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Effect of Three-minute Cold Exposure in Cryosauna at –70°C on the Human Cellular Immune System

S. A. Ponomarev[a], *, A. V. Muranova[a], S. A. Kalinin[a], E. N. Antropova[a], M. P. Rykova[a], M. M. Saltykova[a], and O. I. Orlov[a]

[a] *Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia*

**e-mail: cd147@bk.ru*

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Abstract—Cell components of the adaptive and innate immunity were investigated in essentially healthy volunteers aged 27 to 34 years. Peripheral blood was analyzed for absolute and relative counts of lymphocyte phenotypes $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$, $CD3^-CD16^+CD56^+$, $CD3^+CD16^+CD56^+$, $CD3^+CD25^+$, $CD45RA^+$, $CD4^+CD45RA^+$, and monocytes and granulocytes expressing pattern recognition receptors of the Toll-like family (TLR2, TLR4, TLR6) on the cellular membrane. A single cold exposure at –70°C was found to affect cell factors of the human immune system dramatically. Very low temperatures produce different changes in adaptive and congenital components of immunity representing a complex process triggered by the stress-reaction to a short stay in air cryosauna.

Keywords: air cryosauna, cold exposure, adaptation, lymphocytes, monocytes, granulocytes, pattern recognition receptors, TLR

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In the development of the Arctic region by people who are not adapted to low temperatures, a comprehensive study of the physiological adaptation and the assessment of the reserve capacity of the human body are issues of importance [1, 2]. It is well known that cold exposure has very strong adaptogenic effect on all systems of the human body, including the immune system [3–9]. However, the question of the mechanisms underlying the impact of low temperatures on the human immune system remains open. To date, it is known that the exposure to low and ultra-low temperatures results in a number of changes affecting both the adaptive and innate arms of the immune system [7, 9–11]. Models of low temperature exposure used by the researchers differ both in their physical properties and duration of exposure. Various indices of the immune status are being investigated. In addition, the sites of blood collection vary from researcher to researcher. Therefore, it does not seem possible to compare the data and only the immune response to nonspecific cold exposure may be discussed for each of the models. The approaches to measuring the effects of low temperatures on the human body may be roughly divided into two types: the first one involves cold water immersion; the other, exposure to low-temperature cryosauna or cold air temperatures.

A single 1-h cold water immersion at a temperature of 14°C did not cause statistically significant changes in the human immune system. At the same time, regular 1-h immersions over the period of six weeks lead to a significant increase in the peripheral blood IL-6 level and the level of monocytes and lymphocytes expressing cell surface interleukin-2 (IL-2) receptor (CD25). An upward trend in the total T-lymphocyte ($CD3^+$), T-helper ($CD3^+CD4^+$), cytotoxic-lymphocyte ($CD3^+CD8^+$), and activated T- and B-lymphocyte counts was observed. The level of tumor necrosis factor alpha (TNF-α) significantly increased whereas the concentrations of complement components C3 and C4, C-reactive protein (CRP), immunoglobulins G, M, and A (IgG, IgM, IgA), and the total leucocyte and granulocyte counts did not change [9].

In another study, 40 volunteers were singly exposed to a 170-min water immersion in a bath at a temperature of 14°C with a 10-min break every 20 min, during which the volunteers came out of the bath. This exposure resulted in a significant increase in the total leucocyte, neutrophil, and monocyte counts and a decrease in the total lymphocyte count. An upward trend in the level of anti-inflammatory cytokine IL-6 was also observed. TNF-α level did not change significantly, although one group of the volunteers demonstrated an increasing trend whereas the other, a decreasing trend [6].

The use of low-temperature cryosauna has demonstrated that the five-day course of cryotherapy, which included a 30-s session at a temperature of –60°C followed by 2-min exposure at –110°C led to an increase

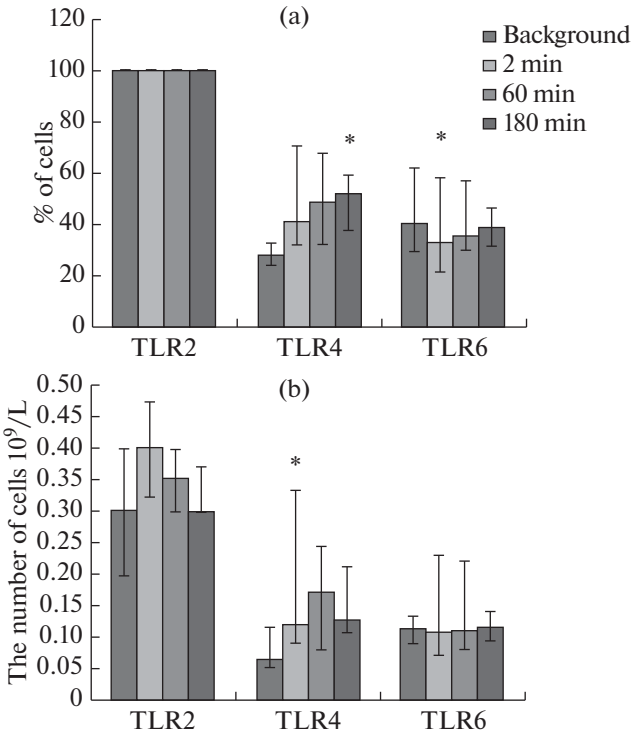

Fig. 1. Changes in the (a) relative and (b) absolute counts of the peripheral blood monocytes expressing TLR2, TLR4, and TLR6 at the cell surface in volunteers participated in the experiment. with cryosauna.
* Here and in Fig. 2, the data are presented as Me ($q25$–$q75$); * significant difference from the background, $p < 0.05$.

in the proinflammatory cytokine IL-10 concentration, a decrease in the concentrations of proinflammatory cytokines IL-8 and IL-2, a downward trend in the level of CRP in the peripheral blood, and an upward trend in IgA, IgG, and IgM concentration. The level of complement components C3 remained nearly unchanged [12].

A ten-day course of cryotherapy with 3-min cryosauna session at a temperature of –130°C led to a significant rise in the IL-6 level and in the lymphocyte, granulocyte, and monocyte counts in the peripheral blood [7].

Evaluation of the blood cytokine level in the blood serum of 45 volunteers has demonstrated that five cryotherapy sessions at a temperature of –130°C led to a significant increase in the IL-6 and IL-10 levels and a decrease in the level of IL-1α. The changes in the levels of these cytokines had the same direction after 10 and 20 sessions of cryotherapy. The level of other cytokines did not change significantly throughout the experiment [8].

Most researchers support the idea that prolonged cold exposure leads the changes in immune function, although there are some studies indicating that even short-term cold exposure may have a profound effect on the indices of the human immunity [6, 7]. It should be noted that the vast majority of studies are focused on the impact of low temperatures on humoral immunity in human. The question about the alterations in the humoral immune response to cold exposure remains open. Therefore, the goal of the present study was to assess the effects of short-term ultra-low temperature cold exposure on the cellular factors of the adaptive and innate divisions of the human immune system.

METHODS

Six apparently healthy men aged 27–34 years with the Quetelet body mass index (BMI) from 22.5 to 32.5 were invited for the prospective study. The participants gained the admission from the medical expert commission and signed an informed written consent to participate in the experiment according to the Declaration of Helsinki as an element of basic research by the Institute for Biomedical Problems, Russian Academy of Sciences. Experimental procedure was adopted at the Academic Board section and approved by the Biomedical Ethics Commission of the Institute for Biomedical Problems, Russian Academy of Sciences (Protocol no. 402 of 17 July 2015).

Venous blood samples for immunological study were collected in the morning on an empty stomach during a background period and also 2 min, 1 h, and 3 h after the termination of cold exposure. The volunteers stayed in a CryoAir cryosauna (Germany) with minimum clothing at a temperature of –70°C for 3 min. In order to avoid psychological stress reaction to the exposure to ultra-low temperature cryosauna, the volunteers attended an introductory session in the cryosauna without blood draw.

Determination of the leucocyte level and the absolute and relative monocyte and granulocyte counts in the peripheral blood was performed by using an automatic Celltac-α MEK 6318K blood analyzer (Japan).

Determination of the surface receptors of immunocompetent cells was performed by the multiparametric immunofluorescence technique using monoclonal antibodies (eBioscience, United States, and Hycult Biotechnology, Netherlands). The following indices were measured in the peripheral blood: the percentage and the absolute number of monocytes, granulocytes expressing TLR2, TLR4, and TLR6 Toll-like receptors at the cell surface, and $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$, $CD3^-CD16^+CD56^+$, $CD3^+CD16^+CD56^+$, $CD3^+CD25^+$, $CD45RA^+$, and $CD4^+CD45RA^+$ lymphocytes.

The results were analyzed by the method of flow cytometry in a FACSCalibur cytofluorometer (Becton Dickinson, United States) using the CellQuest Pro software.

Data processing was performed using the Statistica v.10.0 for Microsoft Windows software. The data were

presented as the median (Me) and interquartile ranges ($q25$–$q75$). The significance of the results obtained was assessed by the nonparametric Wilcoxon's test.

RESULTS AND DISCUSSION

The results of the study have demonstrated a series of changes in adaptive and innate immune systems of the human body. As can be seen in Figs. 1 and 2 and Tables 1 and 2, the changes affect subpopulation pattern and the absolute counts of lymphocytes, monocytes, and granulocytes of different types in the peripheral blood of the subjects who were exposed to 3-min cold exposure in the cryosauna. These data did not go beyond the clinical standards. A significant increase in the total leucocyte count due to a rise in the number of all types of white blood cells was observed 2 min after the termination of cold exposure. However, after 3 h of cold exposure, the lymphocyte level decreases significantly compared to the background values mainly due to a decrease in the $CD3^-$ $CD16^+CD56^+$ natural-killer (NK-cell) count. The levels of monocytes and granulocytes remained high and significantly exceed the background values (Table 1). According to the literature data, 10-day cryotherapy course with 3-min cryosauna exposure at a temperature of –130°C results in a rise in the lymphocyte, monocyte, and granulocyte levels [7]; at the same time, cold water immersion (14°C) for 2 h leads to a decrease in the number of lymphocytes [6].

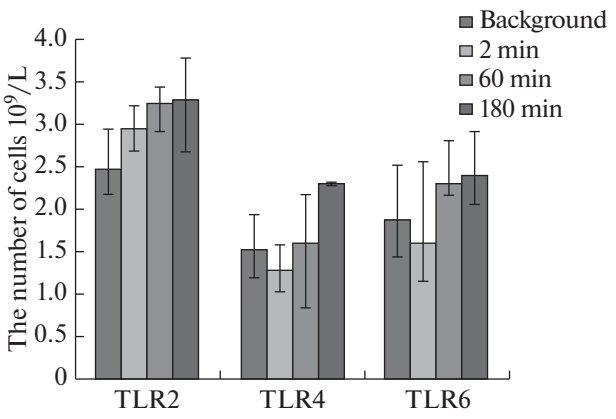

Fig. 2. Changes in the absolute count of the peripheral blood granulocytes expressing TLR2, TLR4, and TLR6 at the cell surface in volunteers participated in the experiment with cryosauna.

To date, this phenomenon cannot be explained satisfactorily in terms of the existing theories. It can only be suggested that a sharp increase in the leucocyte level reflects a robust generalized body reaction to cold exposure through the immune cell mobilization. It should be noted that, normally, the immune system is activated by any nonspecific exposure somehow associated in one way or another with a change in the hormonal status. As such, it was found that the reactions of this type occur not only in response to low-tem-

Table 1. Leucogram indicators of the volunteers participated in the experiment with cryosauna

Indicator	Examination time			
	background	2 min	1 h	3 h
Leucocytes, abs., $\times 10^9$/L	5.45 (5.10–6.02)	6.40 (6.15–6.72)*	5.75 (5.62–6.25)	6.30 (6.30–6.60)*
Monocytes, rel., %	5.00 (4.80–6.40)	5.75 (4.65–7.07)	5.25 (4.47–6.02)	5.45 (4.32–6.35)
Monocytes, abs., $\times 10^9$/L	0.30 (0.20–0.40)	0.40 (0.32–0.47)	0.35 (0.3–0.4)	0.30 (0.30–0.37)
Lymphocytes, rel., %	37.45 (31.87–43.02)	39.55 (37.87–40.32)	32.20 (29.02–35.60)	31.70 (28.2–39.40)*
Lymphocytes, abs., $\times 10^9$/L	1.90 (1.82–2.05)	2.50 (2.35–2.80)	1.80 (1.70–2.27)	1.85 (1.72–2.47)*
Granulocytes, rel., %	52.95 (45.77–59.97)	55.30 (54.75–56.82)	63.65 (59.15–66.50)	62.00 (54.55–67.20)*
Granulocytes, abs., $\times 10^9$/L	3.00 (2.65–3.50)	3.35 (3.15–3.62)	3.80 (3.20–3.95)	3.80 (3.35–4.25)*
CD14, abs.	247.26 (183.15–380.02)	283.43 (223.28–409.56)	240.30 (234.23–375.00)	251.37 (248.74–262.21)
CD14, rel., %	4.51 (4.00–5.83)	4.66 (3.79–6.16)	4.51 (4.13–5.81)	3.99 (3.91–4.73)

* Here and in Table 2, the data are presented as Me ($q25$–$q75$); * significant difference from the background, $p < 0.05$.

Table 2. Indicators of pattern of lymphocyte subpopulations in the peripheral blood of the volunteers participated in the experiment with cryosauna

Indicator	Examination time			
	background	2 min	1 h	3 h
$CD3^+$, rel., %	68.50 (68.00–76.50)	70.50 (67.75–75.50)	71.00 (68.00–77.00)	68.50 (66.00–76.25)
$CD3^+$, abs., $\times 10^9$/L	1.38 (1.28–1.45)	1.81 (1.63–2.21)*	1.41 (1.20–1.56)	1.37 (1.34–1.67)
$CD3^+CD8^+$, rel., %	25.00 (19.75–34.75)	26.50 (22.25–34.5)*	24.00 (19.50–33.75)	24.00 (19.25–31.00)
$CD3^+CD8^+$, abs., $\times 10^9$/L	0.58 (0.38–0.70)	0.83 (0.52–1.01)*	0.52 (0.39–0.69)	0.52 (0.46–0.65)
$CD19^+$, rel., %	12.00 (10.50–15.75)	12.50 (7.75–14.50)	17.00 (12.00–19.75)*	15.50 (12.25–18.75)*
$CD19^+$, abs., $\times 10^9$/L	0.27 (0.18–0.35)	0.33 (0.21–0.37)	0.31 (0.20–0.36)*	0.33 (0.24–0.38)*
$CD3^-CD16^+CD56^+$, rel., %	13.50 (10.75–15.50)	12.50 (12.00–17.50)	8.00 (7.25–11.00)*	8.00 (7.00–13.50)*
$CD3^-CD16^+CD56^+$, abs., $\times 10^9$/L	0.24 (0.20–0.29)	0.33 (0.28–0.41)*	0.17 (0.13–0.21)*	0.18 (0.12–0.25)*
$CD3^+CD4^+$, rel., %	39.00 (34.75–42.50)	39.00 (33.00–42.00)	44.50 (39.25–46.00)	40.50 (38.50–43.25)
$CD3^+CD4^+$, abs., $\times 10^9$/L	0.74 (0.64–0.92)	0.96 (0.92–1.02)*	0.82 (0.73–0.90)	0.77 (0.70–1.04)
$CD3^+CD25^+$, rel., %	4.00 (4.00–6.25)	3.00 (2.25–4.50)	4.50 (3.25–5.00)	5.50 (3.50–6.75)
$CD3^+CD25^+$, abs., $\times 10^9$/L	0.07 (0.06–0.13)	0.10 (0.06–0.12)	0.08 (0.05–0.11)	0.08 (0.06–0.17)
$CD4^+CD45RA^+$, rel., %	20.50 (17.25–23.00)	22.00 (16.25–24.00)	24.50 (15.50–27.50)	21.50 (16.75–24.75)
$CD4^+CD45RA^+$, abs., $\times 10^9$/L	0.42 (0.36–0.48)	0.56 (0.47–0.58)*	0.41 (0.33–0.55)	0.47 (0.42–0.48)
% of CD45RA in CD4	49.75 (42.10–59.27)	49.35 (41.25–60.45)	50.10 (38.57–59.22)	45.70 (41.57–56.87)
$CD45RA^+$, rel., %	70.50 (68.50–75.50)	72.0 (69.50–80.50)*	69.00 (67.50–72.00)*	72.00 (67.25–72.25)
$CD45RA^+$, abs., $\times 10^9$/L	1.45 (1.31–1.50)	1.89 (1.67–2.41)*	1.30 (1.20–1.47)	1.39 (1.30–1.56)
$CD3^+CD16^+CD56^+$, rel., %	9.50 (7.00–13.50)	10.50 (8.50–13.50)	10.50 (6.25–11.75)	9.00 (5.00–11.50)
$CD3^+CD16^+CD56^+$, abs., $\times 10^9$/L	0.18 (0.13–0.24)	0.25 (0.20–0.37)*	0.18 (0.14–0.19)	0.16 (0.11–0.20)

perature exposure, but also to high-temperature exposures [13] and even family disputes [14]. However, as noted above, the question of the polymorphism of the responses of different cell types to the same exposures remains open. In the present experiment, we observed such polymorphism not only among different types of the cells, but also within the lymphocyte subpopulation pattern. If 2 min after the cryosauna session both the absolute and relative counts of lymphocytes from different populations increase, then after 3 h of exposure the dynamic pattern changes: the absolute and relative counts of B lymphocytes ($CD19^+$) significantly increase whereas the NK-cell level in the peripheral blood decreases and the levels of T-helper cells ($CD4^+$), cytotoxic lymphocytes ($CD8^+$), and

$CD3^+CD16^+CD56^+$ T cells exhibiting the properties of natural killers approaches the baseline (Table 2).

Heterogeneity of the response to cold exposure is also indicative for the natural resistance system. As Figs. 1 and 2 show, a single exposure to cryosauna causes a significant rise in the absolute count of peripheral blood monocytes expressing TLR4 at the cellular membrane and a significant reduction in the number of monocytes expressing TLR6. In addition, 3 h after the termination of cold exposure, the percentage of $TLR4^+$ monocytes increases (Fig. 1). The change in the absolute and relative granulocyte counts was not significant (Fig. 2).

A number of researchers believe that these fluctuations in the number of leucocytes are associated with the activation of β-2 adrenergic receptors expressed on the surface of almost all types of immunocompetent cells and activated due to an elevation of the norepinephrine concentration in response to low-temperature exposure [6, 7, 15]. This sets off a complex cascade of reactions which leads to the increase in the synthesis of the proinflammatory cytokines such as IL-6 and IL-10 and the decrease in the IL-12, IL-1α, IL-1β, TNF-α synthesis by the activation of cAMP [7] in the cells which, in turn, modulate the leucocyte level in the peripheral blood. Some researchers think that the increase in the IL-6 concentration may enhance cortisol release which, in turn, contributes to the elevation of blood granulocyte level by inhibiting their adhesion to endothelial adhesion molecules thereby preventing their migration to the tissues [16].

CONCLUSIONS

(1) Exposure to air cryosauna for 3 min at a temperature of −70°C has a significant, mainly activating effect on the cellular components of the adaptive and innate arms of the human immune system.

(2) Polymorphism of the immune reactions emerges 1 h after the termination of cold exposure and 3 h later reaches its maximum.

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