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CHANGES IN PHAGOCYTES AND LYMPHOCYTES IN OBSTRUCTIVE PNEUMONIA

L. V. Yashchenko

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A cytochemical investigation was made of the state of the phagocytes and lymphocytes exposed to the action of a foreign body under septic and aseptic conditions. One of the main mechanisms of the development of an inflammatory-destructive process in the lungs is by local and systemic changes in the ratio between the numbers of phagocytes and lymphocytes, disturbance of the permeability of their lysosomal membranes, and an increase in the number of destroyed macrophages, neutrophils, and lymphocytes, all of which can be regarded as indices of cellular regression.

KEY WORDS: pneumonia; regression; macrophages; leukocytes.

Investigations by Anichkov et al. [1-3] have shown that after partial obstruction of a bronchus (by suturing or introduction of a foreign body) progressive inflammatory-destructive changes develop in the lungs. Their development is associated with activation of the autogenous flora and the arrival of an exogenous flora in the respiratory tract. Macrophages and lymphocytes of the blood have been studied by the writer during this process [6, 8, 9].

EXPERIMENTAL METHOD

A length of nylon thread 6 cm long and 0.6 mm in diameter was introduced into the trachea of 32 rabbits. The distal end of this length of thread lay at the bifurcation of the trachea. Four healthy rabbits acted as the control. The experimental animals (four at each time) were killed 1 and 3 days, 1 and 2 weeks, and 1, 2, 3, and 6 months after introduction of the thread. To assess the action of the foreign body on the phagocytes and lymphocytes under aseptic conditions, a length of sterile nylon thread 1 cm long and 0.2 mm in diameter was introduced intraperitoneally into 43 albino mice. Five animals served as the control group. The experimental

Laboratory of Histochemistry and Electron Microscopy, Department of Pathological Anatomy of Human Tumors, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. Experimental Laboratory, I. M. Sechenov Scientific-Research Institute of Physical Methods of Treatment and Medical Climatology, Yalta.

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TABLE 1. Changes in Morphological Composition, Number of Cells with Diffusion of Acid Phosphatase, and Number of Destroyed Cells in Trachea of Rabbits at Different Times after Introduction of Foreign Body

Index studied	Cells	Control	Time of investigation							
			1 day	3 days	1 week	2 weeks	1 month	2 months	3 months	6 months
Percentage of various cells	Macrophages	73,33±1,23	38,0	21,2	16,0	18,0	46,5	45,68	48,0	32,2±2.94 $P<0,001$
	Neutrophils	16,34±0,53	55,0	66,4	74,25	71,0	47,0	34,66	32,0	28,2±3,36 $P<0,001$
	Lymphocytes	10,33±0,53	7,0	12,4	9,75	11,0	6,5	19,66	20,0	39,6±2,52 $P<0,01$
Percentage of cells with diffusion of acid phosphatase	Macrophages	45,99±3,06	43,94	69,93	67,73	72,44	68,47	53,55	60,24	62,34±1,65 $P<0,001$
	Neutrophils	24,55±2,26	58,32	70,64	63,65	66,29	61,0	63,70	69,23	74,45±2,01 $P<0,001$
	Lymphocytes	25,99±0,69	38,83	49,06	57,99	60,04	57,52	61,37	60,12	62,13±1,24 $P<0,001$
Percentage of destroyed cells	Macrophages	22,66±2,10	54,0	49,66	52,33	56,66	41,33	44,33	—	52,33±2.10 $P<0,001$
	Neutrophils	15,0±1,26	37,33	53,33	62,0	71,66	71,66	75,0	—	70,0±1,68 $P<0,001$
	Lymphocytes	8,33±1,26	34,0	62,33	65,0	63,33	29,66	28,66	28,33	32,0±0,84 $P<0,001$

mice (five at each time, except three at 35 days) were killed 4 and 6 h, and 1, 2, 4, 6, 8, 21, and 35 days after introduction of the thread. Smears taken from the trachea and peritoneal cavity were stained by the Romanovsky–Giemsa and Gram methods. Acid phosphatase activity was demonstrated by Burstone's method (with naphthyl phosphate). To determine the extent of the changes in the phagocytes and lymphocytes in the rabbits, films from the peritoneal cavity (at 6 months) and peripheral blood (at all times) were stained by the Romanovsky–Giemsa method and for acid phosphatase. When films stained by the Romanovsky–Giemsa method were assessed, the cell composition and number of destroyed cells were expressed as percentages. Staining by Gram's method was used to study the microbial flora in the films. During analysis of preparations in which acid phosphatase activity was found the character of distribution of the enzyme was estimated: the granular consistency and the diffuseness of formation of the end product of the reaction. The basis for this assessment is the information in [7] on the lysosomal localization of acid phosphatase, which is responsible for the granular character of formation of the end product of the reaction in the cytochemical test. Conversely, an extralysosomal localization of the enzyme determines the diffuse character of distribution of the end product of the reaction, and from the functional point of view, as has been shown in various pathological processes [4, 5], this is evidence of a disturbance of membrane permeability. In each film from the trachea and peritoneal cavity at least 100 cells, and in each blood film at least 200 cells, were counted, making a grand total of about 40,000 cells. The significance of differences thus revealed was determined by statistical analysis of the material by Student's t test.

EXPERIMENTAL RESULTS

As a result of the action of the foreign body under both septic and aseptic conditions largely similar changes took place in the phagocytes and lymphocytes (Tables 1 and 2).

This was expressed as a decrease in the number of macrophages, an increase in the number of neutrophils and lymphocytes, with a simultaneous disturbance of the permeability of their lysosomal membranes, and an increase in the number of destroyed cells of all three types. The difference observed in the times and character of the cellular changes discovered in the trachea and peritoneal cavity was probably due to the microbial factor. The disturbances discovered in the phagocytes and lymphocytes of the trachea, indicating the development of processes of cellular regression, were connected with the accumulation and activation of a microbial flora and destruction of the mucous membrane, as revealed by the appearance of numerous microbial cells in films from the trachea stained by Gram's method and "sheets" of desquamated epithelium in films stained by the Romanovsky–Giemsa method starting from 1-2 weeks after the beginning of the experiment. The changes described were systemic in character, for as Tables 3 and 4 show, as a result of intratracheal introduction of the thread in the rabbits, the number of cells with diffusion of acid phosphatase and the number of destroyed cells in the peritoneal cavity of the rabbits, and the cell composition and number of cells with diffusion of acid phosphatase in their blood changed. Regression of phagocytes and lymphocytes is evidently an important factor

TABLE 2. Changes in Morphological Composition, Number of Cells with Diffusion of Acid Phosphatase, and Number of Destroyed Cells in Peritoneal Cavity of Albino Mice at Different Times after Introduction of Foreign Body

Index studied	Cells	Control	Time of investigation								
			4 h	6 h	1 day	2 days	4 days	6 days	8 days	21 days	35 days
Percentage of various cells	Macrophages	85,5±3,30	58,66	34,03±7,98 $P<0,001$	62,5	69,5	42,25	73,25	66,75	64,17	77,0±9,40 $P<0,001$
	Neutrophils	7,0±2,24	19,0	53,33±6,30 $P<0,001$	21,5	18,75	18,5	18,0	18,0	19,83	10,0±1,76 $P<0,001$
	Lymphocytes	7,5±2,80	22,33	12,73	16,0	11,75	39,25±1,28 $P<0,001$	8,75	15,25	16,00	13,0±1,76 $P<0,05$
Percentage of cells with diffusion of acid phosphatase	Macrophages	13,91±3,36	54,36	60,51	95,07	90,46	95,77±1,08 $P<0,001$	89,81	90,12	79,97	52,77±11,40 $P<0,001$
	Neutrophils	4,79±0,48	18,79	13,09	74,6	83,9	77,58	85,66±0,86 $P<0,001$	83,77	75,65	29,36±4,33 $P<0,001$
	Lymphocytes	5,1±0,32	13,42	10,84	10,98	36,64	54,22	73,51	87,37±1,54 $P<0,001$	62,62	26,78±3,15 $P<0,001$
Percentage of destroyed cells	Macrophages	12,33±1,26	21,0	39,33	45,66	47,66	50,66	46,66	48,6	65,27	72,0±3,52 $P<0,001$
	Neutrophils	2,66±0,42	14,0	12,33	18,33	20,33	23,33	24,0	29,66	41,99	39,0±1,93 $P<0,001$
	Lymphocytes	2,66±0,42	5,33	5,33	6,66	8,33	9,33	7,0	10,0	31,38	31,0±1,76 $P<0,001$

Legend. Control indices compared with indices of primary maximal variations. The latter were compared with indices obtained on the 35th day.

TABLE 3. Changes in Number of Cells with Diffusion of Acid Phosphatase and Number of Destroyed Cells in Peritoneal Cavity of Rabbits after Intracheal Injection of Nylon Thread

Index studied	Cells	Control	6 months after procedure
Percentage of cells with diffusion of acid phosphatase	Macrophages	23,75±1,05	66,66±3,85 $P<0,01$
	Neutrophils	0	60,0
	Lymphocytes	20,55±0,97	53,84±2,05 $P<0,001$
Percentage of destroyed cells	Macrophages	20,66±2,52	44,0±2,52 $P<0,001$
	Neutrophils	2,0±0,84	7,0±2,10 $P<0,05$
	Lymphocytes	2,66±0,42	11,0±0,84 $P<0,001$

TABLE 4. Changes in Morphological Composition and Number of Cells with Diffusion of Acid Phosphatase in the Peripheral Blood of Rabbits at Various Times after Intratracheal Introduction of Nylon Thread

Index studied	Cells	Control	Time of investigation							
			1 day	3 days	1 week	2 weeks	1 month	2 months	3 months	6 months
Lymphocytes/neutrophils ratio	—	2,71±0,40	3,9	3,31	3,35	5,94	5,96±0,20 $P<0,001$	1,35	—	0,5±0,04 $P<0,001$
Percentage of cells with diffusion of acid phosphatase	Neutrophils	27,13±2,74	25,68	25,98	23,4	33,26±1,44 $P<0,001$	41,83	42,66	52,69	72,38
	Lymphocytes	8,8±0,64	12,79	9,73	13,42	16,27±0,65 $P<0,001$	25,48	26,1	30,57	65,4

in the development of obstructive pneumonia, for it may lead to penetration of the microflora into deeper parts of the bronchial tree. Subsequent autolysis of lymphocytes and phagocytes and liberation of the hydrolytic enzymes contained in them may lead to destruction of the surrounding tissue. Evidence of this is given by the morphological investigation of the lungs of the same rabbits, which showed that the most important component of the developing tissue process was infiltration by phagocytes and lymphocytes followed by the progressive development, starting from the second week, of destructive changes in them. These changes were particularly severe in the later stages when signs of maximal regression of the whole system of phagocytes and lymphocytes were present.

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