# Effect of Rapid Slight Cooling of the Skin in Various Phases of Immunogenesis on the Immune Response

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Slight cooling had no effect on heat emission and heat production, but modulated the immune response to antigen in animals. Changes in the immune response upon rapid slight cooling of the skin (by 1.5°C) depended on the phase of immunogenesis corresponding to cold exposure. When cooling was performed immediately after immunization, antibody production increased in the spleen and blood, while antigen binding in the spleen remained unchanged. Cold exposure on day 5 after antigen treatment as well as immunization at the peak of cooling did not modulate antibody production, but increased antigen binding in the spleen. Our findings attest to an important role of the temperature factor in the formation of the immune response, which should be taken into account during vaccination.

**Key Words:** *skin cooling; immune response*

Various systems of the organism, including the thermoregulatory and immune systems, are usually studied independently of each other and the relationships between these systems remain unknown. The understanding of these interactions is important for evaluation of functional activity of the organism in general and for the development of new methods of effective treatment (*e.g.*, vaccination).

There is no general agreement about the modulatory effect of cooling on the immune response due to differences in the rate, depth, duration, area, and type of exposure. Deep cooling usually decreases immunological reactivity of the organism [5,13]. The effect of this treatment is modulated by various preparations, including activators of carbohydrate and lipid metabolism [1] and vitamins A and E [3]. The absence of the effect of cold and heat exposure on the immune response in birds was also reported [12].

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During cold exposure the afferent temperature signal from thermoreceptors serves as the triggering and organizing factor, which regulates activity and interrelation between various systems. Electrophysiological studies were performed to evaluate pulse activity of thermosensitive skin afferents. Rapid cooling of the skin (more than 0.1-0.2°C/sec) was accompanied by dynamic or static activity of skin thermoreceptors. However, slow cooling of the skin resulted in the appearance of only static activity [7]. Our previous studies showed that as distinct from slow cooling, rapid surface cooling is followed by a significant increase in blood norepinephrine concentration [9]. Skin cooling modulates activity of peripheral thermoreceptors. The afferent signal from these receptors affects function of the endocrine and transmitter systems with immunomodulatory activity. The immune response is a complex dynamic process. Therefore, cold exposure at various stages of immunogenesis induces different changes in this process.

Here we studied thermal homeostasis and immunomodulatory effect of rapid slight cooling of the skin in various stages of immunogenesis.

## MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 248.6±6.2 g. The animals were maintained in a vivarium under standard conditions and had free access to water and food. Cooling and recording of physiological parameters were performed on nembutal-anesthetized animals (40 mg/kg) to exclude the emotional component of stress and suppress locomotor activity. The abdominal area (5×5 cm) was depilated and cooled with a water thermode and thermostats.

The thermoregulatory response to cooling consists of vascular (vasoconstriction) and metabolic reactions (contractile and noncontractile thermogenesis). Ear temperature (vascular reaction), total oxygen consumption (metabolic reaction), electrical activity of neck muscles (contractile thermogenesis during cooling), skin temperature on the abdomen (rate and depth of cooling), and rectal temperature (deep body temperature) were measured continuously. Temperature was measured with a copper-constant thermocouple. Physiological data were recorded and processed using a BIOPAC system.

The abdominal area was cooled at a rate 0.03°C/sec. Cooling was stopped when skin temperature decreased by 1.5°C and then normal temperature was restored. Cooling was performed 20-30 sec after immunization, before immunization (immunization during the peak cooling load), and on day 5 of the post-immunization period (maximum accumulation of antigen-binding and antibody-producing cells). Rectal temperature (core temperature) remained unchanged during slight cooling of the abdominal skin.

The animals were intraperitoneally immunized with sheep erythrocytes ($5\times10^8$ cells in 0.5 ml 0.9% NaCl) and decapitated 5 days after immunization. The immune response was studied routinely. We counted antigen-binding (rosette-forming) and antibody-producing cells (IgM) in the spleen. Hemagglutinin (HA) titer was estimated in blood plasma. The methods to study the immune response were described previously [2]. Control animals were subjected to similar manipulations, narcotization, and immunization without cold exposure. Temporal characteristics of narcotization and immunization corresponded to the scheme of the experiment. Each group consisted of at least 10 animals.

The results were analyzed by Student's *t* test.

## RESULTS

The baseline characteristics of thermal homeostasis before cooling were similar in all animals (Table 1).

Rapid slight cooling of the skin at various terms of immunogenesis was not accompanied by the thermoregulatory response. Parameters of heat emission and heat production remained unchanged during cooling.

Cold exposure 20-30 sec after immunization was followed by a 2-fold increase in the number of antibody-producing cells (APC) in the spleen. The absolute number of these cells in control animals was 529,800.7±12,109.0. Blood HA titer increased by 15% compared to the control (6.5±0.3). However, the number of antigen-binding rosette-forming cells (RFC) in the spleen did not differ from the control (47.5±1.8 RFC per 1000 spleen cells, Fig. 1).

APC number and HA titer remained unchanged under conditions of immunization at the peak of cooling, as well as during cold exposure on day 5 after antigen treatment (Figs. 2 and 3). The number of RFC increased by 1.5 times under conditions of immunization during the peak cooling load (control levels: 529,800.7±121,091.0 APC, 6.5±0.3 HA, 47.5±1.8 RFC per 1000 spleen cells) and after cooling at the peak immune response (control levels: 229,478.6±26,143.0 APC, 6.5±0.3 HA, 66.0±2.3 RFC per 1000 spleen cells).

Our results show that rapid slight cooling not inducing the thermoregulatory reaction considerable modulated the immune response to antigen.

**TABLE 1.** Baseline Parameters of Thermal Homeostasis in Rats before Cooling (*M*±*m*)

| Parameter | | Cooling | | |
|---|---|---|---|---|
| | | after immunization | before immunization | day 5 after immunization |
| Temperature, °C | intracutaneous abdominal | 38.8±0.1 | 38.9±0.1 | 38.7±0.1 |
| | rectal | 38.5±0.1 | 38.4±0.2 | 38.3±0.1 |
| | ear skin | 32.0±0.4 | 31.5±0.6 | 30.7±0.8 |
| Total oxygen consumption, ml/min×kg | | 23.9±1.0 | 22.4±0.7 | 20.4±0.7 |
| Electrical activity of muscles, μV | | 1.5±0.1 | 1.3±0.1 | 1.7±0.1 |

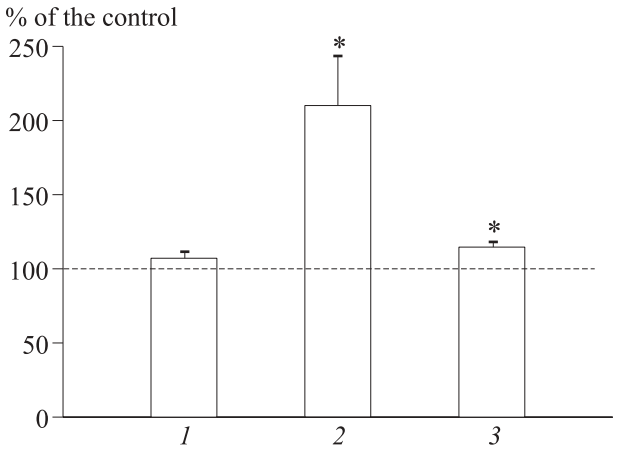


**Fig. 1.** Immune response to cooling 20-30 sec after immunization. *$p<0.05$ compared to the control. Here and in Figs. 2 and 3: RFC (*1*), APC (*2*), and HA ($\log_2$, *3*). Dotted line: control (100%).

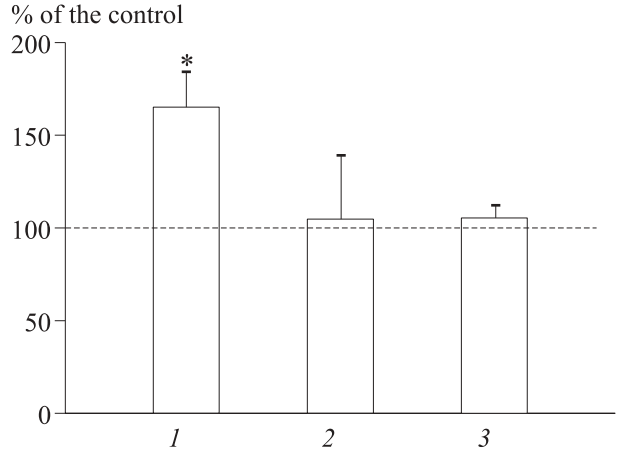


**Fig. 2.** Immune response during immunization at the peak cooling load. *$p<0.05$ compared to the control.

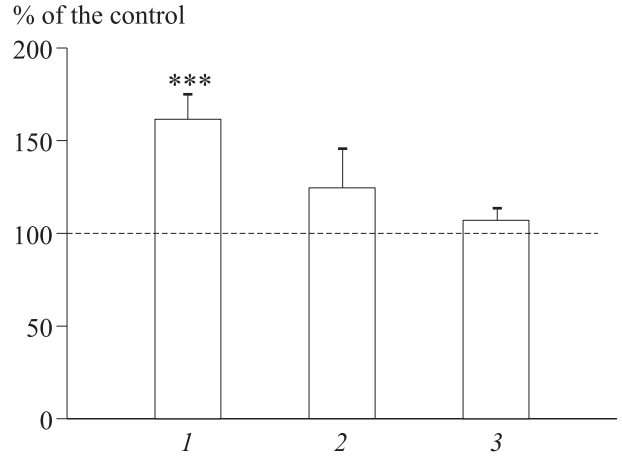


**Fig. 3.** Immune response during cooling on day 5 after immunization. *$p<0.001$ compared to the control.

Slight cooling of the skin was accompanied by stimulation of various components of the immune response. This reaction depended on the phase of immunogenesis corresponding to cold exposure. The immune response is usually suppressed during deep cooling [2,4,5,8]. These data suggest that the immune reaction is suppressed only during deep cooling, but not under any cold exposure.

Our experimental conditions excluded the influence of the emotional component of stress. Therefore, the observed changes in the immune response are related to cold exposure. The modulatory effect of temperature on the immune response depends on the order of immunization and cooling. It can be suggested that the interaction between thermoregulatory and immune systems in various stages of immunogenesis is mediated by various mechanisms. The differences are associated not only with changes in hormonal status induced by cooling and immunization, but also with variations in the composition of active immunocompetent cells at various stages of the immune response.

The immune response is accompanied by changes in noradrenergic activity in specific brain areas and various lymphoid tissues. Products of the immune system modulate the number and expression of adrenergic receptors in the thymus of rats [10, 11]. Protection of the organism from cold exposure is related to activation of the sympathoadrenal system. It manifested in an increase in the concentration of catecholamines in the blood and urine [9]. Postganglionic neurotransmitter norepinephrine plays an important role in the resistance to cold stress [6]. Previous studies showed that suppression of the immune response during deep cooling is realized via β-adrenoceptors [4,8,11]. Modulation of the immune response during cooling is probably associated with the influence of sympathoadrenal neurotransmitter norepinephrine. It was believed that activation of the sympathoadrenal system is followed by suppression of the immune response. Recent studies showed that this system ab increase immune function [8,12]. The mechanisms for stimulation of immunogenesis during slight cooling of the skin require further investigations.

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