BRONCHOALVEOLAR CELL COMPOSITION IN EXPERIMENTAL PNEUMOCONIOSIS

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UDC 616.24-003.661-07:616.231.24-008.8-076.5/-092.9

KEY WORDS: endopulmonary cytogram; alveolar macrophage; pneumoconiosis

Coal dust, on entering the lungs, promotes the development of pneumosclerosis [3]. In contemporary research death of alveolar macrophages (AM), which are responsible for phagocytosis of coal particles, is regarded as a primary mechanism of development of lung fibrosis [2, 4]. It has also been suggested that viable AM and neutrophils play a role in the pathogenesis of lung diseases, including in the development of pneumosclerosis [10, 11, 12]. Mature activated AM secrete a broad spectrum of enzymes such as elastase, collagenase, and acid hydrolases, as well as modulators of cell behavior, inducers of growth and proliferation of fibroblasts and collagen synthesis.

They also form various substances which have a direct cytotoxic and cytolytic action on cells in vitro [5, 9]. Meanwhile it is not yet known whether the absolute number of AM per unit of alveolar surface of the pneumoconiotic lung is increased, or whether there is any change in the absolute and relative numbers of other cells (lymphocytes, neutrophils) in the internal medium of such a lung when the functional load on the macrophagal system of the organ is increased. The aim of the present investigation was to study these problems. No research of this kind has hitherto been conducted on models of chronic anthracosis.

EXPERIMENTAL METHOD

Anthracotic pneumosclerosis was produced by keeping noninbred male albino rats for 6 months in a chamber into which coal dust was injected 6 times a week, for 3 h each time, by means of a dosing device. The coal dust corresponded to stage III of metamorphism and it was not contaminated with silica. The dust concentration in the inspired air was 300 mg/m^3. The animals were allowed access to food and water ad lib. There were two series of experiments. In series I, on 26 intact rats and on 18 rats which had inhaled coal dust, with a body weight of 280-320 g, the volume of the lungs fixed in inspiration through the pulmonary artery [7], the total alveolar surface area by Weibel's method [1], and the collagen content in the lung tissue by Slutskii's method [6] were determined. The lung tissues were studied histologically in semithin sections stained with toluidine blue. In the experiments of series II bronchoalveolar lavage was carried out once under deep pentobarbital anesthesia on 13 control and 19 experimental rats by the method described previously [8]. The number of cells in 1 ml of bronchoalveolar washings (BAW) and the number of nonviable AM after staining with trypan blue were counted in a Goryaev's counting chamber. In films obtained from the cell suspension of BAW, stained by the Pappenheim–Kryukov method, 1000 cells were examined in each case and the result given in the form of an "endopulmonary cytogram" [8]. Rats with no signs of any disease other than anthracosis were used in the experiment. The results were subjected to statistical analysis by the Fisher–Student method. Differences were considered significant at the $p \leqslant 0.05$ level.

EXPERIMENTAL RESULTS

The collagen content in the lung tissue 6 months after inhalation of coal dust was increased by 63%, the weight of the lung was increased, and the total alveolar surface area was reduced as a result of overstretching of some alveoli. These data are evidence of the develop-

Laboratory of Pulmonology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Department of Operative Surgery and Topographic Anatomy, Karaganda Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 726-728, December, 1988. Original article submitted October 2, 1987.

 0007-4888/88/0012-1768$12.50

TABLE 1. Characteristics of Lung and BAW of Rats after Inhaling Coal Dust (M ± m)

Parameter	Control	Experiment
Collagen content in lung tissues, mg/g wet weight	41.1 ± 0.93 (10)	65.5 ± 1.1 (7)
Volume of lung fixed at inspiration, cm^3	11.1 ± 0.4 (10)	10.2 ± 0.8 (6)
Total alveolar surface area (S), m^2	0.296 ± 0.001 (6)	0.271 ± 0.001* (5)
Volume of lungs according to degree of filling with physiological saline during obtaining of BAW, ml	14.7 ± 0.09 (13)	15.3 ± 0.06 (19)
Volume of BAW obtained, ml	9.8 ± 0.7 (13)	9.2 ± 0.7 (19)
Number of cells ($\cdot 10^6$) in 1 ml of BAW	0.22 ± 0.2 (13)	0.35 ± 0.02* (19)
Number of cells ($\cdot 10^6$) in total BAW (C)	2.27 ± 0.29	3.19 ± 0.23*
Total number of AM in BAW**	2.02 ± 0.3	2.89 ± 0.2
Number of nonviable AM, %	3.7 ± 0.6 (13)	4.9 ± 0.6 (19)
Total number of neutrophilic granulocytes in BAW**	0.11 ± 0.05	0.13 ± 0.03
Total number of lymphocytes in BAW**	0.12 ± 0.04	0.15 ± 0.04
Cell density coefficient (C/S)	7.6	11.7

Legend. *) Results in control and experiment differ significantly ($p \leqslant 0.05$), **) calculated data obtained from relative percentages of cells in endopulmonary cytogram; number of animals shown in parentheses.

ment of anthracotic fibrosis of the lung, accompanied by emphysema. The volume of the lungs was almost unchanged compared with the control (Table 1).

AM, responsible for phagocytosis of dust, are located in the lumen of the bronchi and alveoli and in the lung tissue, where as a rule they are surrounded by cells of the lymphoid series. An increased number of cells was observed in BAW of rats which had inhaled coal dust: the number of cells in 1 ml of BAW was increased by 59%, and in the washings as a whole by 40% (Table 1). An increased cell count ($0.40 \cdot 10^6$ cells in 1 ml) in the control was observed in only one of 13 rats, but in the experiment, in seven of 19 rats [$(0.37\text{-}0.57) \cdot 10^6$ cells in 1 ml]. The BAW contained 60-85% of AM containing coal particles in their cytoplasm, i.e., activated cells predominated. The number of nonviable AM in each case did not exceed 9.5%, with a mean value of 4.9% (Table 1). Thus massive death of AM in the dust-affected lung was observed.

Cells of the monocytic-macrophagal series predominated in the endopulmonary cytogram of both control and experimental rats: In the control they accounted for 90.1 ± 1.6%, and in the experiment, for 90.9 ± 1.2%. Lymphocytes accounted for 5.0 ± 1.3 and 4.5 ± 1.0%, and neutrophilic granulocytes for 4.6 ± 1.3 and 4.4 ± 0.8% respectively (Fig. 1). In both control and experiment two cases were found in which the number of lymphocytes in BAW was increased (11.3 and 17.6% in the control, 17.3 and 14.8% in the experiment). Eosinophils were present in BAW of individual animals (Fig. 1). An increased proportion of neutrophils (18%) was observed in BAW of only one intact rat and of two rats which inhaled coal dust (10.4 and 11.1%).

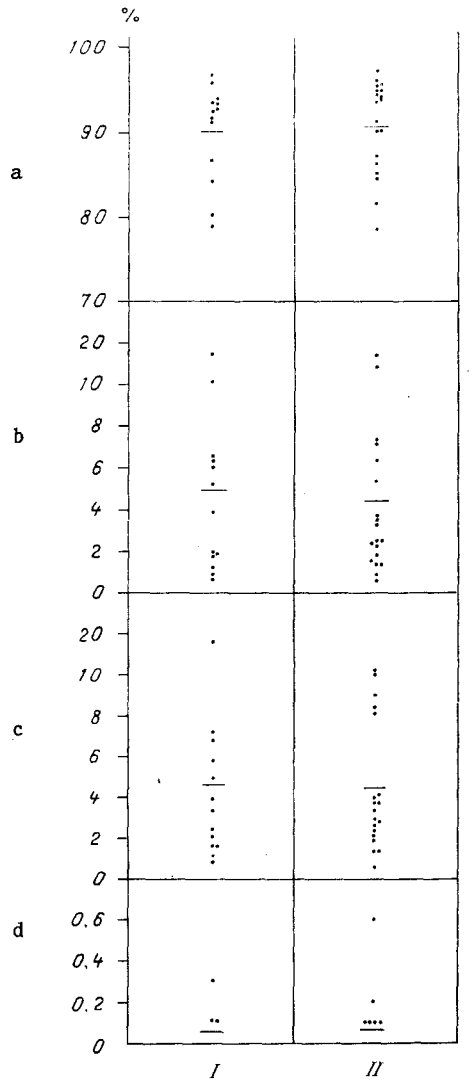

Fig. 1. Endopulmonary cytogram of BAW of control and experimental animals. Vertical axis: monocyte-macrophages (%); b) lymphocytes (in %); c) neutrophils (in %); d) eosinophils (in %). I) Control; II) anthracosis.

The increase in the cell count of BAW from the dust-affected lungs took place parallel with an almost proportional inflow of various cells into their respiratory part. The absolute number of AM in anthracosis was significantly ($p<0.05$) increased by 43%, of lymphocytes by 25%, and of neutrophils by 18% (Table 1). In addition, the number of cells per unit area of alveolar surface was increased in the dust-affected lung. This was shown by an increase in the value of the coefficient reflecting the cell density in the alveoli of the affected lung.

The results are in agreement with those obtained by other investigators, who showed that the cell count of BAW is increased in miners [13] and in rats and guinea pigs with experimental silicosis, but there is no increase in the number of lymphocytes and eosinophils; significant mortality among AM likewise is not observed [10, 11]. However, in silicosis, neutrophilia is observed as a rule in BAW, evidence of inflammation in the lung.

Thus during development of anthracotic pneumosclerosis the number of AM, lymphocytes, and neutrophils per unit area of alveolar surface increases; the increased cell count in BAW against the background of a "normal" ratio of the different kinds of cells and the moderate level of death of AM, may be a sign of an increased functional load on the macrophagal system during development of fibrosis of the lung and active migration of cells into its internal medium.

The possibility cannot be ruled out that the increase in the absolute number of mature viable activated AM in the respiratory part of the lung during inhalation of "inert material" (coal particles) is a key factor in the initiation of interstitial fibrosis. The population of activated AM, in our opinion, may play a direct part in the pathogenesis of anthracosis.

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TIME COURSE OF STRUCTURAL AND FUNCTIONAL RESTORATION OF THE SCIATIC NERVE AND OF SKIN RECEPTORS FOLLOWING REINNERVATION OF THE ALBINO RAT HIND LIMB

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UDC 616.833.58-001-092.9-003.9-07

KEY WORDS: regeneration; nerve; skin receptors; morphology; function

The problem of restoration of limb function after trauma to a nerve trunk is at the center of attention of morphologists, pathophysiologists, and clinicians. An abundance of experimental data has been gathered and is evidence of differences in the degree of effective repair of nerves depending on the techniques and materials used for reposition of the injured nerve [2-5, 14]. Data on the morphological features of regeneration of nerve fibers in different regions of the nerve after its injury and subsequent neuroplasty [1, 4] with replacement of the gap by various conducting sheaths [1, 6-8, 11-13], have been described in several publications. Attempts at morphometric analysis have been undertaken in order to explain the nature of the regenerating fibers after crushing and division [9, 10]. Chiu and co-workers [8], who used a method of joining together ends of a nerve by means of a venous autograft, carried out morphological investigations of the regenerative process together with electrophysiological observations. The authors cited studied and characterized the development of functional activity of the muscles in the injured animal limb by recording M responses. However, no comprehensive study of morphological and functional restoration of the conducting part of nerve trunks and of their receptor endings in the skin has yet been carried out during reparative

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 728-732, December, 1988. Original article submitted March 24, 1988.