal factors are limited in many respects.

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0007-4888/94/0005-0499$12.50

TABLE 1. Proliferative Response of SC and Number of APC for Long-Term Discontinuous Overheating ($M \pm m$)

Parameter	Time of investigation, days					
	3		5		10	
	control	experiment	control	experiment	control	experiment
Proliferative response to:						
PHA	11.17±1.08	14.67±1.61*	4.88±0.66	7.85±1.81	11.85±1.66	6.83±1.45*
ConA	10.79±0.93	12.56±1.09	11.38±1.52	13.49±1.84	22.34±2.22	12.49±1.93*
Alloantigens	14.72±2.42	20.48±4.37	12.85±3.97	19.27±3.29	54.16±9.47	28.59±7.47*
LPS	2.82±0.29	3.17±0.30	3.17±0.36	3.36±0.35	3.71±0.18	1.62±0.15*
PWM	5.02±0.33	6.29±0.91	4.55±0.58	4.89±0.34	5.66±0.46	3.80±0.18*
Number of APC per 10^6 SC	499.0±29.3	579.8±33.1	571.0±48.5	317.0±92.8*	494.6±21.6	367.6±55.9*

Continue

	20		30		40	
	control	experiment	control	experiment	control	experiment
Proliferative response to:						
PHA	5.68±0.33	4.52±0.31*	6.94±1.33	5.40±0.50	13.83±2.19	13.69±2.24
ConA	17.45±1.12	12.25±1.50*	13.68±1.37	8.48±0.84*	25.49±3.58	21.06±3.82
Alloantigens	30.70±5.40	15.07±4.81*	39.58±8.43	9.17±4.10*	55.35±20.04	48.97±15.48
LPS	3.49±0.28	2.29±0.31*	3.41±0.25	2.51±0.24*	6.04±0.83	4.63±0.75
PWM	5.33±0.41	3.19±0.26*	5.83±0.37	5.01±0.21*	4.79±0.23	4.97±0.72
Number of APC per 10^6 SC	570.4±66.2	174.7±38.1*	445.0±29.7	431.6±24.8	393.8±25.6	420.8±16.5

Note. The data on the proliferative activity are presented as SI. Reliable ($p<0.05$) differences of parameters as compared with the control are denoted by an asterisk.

The aim of the present study was to investigate the immunological responses during adaptation of the organism to a dosed thermal exposure.

MATERIALS AND METHODS

First-generation (CBA×C57Bl/6)F_1 hybrid mice weighing 24-26 g were used in the study. The immune responses were studied over the time course of adaptation to a dosed thermal factor, using long-term discontinuous overheating of the animals (daily exposures of mice in the heat chamber at 43-44°C for 20 min). The animals were exposed to heat for 3, 5, 10, 20, 30, and 40 days. The rectal temperature for the first overheating rose to 42°C on average. The cell immunity was assessed by measuring the proliferative activity of splenic cells (SC) in response to alloantigens in a unidirectional mixed lymphocyte culture (MLC) [1] and in the test of lymphocyte blast transformation (LBT) [3] in response to stimulation with the polyclonal T-cell mitogens phytohemagglutinin (PHA, Difco) and concanavalin A (ConA, Serva). The humoral immune response was studied by measuring the proliferative activity of SC and LBT in response to the B-cell mitogens lipopolysaccharide of *Escherichia coli* (LPS, Difco) and pokeweed mitogen (PWM, Grand Island Biol. Company) and quantitating the number of antibody-producing cells (APC) in the spleen on day 5 after injection of antigen [5]. The optimal concentration of PHA, ConA, LPS, and PWM was 30, 12.5, 40, and 10 μg/ml culture medium, respectively. Sheep erythrocytes (SE) injected to mice in a dose of 5×10^8 cells intraperitoneally 24 h after the corresponding overheating were used as the antigen. For investigation of the proliferative response of SC the test samples were taken 24 h after the last overheating. SC of the control and experimental F_1 hybrid mice served as the responsive cells in MLC. Irradiated SC of BALB/c mice were used as stimulators. Cells were cultured in RPMI-1640 medium (Serva) containing 5-10% human serum, 2 mM L-glutamine (Serva), 5×10^{-5} M 2-mercaptoethanolamine (Sigma), and 10 mM HEPES (Flow Lab.). Sixteen hours before the end of incubation 1 μCi

of ^{3}H-thymidine was added to each well of Linbro microplates. The cell response was determined as ^{3}H-thymidine incorporation in DNA of proliferating cells, with calculation of the stimulation index (SI):

$$SI = \frac{\text{cpm after stimulation}}{\text{cpm in the control}}.$$

From 6 to 15 animals were involved in each experimental series. Each variant comprised 6-9 tests. The number of splenic colony-forming units (CFUsp) in the bone marrow was determined by the method of exogenous cloning [6]. Bone marrow cells (5×10^4) of experimental and control animals in 0.5 ml medium 199 were intravenously injected to lethally irradiated syngeneic recipients. The total dose of radiation was 9.2 Gy. The number of macrocolonies on the surface of the spleen was counted on day 8 postirradiation. The results were statistically processed using Student's t test.

RESULTS

Long-term discontinuous overheating of animals was attended by changes in the functional activity of lymphocytes, the severity and trend of changes depending on the time of overheating (Table 1). For instance, overheating of animals for 3 days was only attended by a rise of the proliferative activity of SC in response to stimulation with PHA. We failed to reveal any other changes in lymphocyte function.

No changes of proliferative activity of SC in response to stimulation with mitogens and alloantigens were observed for a 5-day overheating. However, the number of APC in the spleen dropped for this period of overheating, which may indicate that under conditions of thermal exposure the mechanisms of B-lymphocyte differentiation are disturbed while their proliferative potential is preserved.

Exposure of the animals to a dosed thermal factor for 10 and 20 days led to the development of pronounced immunosuppression, evidence of which was suppression of the proliferative activity of SC in response to stimulation with mitogens and alloantigens, as well as a reduced number of APC in the spleen.

Thermal conditioning during 30 days resulted in restoration of the number of APC in the spleen and of the proliferative activity of SC in response to stimulation with PHA. The proliferative activity of SC in response to stimulation with ConA, LPS, PWM, and alloantigens was still reduced.

TABLE 2. Exogenous Cloning for Long-Term Discontinuous Overheating ($M\pm m$)

Time of investigation, days	Number of macrocolonies per spleen	
	control	experiment
3	20.30±1.29 (10)	21.00±1.06 (11)
5	20.30±1.29 (10)	18.8±1.34 (11)
10	16.18±0.87 (11)	16.60±1.21 (10)
20	16.18±0.87 (11)	15.40±0.92 (10)
30	15.50±1.09 (12)	16.75±1.09 (12)
40	15.50±1.09 (12)	16.30±0.86 (12)

Note. The data are presented as the number of macrocolonies per spleen. Number of animals in the group shown in parentheses.

As the animals adapted to the given thermal exposure during long-term discontinuous overheating, the functional activity of lymphocytes was restored, which was indicated by the fact that on day 40 after the start of overheating the proliferative activity of SC in response to stimulation with B- and T-cell mitogens reverted to the normal level, and the number of APC remained at the control level.

Despite the functional recovery of the immune system during adaptation of animals to thermal exposure, pronounced immunosuppression was preserved in the organism for a long time. Since the efficacy of the immune system largely depends on the function of hemopoietic stem cells (HSC), the colony-forming activity of HSC was studied over the time course of overheating. The experiments demonstrated that long-term discontinuous overheating was not attended by changes in CFUsp (Table 2). The absence of changes in the colony-forming activity of HSC-precursors of immunocytes under conditions of immunosuppression may attest to preservation of the immune system potential and is evidently largely responsible for its functional recovery during adaptation of the organism to a high external temperature.

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