Diurnal Fluctuations in the Mitotic Activity of Alveolar Macrophagal Monocytes from Bronchoalveolar Washings-Off in Mice

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Previous experimental and clinical studies have demonstrated that human and animal alveolar macrophagal monocytes obtained as a result of bronchoalveolar lavage can divide directly in alveoli both in health and in pulmonary diseases [1-8], but there are virtually no reports on the effects of circadian rhythms on the rate of proliferation of these cells. We have found only one relevant paper [7].

The task of our research was to find out whether the level of mitotic activity of alveolar macrophagal monocytes is subject to circadian fluctuations.

MATERIALS AND METHODS

Twenty male F_1(BALB×Black 6) mice weighing 18-20 g were used in the experiments. The mitotic index (MI) of alveolar macrophagal monocytes obtained from tracheobronchial washings-off was assessed in February twice daily, at 08:00 and 20:00 h, each time in 10 animals. The animals were sacrificed by cervical dislocation, after which 1 ml of sterile normal saline heated to 37°C was injected through an incision in the trachea into the lungs. Two minutes later the wash was sucked off and the resultant cell suspension passed through a capron filter. Cell viability was then assessed using 1% trypan blue in a Goryaev chamber, and cytological preparations were made using an original method [1]. The preparations were fixed in methanol and stained after Romanovskii-Giemsa. After the washing-off, the viscera were inspected, and animals with signs of any abnormalities were discarded. The mitotic activity of macrophagal monocytes was assessed after examination of 2000 mononuclear cells in each case. The MI was expressed in promille. The cellular composition of a pulmonary lavage was analyzed in each case with assessment of the percent share of various cellular elements - the endopulmonal cytogram (EPC) - per 1000 cells. Animals with an EPC differing from the norm were rejected. The results were processed using the Fisher-Student test. Differences were considered reliable at $p<0.05$.

RESULTS

Two animals were rejected before assessment of alveolar macrophagal monocyte mitotic activity: one had an enlarged spleen, the other neutrophilia (16%) and lymphocytosis (21%) in the EPC. Alveolar macrophagal monocyte mitoses were detected in tracheobronchial washings-off of 17 out of 18

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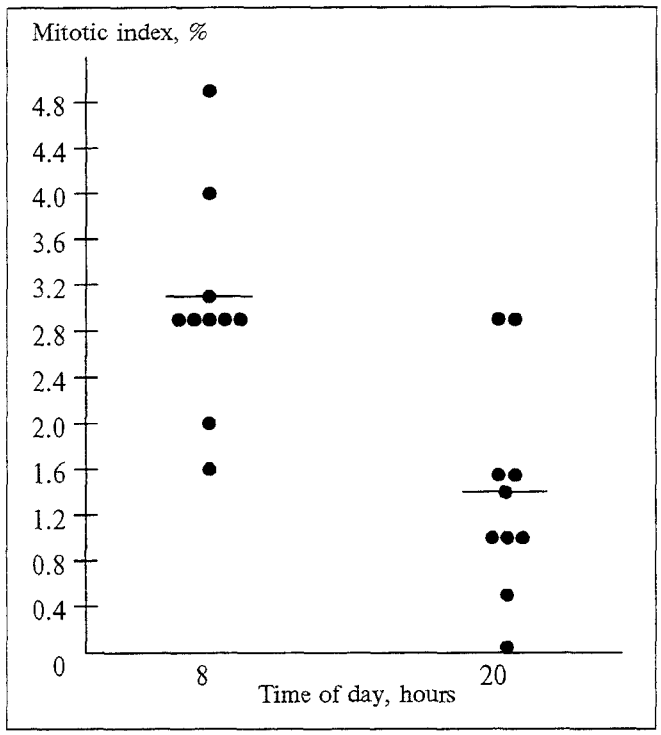

Fig. 1. Mitotic activity of murine alveolar macrophagal monocytes at various times of day.

mice, being absent in only one animal tested in the evening. All phases of mitosis occurred, from prophase to late telophase. The levels of individual mitotic activity varied from 1.6 to 5.0‰ in the morning hours, being 3.07±0.3‰ on average, and from 0 to 3.0‰ in the evening hours, 1.39±0.3‰ on average (Fig. 1). We see that there was a higher mitotic activity (2.2-fold) of alveolar macrophagal monocytes in the morning than in the evening hours, the differences being statistically reliable ($p<0.002$).

Hence, using cytological preparations of tracheobronchial washings-off from intact F_1(BALB×Black 6) mice without cytostatics, we showed that the level of proliferation of alveolar macrophagal monocytes changes over 24 h under normal conditions. This level is reliably higher in the morning (08-10:00 h) than in the evening (20-22:00 h).

Our data are at variance with the results of an earlier study [6] of 24-hour colchicine-arrested mitoses from Fisher 344 rat bronchoalveolar washings-off without pathogenic microflora. According to these authors, 1.83% of alveolar macrophages had mitoses over 24 hours; they came to the conclusion that populations of these cells in intact rats may be maintained only due to their proliferation directly in alveoli. No noticeable circadian fluctuations in mitotic activity were revealed. Unfortunately, the time of mitosis assessment (morning or evening hours) was not specified in this paper. It is possible that the discrepancy of the results is explained by differences in the species of animals used. Methodological specificities should not be neglected either: the authors in question counted colchicine-arrested mitoses, whereas we used no cytostatics.

Our data on diurnal fluctuations in the mitotic division of alveolar macrophagal monocytes in the lungs should be taken into consideration in studies of the physiological regeneration of the population of these cells and when analyzing the extent of their proliferation in various pulmonary diseases.

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