GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of Single and Multiple Cold Stimuli on Nonspecific Resistance of Rats

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Acute cold stress induced lymphocyte activating factor production by peritoneal macrophages and elevation of blood corticosterone concentration in the rat, while lysosome-cation indices significantly decreased. Multiple cold stimuli initiated lymphocyte activating factor production by peritoneal macrophages in the rat, but corticosterone blood concentration decreased and lysosome-cation indices remained unchanged. The difference in lymphocyte activating factor production in response to single and multiple cold stimuli has been demonstrated for the first time.

Key Words: *intereleukin-1; lymphocyte activating factor; lysosome-cation proteins; stress*

Effects of the cold on nonspecific and immune resistance have been studied in the framework of the investigation of factors and mechanisms responsible for immune impairments in conditions of the North.

Modern analytical methods for assessment of antibacterial and immune system activity as well as of hormonal reactions are helpful in the investigation of the effects of cold on the organism's resistance.

The interactions between immune and neuroendocrine systems have been recently established. They modify functional activity of both systems, particularly in the presence of environmental negative factors [2,3].

Specific and nonspecific resistance are controlled by cytokines (interleukines (IL), interferons, tumor necrosis factor, etc.) produced by the immune system cells [7]. IL-1, which has a wide range of activity, is one of the main factors mobilizing these systems. This cytokine plays a keyrole in stimulating immune reactions and inflammation and exhibits pirogenic activity [8].

IL-1 transmits information between neuroendocrine and immune and nonspecific resistance systems. Affecting T and B lymphocyte activities, endothelium and connective tissue functions, and hemocytoblast genesis in the bone marrow, IL-1 stimulates resistance reactions [6].

It is involved in neuroendocrinal system activation and stress-induced reactions [6].

A variety of stressors modulate IL-1 production, increasing its blood concentration at early stages after stimulation [9].

While studying the mechanisms of the low temperature effects on the organism's resistance, we measured the level of lymphocyte activating factor (LAF), an indicator of IL-1 synthesis intensity. LAF was discovered in 1972 as a product of macrophage activity. It stimulates mice thymocyte proliferation in the presence of submitogenic amounts of lectines [11]. LAF stimulates lymphocyte proliferation and antibody synthesis. In other words, it exhibits the activities of a macrophagal factor that activates B

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cells. It acts as an endogenous pirogen. LAF contains IL-1, IL-6, and tumor necrosis factor [12].

Nonspecific resistance mechanisms, for instance, neutrophilic granulocyte (NG) antibacterial activity, are affected by various stress factors [4].

The lysosome-cation test (LCT) gives an informative index of neutrophilic antibacterial activity that reveals cationic proteins in peripheral blood NG with considerable antimicrobial activity [4,5]. Some of them, in particular, defensins modify the function of endocrine system because they are the corticostatins with a wide range of biological activity.

Neutrophilic granulocyte counts in humans from the Far North are 2-3 folds lesser than in humans from the European part of Russia [4].

According to Selye's theory, any stress increases glucocorticoid and adrenalin levels and NG count in peripheral blood. An increase in the level of IL-1 has been demonstrated in stress [8,9]. We studied LAF production by peritoneal macrophages, plasma corticosterone level, and the number of cationic proteins in NG in rats exposed to single and multiple cold stimuli.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weghing 170-180 g. Acute cold stimulus was a 20-min exposure to cold in individual chambers at -20°C (12 rats). Multiple cold stimuli were a series of such exposures repeated every day for 10 days (12 rats). Immediately after stress, peritoneal macrophages were separated and incubated for 18 h without additional stimulation in the presence of *St. aureus,* a conventional IL-1 production inductor.

The determination of LAF level in the incubation medium was based on comitogenic effect of LAF on murine thymocyte proliferation induced by conA in suboptimal doses.

The concentration of corticosterone was measured by direct rado immune assay [1]. The number of cationic proteins in NG was determined by the mean cytochemical coefficient [5]. The differences between the test and control groups were statistically analyzed by Student's *t* test.

RESULTS

Macrophages do not produce LAF in intact animals; however, after stimulation with *St. aureus* they produced LAF.

Peritoneal macrophages in rats exposed to the acute cold stimuli started producing LAF without any additional stimulation *in vitro.* No change in LAF level was observed after stimulation of the cells with *St. aureus* (Table 1).

Serum concentration of corticosterone after acute cold stimulus was significantly higher than in the control group ($p<0.05$) or in rats subjected to multiple cold stimuli ($p<0.05$). The acute cold stimulus increased LAF production by peritoneal macrophages. The multiple cold stimuli also augmented LAF production by rat macrophages without activation by staphylococcus. After addition of staphylococci, LAF production by macrophages increased in rats exposed to multiple cold stimuli in comparison with the control ($p<0.05$) or rats exposed to a single cold stimulus ($p<0.05$). Serum concentration of corticosterone in rats subjected to multiple cold stimuli decreased significantly in comparison with rats exposed to acute cold stimuli and with controls.

These findings demonstrate that both single and multiple cold stimuli induce LAF production by rat macrophages as well as bilateral shifts of serum corticosterone concentration. A comparison of the LCT parameters in different experimental conditions shows a decrease in the mean cytochemical coefficient for cationic proteins in rats after the single cold stimuli (1.23 arb. units, $p<0.01$), while no change was observed after multiple cold stimuli (1.61 arb. units) compared with the control (1.58 arb. units).

Thus, both single and multiple cold stimuli induce LAF production by rat peritoneal macrophages. In experiments with the multiple cold stimuli, it was

Table 1. Corticosterone Levels in the Blood Serum and Peritoneal Macrophage LAF Activities in Rats Exposed to Cold Stimuli (*M±m*)

Experimental groups (n=12)	Corticosterone concentration in serum, ng/ml	LAF activity, units/ml×10^{-3}	
		without stimulation	after stimulation by *St. aureus*
Control	232.6±32.37	0	4.12±0.08
Cold stimuli			
single	349.167±16.95*	3.2±0.86	3.1±0.07
multiple	142.5±17.2**	4.5±0.9+	7.7±0.7*+

Note. $p<0.05$: *compared with control; +compared with single stimuli.

established that LAF production by cells of mononuclear phagocyte system activated or not by *St. aureus in vitro* is significantly higher than in the control or in rats exposed to a single cold stimulus. LAF production initiated by single cold stimulus is accompanied by an increase in corticosterone level in the blood. At the same time, augmented LAF production by macrophages in case of the multiple cold stimulus is accompanied by a decrease in corticosterone level compared with the control rats.

Taking into account complex regulatory feedback relations between IL-1 (major LAF component) and glucocorticoid hormones [9] and the fact that a decrease in blood corticosterone level in adrenalectomized mice induces LAF production by mononuclear phagocytes, it seems that a possible mechanism responsible for increased LAF production by multiple cold stimuli is associated with the fall of blood corticosterone level in stressed animals.

A considerable decrease in LKT parameters in the singe cold stimuli conditions is consistent with the data on rat NG cationic proteins under acute stress [5]. After 10-day multiple cold stimuli, LKT parameters restored and practically did not differ from those in the control group.

Thus, no changes in blood levels of glucocorticoid hormones, of the number of NG, and cationic proteins in NG were observed in rats after multiple cold stimuli, while LAF production by macrophages remains enhanced. These findings suggest an adaptation to multiple cold stimuli.

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