Bronchoalveolar Lavage Cells and Mitotic Activity of Monocytes and Macrophages in Rats after Long-Term Intermittent Hypoxia

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Long-term (1.5 months) intermittent hypoxia promoted desquamation of bronchial epithelial cells, decreased the relative content of alveolar macrophages and monocytes in bronchoalveolar lavage, stimulated lymphocyte migration to the lungs, and increased the relative content of lymphoid cell in rats. Mitotic activity of monocytes and macrophages decreased against the background of intraalveolar lymphocytosis (lymphocytic alveolitis).

Key Words: *hypoxia; lungs; intrapulmonary cell count*

Repeated normobaric hypoxia (NH) is used in clinical practice for the therapy of chronic bronchitis and bronchial asthma [2,7,8]. Low oxygen content in inspired air causes a variety of adaptive reactions in the body, including hyperventilation, enhanced blood circulation, constriction of pulmonary vessels, and increased cell membrane permeability.

It was shown that repeated NH (30 min a day for 10 days) with a gas mixture containing 10% O_2 elevates the content of epinephrine and norepinephrine in the peripheral blood and induces immediate adaptive reactions in Wistar rats: pronounced desquamation of the bronchial epithelium (BE), intense migration of neutrophils to the lung parenchyma, and degranulation of connective tissue mast cells [5,6,9]. These changes are accompanied by an increase in the relative content of neutrophils and lymphocytes (by more than 2 times) in bronchoalveolar lavage fluid (BALF) and the rise of phagocytic activity of neutrophils and macrophages. Morphometry revealed no signs of lung inflammation except for focal dystelectases. Repeated NH causes hyperplasia of lymphoid follicles in the mucosa of large and lobar bronchi [5,6,9]. It was suggested that the high content of effector cells (neutrophils and lymphocytes) in BALF reflects adaptation of the respiratory system to short-term intermittent hypoxia.

Here we studied the response of effector cells (macrophages, lymphocytes, and neutrophils) to NH and estimated mitotic activity of monocytes and macrophages under conditions of intraalveolar lymphocytosis.

MATERIALS AND METHODS

Experiments were performed in summer on male outbred albino rats weighing 200-280 g kept in a vivarium. Experimental rats were exposed to NH (n=8) for 20 min (10% O_2, 6 times a week, 1.5 months). Twelve intact animals served as the control. BALF was obtained 1.5 months after the start of the experiment. The rats were *in situ* intraperitoneally narcotized with nembutal. Warm physiological saline was pumped intratracheally (37°C, 20 mm Hg) to complete filling of the lungs and then aspirated with a syringe. We assayed BALF samples (n=16) obtained at 13.00 from 12 control and 4 experimental rats without signs of diseases. The number of cells per 1 ml BALF was calculated in a Goryaev chamber. The ratio between dead and viable cells was estimated by 1% trypan blue exclusion. BALF samples were centrifuged on a Heraeys Christ centrifuge, stained by the method of Romanovskii—Giemsa, and studied under a light immersion

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microscope (500 cells from each sample, ×1000). Intrapulmonary differential cell count (percent of various cell types) was then evaluated [1].

Proliferative activity of monocytes and macrophages was estimated by calculating the number of mitosis per 4000 mononuclear alveolar monocytes and macrophages in BALF. The mitotic index was expressed in promiles.

The results were analyzed by Fischer—Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Histological assay of the lungs from control and experimental animals revealed no pathological changes. The total cellularity of BALF did not differ between control and experimental rats (Table 1). Repeated NH induced desquamation of BE cells (Table 1). The relative content of BE cells in BALF from experimental rats increased to 47%. The mean number of BE cells in 1 ml BALF from these animals was higher than in the control (Table 1). All cells were stained with trypan blue and, therefore, appeared to be dead. These results are consistent with published data that single and repeated NH are accompanied by marked desquamation of BE cells [5,8,9].

Repeated NH led a considerable decrease in the relative content of alveolar macrophages and monocytes (Table 1). However, the percent of dead alveolar macrophages did not differ between the control and experimental animals (Table 2). Dead lymphocytes (100%) were found only in 1 control rat, while bronchial epitheliocytes (100%) were dead in all control rats and in 4 of 5 experimental rats. Mitotic activity of monocytes and macrophages in experimental rats was 2-fold below the control value (Table 2).

The relative content of neutrophils in BALF from control and experimental rats was the same, which indicated the absence of neutrophilic alveolitis in the majority of animals exposed to long-term intermittent hypoxia. At the same time, in some experimental rats the number of BALF neutrophils increased to 2.5%, which was probably determined by individual reactivity. Our results contradict the published data that the relative content of BALF neutrophils in Wistar rats increases by 16 times after repeated NH (10 exposures) [5,6]. It can be suggested that prolonged intermittent NH (1.5 months, 36 exposures) promotes adaptation of the bronchoalveolar system, which manifests in normalization of cell composition.

The relative content of lymphoid cells in BALF from experimental rats increased by 7 times. Apart from small lymphocytes, BALF contained large cells with 1 centrally positioned nucleus with invaginations, light ring around the nucleus, and bright-blue granular cytoplasm with solitary light vacuoles. These cells were referred to plasma cells. They were only occasionally seen in the control, but constituted 2-8% BALF cells in experimental animals (Table 1). The presence of plasma cells in BALF from animals ex-

TABLE 1. BALF Cellularity and Intrapulmonary Cell Count in Male Outbred Rats (*M±m*)

Parameter	Control (n=12)	Experiment (extreme values, n=4)
Number of cells in BALF, 10^6/ml	0.13±0.02	0.11±0.02 (0.06-0.17)
Relative content of cells, %		
alveolar macrophages and monocytes	95.20±1.05	78.9±4.7* (71.5-95.0)
lymphocytes	2.5±0.7	17.5±3.9* (4.5-24.5)
neutrophils	1.95±0.66	0.8±0.4 (0-2.3)
plasma cells	0	2.7±1.7* (0-8.5)
Number of bronchial epithelial cells, 10^{10}/ml	0.0130±0.0019+	0.045±0.009*
%	13.18±3.70	17.4±4.9 (4.50-42.63)

Note. Relative contents of alveolar macrophages, lymphocytes, and polymorphonuclear leukocytes were estimated without and with BE cells. BE cells were not calculated in evaluating intrapulmonary cell count. +n=5. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Mitotic Activity and Number of Dead Monocytes and Macrophages in BALF from Male Outbred Rats (*M±m*)

Parameter	Control (extreme values, n)	Experiment (extreme values, n)
Number of dead alveolar macrophages and monocytes, %	4.10±3.73 (0-9.52; 12)	5.16±3.56 (0-9.28; 5)
Mitotic index, %	2.70±0.41 (1.0-4.5; 8)	1.25±0.23* (0-2.0; 8)

posed to NH is the sign of activation of the bronchoalveolar immune system. It can not be excluded that mitoses in BALF monocytes and macrophages are suppressed during lymphocytic alveolitis. Hypoxia is probably accompanied by activation of lymphocytes, whose cytokines inhibit mitoses in macrophages and monocytes.

Hence, long-term repeated intermittent NH (similarly to short-term NH) stimulates migration of lymphocytes to the lungs. Lymphoid cells probably migrate from hyperplastic follicles of bronchial lymphoid tissue [3,6] and from pulmonary blood capillaries, whose permeability increases under hypoxic conditions. These changes reflect an adaptive response of the bronchoalveolar immune system. Activation of the bronchoalveolar immune system during long-term intermittent hypoxia is not only the structural and functional mechanism responsible for adaptation to stress, but also the factor preventing hypoxia-induced damages [4]. Under conditions of NH, physiological regeneration of alveolar macrophages during activation of the bronchoalveolar immune system is realized via recruitment of bone marrow precursors and migration of circulating monocytes to the lungs followed by their differentiation into macrophages [10]. Proliferation of monocytes and macrophages in the alveoli probably plays a secondary role in this process.

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