Specific and Nonspecific Reactions of Mouse Immune System under the Effect of Short-Term Exposure in Warm and/or Cold Water

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Transient changes in environmental temperature produce a short-term, but significant effect on the immune system reactions in laboratory mice. Activities of nonspecific resistance factors (peritoneal macrophages) in mice exposed in warm or cold water were characterized by similar reactions, while the reactions of cellular and humoral immunity were opposite. Exposure to cold water activated cellular immunity, while warm water activated humoral immune system. The most significant changes in the immune system reactions were observed during the first 3 days of thermal exposure. Temperature alteration from cold to warm leads to activation of cellular and suppression of humoral components of the immune system. Alteration of water temperature from warm to cold leads to activation of nonspecific resistance factors, cellular and humoral immunity.

Key Words: *nonspecific resistance; cellular and humoral immunity; exposure in warm and cold water*

Close relationship between immunogenesis and thermoregulation systems was demonstrated in numerous studies. The formation of specific immune response to infectious antigen, specifically, production of IFN-γ responsible for the formation of many cellular immunity reactions by Tx1 lymphocytes, is often associated with fever [1], while artificial hypothermia can lead to activation or inhibition of specific immune reactions to the antigen [2]. The available data mainly indicate the predominant suppressive effect of low environmental temperature on functional activity of the immune system. Elevation of environmental temperature can suppress the immune system, *e.g.* through activation of apoptosis and impairment of thymocyte maturation and their selection in the thymus [2-5]. The decrease of environmental temperature leads to a decrease in activity of phagocytosis system cells, intensity of cellular and humoral immunity reactions. The relationship between thermoregulation and immunogenesis systems can be realized through neuroendocrine regulation [1]. A direct correlation between increased concentration of norepinephrine and IL-6 concentration in the plasma was detected in humans exposed to cold [5]. *In vitro* studies showed that low temperature modified production of IL-1 by macrophages and suppressed the function of T-helpers (the most thermosensitive lymphocytes) [6,7].

Studies aimed at detection of thermal conditions promoting selective activation of cellular and/or humoral immune systems are extremely rare.

We studied the effects of short-term exposure to cold and heat on nonspecific and specific reactions of the immune system in laboratory animals.

MATERIALS AND METHODS

Experiments were carried out on 496 laboratory mice weighing 20-25 g. The animals were divided

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into 5 groups: 1) intact controls; 2) exposure in warm water; 3) exposure in cold water; 4) contrast alteration of water temperature from cold to warm (1 cold+heat cycle); 5) contrast alteration of water temperature from warm to cold (1 heat+cold cycle). The temperature of warm water was 40-42°C, that of cold water 7-9°C. The duration of exposure in warm water was 30 sec, in cold water 5 sec. Controls were fixed on the table during the same period without plunging into water (stress control). Manipulations with the animals (exposure in water or on the table) were carried out individually, once a day for 5 consecutive days. The animals of groups 2 and 3 were divided into 5 subgroups, depending on the number of water procedures (from 1 to 5). Immunization was performed after water exposures. The mice were sacrificed by cervical dislocation.

Immunoreactivity was evaluated by functional activity of nonspecific resistance factors (peritoneal macrophages), cellular and humoral immunity. The macrophage capacity to adhesion and absorption of opsonized sheep erythrocyte (SE) IgG and metabolic activity of macrophages in spontaneous NBT test were evaluated. Activity of humoral immunity was evaluated by Cunningham test. The count of antibody producing cells (APC) in the spleen was evaluated on day 5 after intraperitoneal immunization of animals with SE (4×10^8). Activity of cellular immunity was evaluated by delayed-type hypersensitivity (DTH) reaction to SE after Crowle. The animals were sensitized with 0.25% SE suspension in 0.5 ml saline intraperitoneally; on day 6 the mice were injected with a resolving dose of 50% SE in normal saline (50 μl) into the right hind paw pad; 50 μl saline was injected into the contralateral paw. The reaction was evaluated 24 h after injection of the resolving dose of SE by measuring edemas in the left and right paws and estimating the percentage of the right paw enlargement in comparison with the left paw.

The results were evaluated by methods of variation statistics using the SPSS 11.5 for Windows software.

RESULTS

First we evaluated changes in immunoreactivity induced by short-term exposure of animals in warm (Fig. 1) and cold (Fig. 2) water for 5 days. The values in the control group were taken for the "0" and the percentage of deviation from it in experimental groups was estimated.

Exposure to warm water (Fig. 1) modulated receptor, absorption, and metabolic activities of macrophages. The expression of receptors to aggregated IgG on macrophages somewhat increased ($p<0.05$) after the 1st exposure and decreased ($p<0.01$) compared to the control after the 2nd, 3rd, and 4th exposures, while after the 5th exposure it returned to the control level. Absorption of opsonized SE by macrophages changed in a wave-like mode: decreased after the 1st exposure ($p<0.05$), returned to the control level after the 2nd, increased after the 3rd exposure ($p<0.05$), decreased after the 4th ($p>0.05$), and increased significantly ($p<0.01$) compared to the control level after the 5th exposure. The metabolic activity of macrophages in the NBT test increased significantly on days 1-3 ($p<0.001$). After the 4th exposure the number of activated ma-

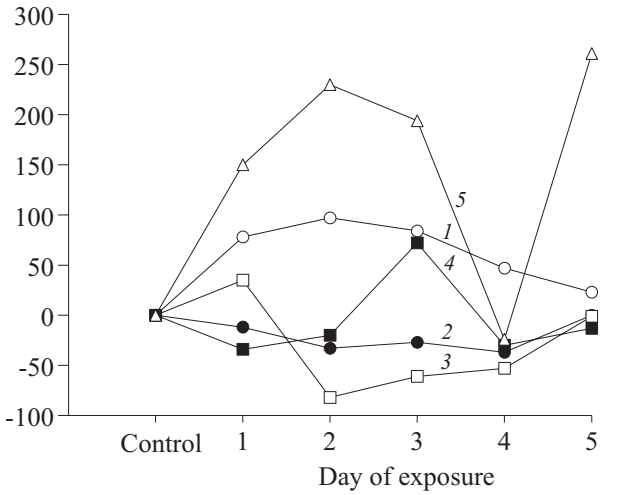

Fig. 1. Effect of warm water on functional activity of immune system of mice. *1*) count of antibody producing cells (APC) in the spleen; *2*) delayed-type hypersensitivity (DTH) reaction; *3*) macrophage adhesive activity; *4*) macrophage absorption activity; *5*) macrophage metabolic activity.

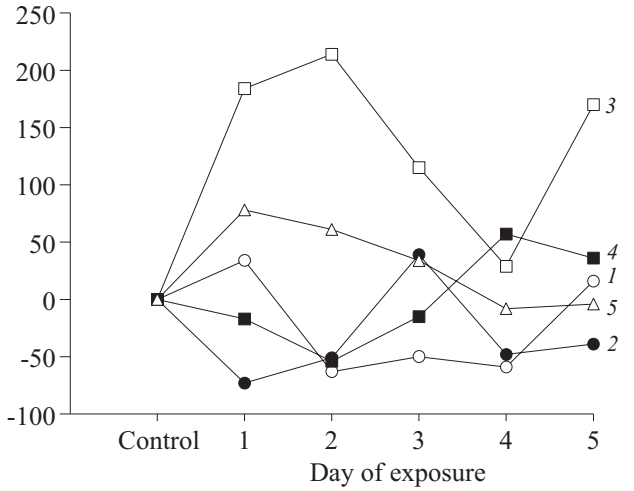

Fig. 2. Effect of cold water on functional activity of mouse immune system. *1*) adhesive activity of macrophages; *2*) absorption activity of macrophages; *3*) metabolic activity of macrophages; *4*) APC count in the spleen; *5*) DTH level.

TABLE 1. Effects of Contrast Alternation of Water Temperature on Functional Activity of Mouse Immune System

Group	ACM, %	PCM, %	NBT, %	APC/spleen	DTH, %
Control	39.4±2.6	13.9±1.1	6.6±0.5	42 563±3123	28.5±1.2
cycle cold—heat	37.2±2.5	13.0±1.3	8.2±0.9	26 471±2533**	40.8±2.4*
cycle heat—cold	42.5±2.7	21.5±1.3*	10.5±0.9*	58 297±3857**	34.9±1.9*

Note. ACM: adhesive capacity of macrophages; PCM: phagocytic capacity of macrophages. *$p<0.05$, **$p<0.01$ compared to the control.

crophages decreased to the control level, and after the 5th exposure spontaneous metabolic activity of macrophages increased again ($p<0.001$).

Short-term exposure in warm water modulated activity of specific humoral (APC count/spleen in response to SE) and cellular immunity (DTH reaction to SE). The count of APC per spleen was above the initial level after the 1st-4th exposure in warm water ($p<0.001$) and returned almost to the control level after the 5th exposure ($p>0.05$). DTH reaction to SE was below the control after the 1st ($p>0.05$), 2nd ($p<0.05$), 3rd ($p>0.05$), and 4th ($p<0.05$) exposures and returned to the control level after the 5th exposure.

After short-term exposure in cold water (Fig. 2) the expression of receptors to aggregated IgG on macrophages increased after the 1st session ($p<0.05$), decreased after sessions 2-4 ($p<0.01$), and returned to the control level after the 5th exposure. The capacity of macrophages to absorption of opsonized SE changed in a wave-like mode: decreased after exposures 1 and 2, increased after exposure 3, and decreased after exposures 4 and 5 ($p<0.05$). Metabolic activity of macrophages in spontaneous NBT test increased significantly from the 1st to the 3rd exposure ($p<0.001$). After exposure 4 the number of activated macrophages decreased almost to the control level. After the 5th exposure metabolic activity of macrophages increased ($p<0.001$).

Exposure in cold water suppressed humoral immunity (APC /spleen) below the control level after exposures 1 ($p>0.05$), 2 ($p<0.01$), and 3 ($p<0.01$); after exposures 4 and 5 this parameter returned to the control level. Activity of cellular immunity (DTH reaction to SE) increased significantly after the 1st, 2nd ($p<0.01$), and 3rd ($p<0.05$) short-term cooling session and returned to the control level after the 4th and 5th cooling session.

At the next stage of the study we evaluated the effect of contrast alternation of water temperature (cold to warm and warm to cold) on the immune system (Table 1). Single immunization of animals and testing with subsequent autopsy were carried out after 5 days of water exposure.

After 5 days of exposure in cold→warm water activity of macrophages virtually did not change, humoral immunity decreased (APC/spleen), and activity of cellular immunity increased in comparison with the control. After 5 days of exposure in warm→cold water the absorption and metabolic activities of macrophages increased ($p<0.05$), as well as activities of humoral (APC/spleen) and cellular immunity.

Hence, even a short-term thermal exposure had a significant impact on the functional activity of the immune system. Macrophages reacted similarly to decrease and increase of water temperature. Activities of cellular and humoral immunity changed in opposite directions. Exposure in warm water led to activation of mainly humoral immunity, while the decrease in water temperature led to activation of mainly cellular immunity. Contract alternation of water temperature from cold to warm promoted activation of humoral immunity, while alternation from warm to cold water activated all components of the immune system.

These results indicate that despite high specificity of the immune response, its intensity can be nonspecifically, but selectively regulated by specially chosen thermal conditions. The most significant changes in the immune system reactions were observed during the first 3 days of thermal exposure, and hence, this phenomenon can be used in vaccine prevention and immunocorrection for increasing the efficiency of these measures. Detection of the mechanisms of thermal effects on the development of specific immune response deserves a special study.

REFERENCES

1. Yu. V. Vetrova, O. V. Gus'kova-Alekseeva, V. N. Morozov, and A. A. Khadartsev, *Vest. Novykh Med. Tekhnologii*, No. 3, 100-105 (2000).
2. T. V. Kozyreva, L. S. Eliseeva, and S. V. Zlygosteva, *Ros. Fiziol. Zh.*, No. 1, 83-88 (2003).
3. S. V. Kruglov, L. A. Baida, M. G. Pshenichnikova, *et al.*, *Byull. Eksp. Biol. Med.*, **134**, No. 10, 374-378 (2002).
4. F. Z. Meerson, *Adaptation Medicine. Concept of Long-Term Adaptation* [in Russian], Moscow (1993).
5. K. M. Brenner, J. W. Castellani, C. Gabaree, *et al., J. Appl. Physiol.*, **87**, No. 2, 699-710 (1999).
6. D. F. Hanson, *J. Immunol.*, No. 151, 436-442 (1993).
7. D. F. Hanson, *Ann. NY Acad. Sci.*, No. 813, 453-464 (1997).