Immune Response and Corticosteroid Content in Different Regimens of Cooling

T. V. Kozyreva and L. S. Eliseeva

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The effects of different cooling regimens modulating thermal afferent signal on the immune response were experimentally studied on rats. Immunization at low body temperature changed the immune response to the antigen. These changes depended on the depth and rate of previous cooling. The presence of dynamic activity of peripheral thermosensitive afferents potentiated the immune response after surface cooling and reduced the degree of suppression of the immune response after deep cooling. No clear-cut relationship between changes in the immune response and blood concentration of corticosterone during cooling was found.

Key Words: *cold; immune response; corticosterone*

Reaction of the organism to external factors is a combination of responses of different physiological systems. This combination is determined by the type of afferent information ensuring constant and precise interactions of these systems and the reaction of the whole organism to environmental conditions. It can be assumed that the development of the immune response also depends on the type of afferent thermal signaling.

The starting point of afferent information during cooling is peripheral skin receptors (afferent nerve endings). The signal from thermosensitive skin afferents depends on the magnitude, rate, and site of thermal exposure. Each thermoreceptor is characterized by static activity at a certain temperature range. Rapid heating or cooling (more than 0.01-0.02°C/sec) causes a dynamic reaction of thermoreceptors. Cold receptors respond to rapid cooling by a transient increase in firing rate followed by its decrease to a value corresponding to a new temperature.

We investigated the effects of different modes of cooling differently modulating the thermal afferent signal on the immune response of splenocytes to antigen.

Institute of Physiology, Siberian Division of the Russian Academy of Medical Science, Novosibirsk

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats. Cooling and immunization were carried out under Nembutal narcosis (40 mg/kg) in order to avoid emotional stress.

A 25-cm^2 shaven area on the abdomen was cooled using a water thermod and thermostats. Intracutaneous temperature of the cooled abdominal area and rectal temperature were constantly measured by thermocouples throughout the experiment. The intracutaneous temperature within the cooled area decreased with rates of 0.05°C/sec (rapid cooling) or 0.005°C/sec (slow cooling). The first regimen *a priori* determined the presence of a dynamic component in cold receptor activity, and the second regimen ensured the absence of this component. The duration of cold exposure determined the degree of cooling: surface cooling (decrease in skin but not rectal temperature), mild (decrease in rectal temperature by 1°C), and deep cooling (decrease in rectal temperature by 3-4°C). The animals were immunized after attaining of a certain skin or rectal temperature and then warmed to the initial temperature. The temperatures were recorded and processed on a computer. Each group of experimental and control animals consisted of at least 10 rats.

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The animals were immunized intraperitoneally with sheep erythrocytes (5×10^8 in 0.5 ml normal saline). The antigen-binding function of splenocytes was evaluated by the number of rosette-forming cells (RFC) in splenocyte suspension (per 10^3 examined cells) on day 5 after immunization [3]. In some experimental series RFC were counted 30 sec after immunization for evaluation of the early stage of the immune response. Antibody-producing function was evaluated by the number of plaque-forming (antibody-producing) cells (PFC) in the spleen on day 5 after immunization [4].

The plasma level of corticosterone was measured by high-pressure chromatography [10]. Blood was collected from the decapitation wound on day 5 or 30 sec after experimental exposure.

Controls were subjected to similar manipulations without cooling. The results were statistically processed using Student's *t* test.

RESULTS

The initial rectal and skin temperatures were 37.2±0.19 and 37.7±0.26°C, respectively. The number of RFC in the spleen of control animals on day 5 after injection of the antigen was 51.2±3.5 per 10^3 splenocytes and the number of PFU 349,125±42,928 per spleen.

Rapid surface and mild cooling associated with dynamic activity of skin thermoreceptors led to marked stimulation of the immune response (Fig. 1, *a*). The stimulation was more pronounced when only skin temperature decreased: the number of RFC increased more than 2-fold. Further cooling resulting in a decrease of rectal temperature by 3-4°C produced an immunoinhibitory effect: the number of RFC in the spleen decreased.

The number of PFU in the spleen after rapid mild cooling increased by 68% ($p<0.05$), while after rapid deep cooling (rectal temperature decrease by 3-4°C) decreased by 36% ($p<0.05$). Hence, changes in the number of antibody-producing cells under these regimens were similar to changes in the number of antigen-binding cells in the spleen.

Slow cooling of the skin did not modify the immune response to the antigen: the number of RFC in the spleen remained unchanged (Fig. 1, *b*). Slow mild cooling (rectal temperature decrease by 1°C) stimulated the immune response similarly to rapid cooling. Slow deep cooling with rectal temperature decrease by 3-4°C gave an opposite effect and suppressed the splenocyte response to the antigen. This suppressive effect was more pronounced than that produced by rapid deep cooling.

Stimulation of the immune response after surface and mild cooling and its suppression after deep cooling suggest that changes in the immune response depend on the severity and duration of cold exposure. This is in line with published reports on the significance of the strength and duration of stress exposure for the modulation of immune reactions [9].

The relationship between the rate of cooling and changes in the immune response attracts special attention. Our experiments showed that different effects of rapid and slow cooling are determined by different functional states of skin cold receptors: dynamic activity of these receptors during rapid cooling and the absence of this activity during slow cooling. The effect of dynamic activity of thermoreceptors was particularly demonstrative during surface cooling with a decrease of skin temperature by 2°C. The presence of dynamic activity during rapid cooling was associated with pronounced stimulation of the immune response (increased level of rosette formation), while slow sur-

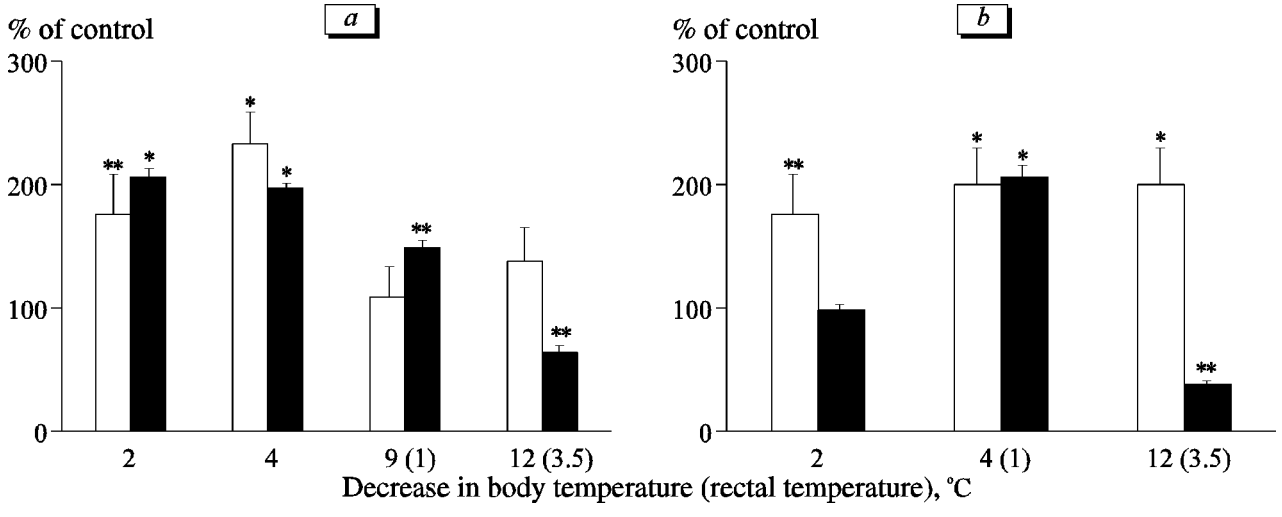

Fig. 1. Changes in blood corticosterone concentrations (light bars) and immune response to antigen (dark bars) on day 5 after immunization under conditions of rapid (*a*) and slow (*b*) cooling. Here and in Fig. 2: *$p<0.01$, **$p<0.05$ compared to the control.

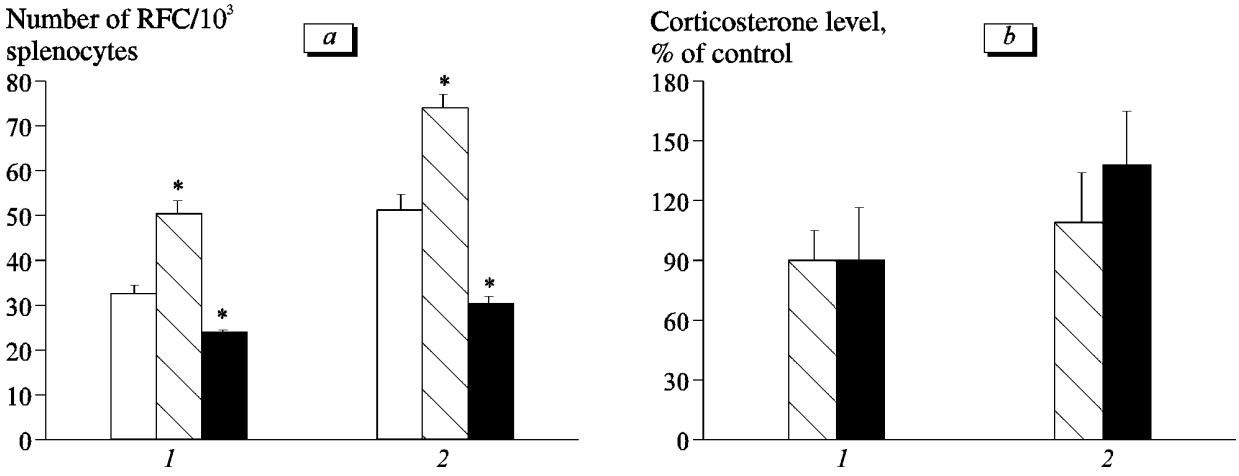

Fig. 2. Effect of preliminary rapid cooling on immune response (number of rosette-forming cells, RFC, *a*) and blood corticosterone concentration (*b*) 30 sec (*1*) and 5 days (*2*) after immunization. Light bars: control without cooling; cross-hatched bars: rapid mild cooling with rectal temperature decrease by 1°C; dark bars: rapid deep cooling with rectal temperature decrease by 3-4°C.

face cooling in the absence of this activity did not modulate the immune response (Fig. 1).

Both immunization and cold exposure can lead to changes in blood glucocorticoid concentrations [1,2]. We observed an increase in blood corticosterone concentration (from 0.100±0.012 to 0.150±0.013 μg/ml, $p<0.05$) in animals immunized without cooling. Changes in the plasma corticosterone depended on the protocol of additional cold exposure, which attests to the formation of different hormonal trace effects during cooling with different rate and depth (Fig. 1). However we found no detect relationship between changes in antigen-stimulated immune response (increased or decreased rosette formation) and blood corticosterone on day 5 after immunization and cooling.

The direction of changes in the immune response can manifest in the early period after immunization (Fig. 2, *a*). Both on day 5 and 30 sec after injection of the antigen and preliminary cooling, mild rapid cooling (decrease in rectal temperature by 1°C) stimulated , while deep rapid cooling (decrease in rectal temperature by 3-4°C) suppressed the immune response compared to the control. However the absolute count of RFC in the spleen 30 sec after immunization was much lower than on day 5 in both control and experimental animals. Plasma corticosterone remained unchanged 30 sec after immunization and cooling (Fig. 2, *b*), *i. e.* there was no relationship between corticosterone level and immune response, similarly to day 5 after immunization. This suggests the involvement of other hormone systems, *e. g.* sympathoadrenal system, in the formation of immune changes during thermal exposure.

It was previously shown that dynamic activity of skin cold receptors during rapid cooling determined more pronounced activation of the sympathoadrenal system [6-8]. These data are of particular importance for elucidation of the mechanisms underlying different effects of rapid and slow cooling on the immune reactions. The difference in the temporal parameters and degree of activation of the sympathoadrenal system during rapid and slow cooling can contribute to variations in immune reactivity. There is no consistent theory on the interactions between the sympathoadrenal and immune systems, but there are data on the effects of catecholamines on the immune system. These effects depend on their blood concentration at the moment of perception of antigenic information [5,9].

Hence, cooling during immunization modulates the immune response. These changes depend on the depth and rate of cooling. Surface cooling (decrease in skin temperature) stimulated the immune response only in case of rapid cooling associated with dynamic activity of cold receptors. Mild cooling with body temperature decrease by no more than 1°C stimulated the immune response both after slow and rapid cooling. By contrast, deep cooling suppressed the immune response, and this effect was more pronounced after slow cooling; the dynamic activity of skin thermoreceptors was absent in this case. The presence of dynamic activity of peripheral thermosensitive afferents potentiated the immune response after surface cooling and reduced the degree of suppression of the immune response after deep cooling. No relationship between changes in the immune response and blood corticosterone concentration during cold exposure was found.

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