PHAGOCYTIC ACTIVITY AND CYTOCHEMICAL PROPERTIES OF POLYMORPHONUCLEAR LEUKOCYTES AND MONOCYTES IN LUNG TISSUE OF ALBINO MICE INFECTED WITH Mycobacterium tuberculosis

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Many facts have now been accumulated as a result of the study of the phagocytic activity of the cells of the liver, spleen, lymph glands, and peritoneal exudate toward Mycobacterium tuberculosis [1-3, 5, 6]. Phagocytosis of M. tuberculosis in lung tissue has received less study, and the biochemical changes taking place in phagocytic cells in tuberculosis have hardly been investigated at all.

The object of the present investigation was to study phagocytosis of M. tuberculosis in the lung tissue of albino mice and, at the same time, to determine intracellular carbohydrates in the phagocytes as an indicator of their metabolic activity.

EXPERIMENTAL METHOD

Experiments were conducted on albino mice infected intranasally with a 2 week culture of M. tuberculosis (strain $H_{37}R_V$) in a dose of 400 million bacterial cells. To obtain a homogeneous suspension, the culture was ground in a mortar with physiological saline and centrifuged for 3 min at 3000 rpm. The bacterial suspension was prepared immediately before infection and its density was determined against a standard bacterial suspension. Mice were killed with ether 0.5, 2, 6, 18, 48, and 120 h after intranasal infection with M. tuberculosis. The lungs were removed from the thorax and washed in distilled water. Each lobe of the lung was cut through the middle and impression films made from the cut surface on four slides: two films fixed in Nikiforov's mixture and stained by the Ziehl-Neelsen method were used for counting the phagocytic number and the number of active phagocytes. These indices were determined separately for the polymorphs and monocytes. Intracellular carbohydrates were detected in two impression films, one of which was incubated with amylase for 30 min at 37°, after which both were treated by Shabadash's method: they were fixed in 96° alcohol for 24 h, treated with 0.8% potassium periodate solution for 30-35 min, washed in three changes of distilled water, treated with Schiff's reagent for 50-60 min, and counterstained with a weak solution of methylene blue. The amylase control showed that the polymorphonuclear phagocytes contained glycogen split by amylase. These cells, after treatment with amylase, when stained by Shabadash's method had only an almost invisible cherry-red border of glycogen around the edge of their cytoplasm. The cytoplasm of the monocytes stained by Shabadash's method contained a small quantity of fine granules of pale pink polysaccharide in the perinuclear zone, and as the amylase control showed, this was not glycogen. Since both carbohydrates revealed were polysaccharides, the intracellular carbohydrates found in the polymorphs were called glycogen and the carbohydrate in the monocytes was called simply polysaccharide.

In each impression film 100 polymorphs were counted and distributed into three groups depending on the number of stained granules (+, ++, and +++). During analysis of the results, only cells with average intensity of staining and intensively stained cells (++ and +++) were counted as polymorphs containing glycogen. Analysis of the polysaccharide in the monocytes showed that changes in their phagocytic activity were not accompanied by fluctuations in the content of polysaccharide with the cells, but by an increase or decrease in the number of cells containing polysaccharide and this was expressed in percent. The phagocytic indices and cytochemical properties of the lung tissue cells were studied in 90 albino mice weighing 18-20 g.

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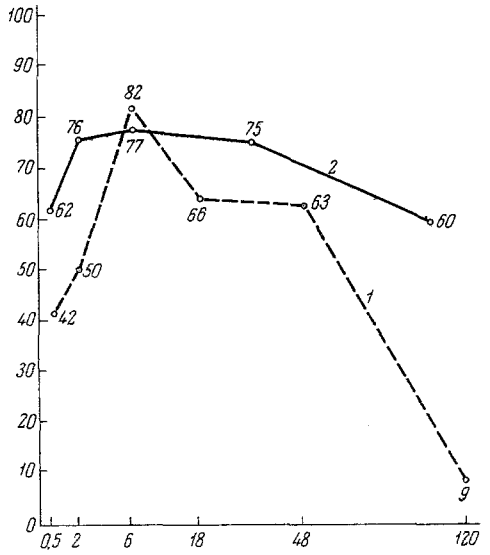
Changes in Phagocytic Activity of Monocytes and Polymorphs at Various Times After Infection (M ± m)

Time after infection (in hours)	Phagocytic number		No. of active phagocytes (in %)	
	monocytes	polymorphs	monocytes	polymorphs
½	1,65±0,11	0,10±0,01	38±2,49	5±1,04
2	2,13±0,16	0,13±0,02	44±5,25	5±1,03
6	2,28±0,17	0,28±0,06	46±2,66	10±2,35
18	3,19±0,19	0,28±0,04	58±3,22	14±2,20
48	3,75±0,19	0,15±0,04	65±2,82	8±2,41
120	1,82±0,15	0,06±0,01	48±2,10	3±0,84



Dynamics of changes in numbers of polymorphs and monocytes containing glycogen and polysaccharide after infection. 1) Polymorphs; 2) monocytes. Ordinate) number of phagocytes (in percent), abscissa) time after infection (in h).

Thirty albino mice were infected at the same time, and distributed into groups, each containing five animals, for each time of sacrifice. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

At the first investigation the phagocytic number of the monocytes was 1.65 (see table), rising after 18 h to 3.19 and reaching its maximum of 3.75, i.e., 2.3 times the initial value, 48 h after infection. Subsequently the phagocytic number fell and 120 h after infection it was almost back at its original level – 1.82. A similar pattern of changes was found in the number of active monocytes, with the exception that their percentage 120 h after infection was still much above the initial level.

Statistical analysis of the significance of the difference between the results obtained initially and those obtained after 18 h ($P < 0.01$) and 48 h ($P < 0.01$) confirmed these conclusions. Results of comparison of the indices after 48 and 120 h were also statistically significant ($P < 0.01$).

The study of phagocytosis of M. tuberculosis by polymorphs showed that their phagocytic activity was less than that of the monocytes (see table). The phagocytic number of the polymorphs ½ h after infection was 0.1, i.e., one-sixteenth of the phagocytic number for the monocytes. However, the phagocytic indices of the polymorphs rose slightly in the course of development of experimental tuberculosis to reach a maximum 6-18 h after the beginning of the experiment. Phagocytosis of the bacterial cells by the polymorphs then diminished and by 120 h it was almost down to its initial level.

Parallel determination of the cytochemical properties showed that the increase in phagocytic activity of the polymorphs was accompanied by an increase in the content of intracellular glycogen (see figure). The number of polymorphs with an increased (++ and +++) content of intracellular glycogen was maximal (82%) 6 h after infection, when it was almost twice its initial value. Subsequently the glycogen content in the polymorphs fell. A sharp decrease in glycogen content took place 120 h after the beginning of the experiment (9%).

In the course of development of phagocytosis a regular pattern was observed in the changes in the number of monocytes containing polysaccharide. However, these changes were less marked than those for the polymorphs (see figure).

The investigations described above thus revealed some general principles governing the dynamics of development of phagocytosis of M. tuberculosis by the polymorphs and monocytes in the lung tissues of albino mice infected intranasally. During the first hours after infection a phagocytic reaction develops, involving both polymorphs and monocytes, but the latter are much more active in this respect. Phagocytosis by monocytes reaches its maximum 48 h, and by polymorphs 6 h after infection with M. tuberculosis. The increase in phagocytic activity of the cells is accompanied by an increase in the content of intracellular carbohydrates in the polymorphs and by an increase in the percentage of monocytes containing carbohydrate.

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