EFFECT OF LOW TEMPERATURE ON LIPID PEROXIDATION IN THE LUNGS AND ON PULMONARY MACROPHAGE ACTIVITY IN RATS

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It was shown previously that the content of lipid peroxidation (LPO) products in the lungs is increased in rats exposed to the harmful action of low temperatures [4].

Among the possible producers of activated forms of oxygen, which trigger LPO, are macrophages. Accordingly, it was decided to study what structural changes take place in pulmonary macrophages during exposure to cold and to examine correlation between activity of the pulmonary macrophages, intensity of LPO, and the antioxidant potential of lung tissue at different stages of cold stress.

EXPERIMENTAL METHOD

Experimental were carried out on 60 male Wistar rats weighing 160-180 g. The animals of group 1 (30 rats) were kept at −7°C in a cold chamber, the animals of group 2 (30 rats) under normal conditions (20°C). The animals were killed after 2 h and 1 and 8 days. The LPO level was estimated from the accumulation of malonic dialdehyde (MDA) [10] and of diene conjugates (DC) [3]. Antioxidant potential was determined from the concentration of reduced glutathione [5] and α-tocopherol [11] in the lung tissue. Protein was determined by Lowry's method. Alveolar macrophages were obtained by washing out the bronchopulmonary tract three times [9] and they were counted in a Goryaev's chamber. For differential counting, the pool of washed out cells was deposited on a slide, fixed with methanol, and stained with azure II-eosin. Alveolocytes and macrophages were differentiated morphometrically [6]. The ingestive capacity of the pulmonary macrophage system was determined from the uptake of colloidal carbon (from "Wagner G."). The total number of interstitial macrophages loaded with carbon particles was counted in lung sections stained with hematoxylin and eosion in 10 fields of vision by means of a morphometric grid [2] under a magnification of 2500. Activity of cathepsin D and acid phosphatase (AP) was determined in a homogenate of the lungs [1] after preliminary flushing of the vascular bed of the lungs with cold physiological saline through the right ventricle.

EXPERIMENTAL RESULTS

During exposure of the rats to low temperatures the level of LPO products and activity of the antioxidant systems in the lungs underwent phasic changes (Table 1). The DC concentration in the lung tissue 2 h after the rats were placed in the cold chamber, for instance, showed a tendency to fall whereas the MDA concentration was unchanged. This was accompanied by a significant rise in the level of antioxidants: reduced glutathione and α-tocopherol. After 24 h the concentrations of LPO products in the lungs rose sharply whereas activity of the antioxidant systems fell significantly.

By the 8th day of exposure to low temperatures the DC and MDA levels remained high, despite an increase in the reduced glutathione and α-tocopherol concentrations in the lung tissue to control values. Whereas after exposure to cold stress for 2 h no significant changes were found in the parameters of the pulmonary macrophage system investigated, exposure to cold for 24 h led to an increase in the number of macrophages ingesting carbon particles in the inter-

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TABLE 1. Parameters of LPO and Activity of Antiperoxide Protection System during Cooling (M ± m)

Group of animals	Diene conjugates, nanomoles/g lipids	MDA, nanomoles/g protein	Reduced glutathione, μmoles/tissue	α-Tocopherol, μg/g tissue
Control	4,8±0,3	1,1±0,07	11,7±1,3	13,4±1,7
Cooling				
2 h	3,9±0,2	1,1±0,06	16,6±1,6	17,8±1,2
1 day	10,7±0,5**	2,1±0,09*	8,5±0,6*	7,4±0,6*
8 days	9,7±0,9*	2,0±0,2*	14,0±1,3	14,0±1,0

Legend. Each group consisted of 12 rats. Here and in Table 2: *P < 0.01, **P < 0.001 compared with control.

TABLE 2. Number of Washed Out Alveolar Macrophages, Number of Macrophages in the Interstices of the Lungs Ingesting Colloidal Carbon, and Activity of Cathepsin D and AP in Lung Homogenate (M ± m)

Group of animals	Number of macrophages washed out of alveoli (cells/g weight of lungs)	Number of macrophages in interstices loaded with carbon in 10 fields of vision	Cathepsin D activity, μmoles tyrosine/min/g protein	Acid phosphatase activity, μmoles tyrosine/min/g protein
Control	6,0±0,4	24,0±1,4	0,3±0,02	15,0±1,0
Cooling				
2 h	5,2±0,1	26,8±1,2	0,35±0,03	16,6±1,8
1 day	8,3±0,4	59,0±2,0*	0,91±0,02**	27,0±2,0*
8 days	3,6±0,4*	70,4±1,9**	0,8±0,02*	26,0±2,0*

Legend. Each group consisted of seven rats.

stices of the lungs to twice the number at the previous time (Table 2). The number of macrophages flushed out of the alveoli was increased by 1.4 times. Total cathepsin D and AP activity in the lung homogenate was increased by three and by almost two times respectively by the end of the first day.

After 8 days of cold stress the number of interstitial macrophages ingesting carbon showed a further increase although activity of the hydrolytic enzymes remained at the same level. There was a marked decrease in the number of alveolar macrophages in washings from the bronchopulmonary tract by 1.8 times (Table 2). Analysis of the adherent cell pool revealed a decrease in the number of fixed macrophages (76.0 ± 2.5 in the control, 33.0 ± 0.2 cells in the experiment), whereas the number of alveolocytes was increased (12.4 ± 0.5 in the control, 7.0 ± 0.8 cells in the experiment). Incidentally, the number of leukocytes in the peripheral blood of the control and experimental animals was almost identical: $(8.8 \pm 0.3) \cdot 10^3$ and $(9.7 \pm 0.5) \cdot 10^3$, respectively.

The increase in ingestion of inert colloid by the pulmonary macrophages, the increase in the alveolar pool of macrophages 24 h after the beginning of exposure to cold, and the increase in hydrolytic enzyme activity in the lung tissue, the main sources of which are pulmonary macrophage [8], thus suggest activation of the latter during cold stress. The increase in the concentration of LPO products in the lung tissue could be the result of depression of the antioxidant systems and of activation of catabolic processes which, in turn, stimulate the macrophagal response. LPO products in this case may act as agents causing primary injury to the tissue structures, and thereby "triggering" a complex of cellular responses directed toward repair of the injury. Phagocytic macrophages utilize more oxygen than resting macrophages, and generate activated forms of oxygen, which are precursors for peroxide reactions. Excessive entry of oxidants from activated macrophages into the medium sharply intensifies lipid peroxidation processes [7] and thereby contributes to the formation of a "vicious circle" with a decrease in resistance of the tissue structures of the lungs and the appearance of functional and morphological changes that are typical of cold injury [4].

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RELATIONS BETWEEN NATURAL KILLER CYTOTOXICITY AND INTERFERON SYSTEMS IN IMMOBILIZATION STRESS

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Normal killer (NK) cells constitute a lymphoid cell population with cytotoxic activity against various tumor cells and also cells infected with viruses or microbial agents [6]. Consequently, NK cells may play an important role in resistance to many diseases. Since these cells do not require preliminary antigenic sensitization for their function and, consequently, do not require time to develop their specific immune response, NK cells are rightly considered to be the "first line of defense" against malignant or virus-infected cells [7].

The writers showed previously that emotional-painful and immobilization stress regularly induce phasic changes in NK cell activity [2]. The first phase of deep depression of NK cell activity, corresponding to the stage of inhibition of nucleic acid and protein synthesis in stress, is followed by a second phase of increased NK cell activity, corresponding to the anabolic stage, i.e., to poststressor activation of nucleic acid and protein synthesis [1]. Since interferons and their inducers play a decisive role in the regulation of NK cell activity [5, 10] and since the serum interferon level falls in the initial phase of the stress response [9], it has been suggested that changes in interferon formation in the body may participate to some degree in the mechanism of the change in NK cell activity observed in stress. One way of testing this hypothesis is evidently to study the dynamics of the serum level of interferon and its formation by lymphocytes during development of the stress response.

The aim of this investigation was accordingly to study the dynamics of the serum interferon level and of interferon production by splenocytes and to compare them with depression of NK cell activity in immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on inbred male CBA mice weighing 16-18 g. Immobilization stress was produced in animals in the supine position with their limbs fixed for 6 h. NK cell activity was determined 1, 3, 5, 7, 14, and 21 days after exposure to stress. The mice were

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