CYTOCHEMICAL STUDY OF RABBIT BLOOD MICROPHAGES DURING PHAGOCYTOSIS

E. A. Venglinskaya and M. G. Shubich

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Experiments on rabbits showed that phagocytosis of bacteria leads to a decrease in the content of cationic protein and in the myeloperoxidase activity in the microphages (pseudosinophilic leukocytes) of rabbits' blood. In the final stage an increase in acid phosphatase activity, explained by increased permeability of the lysosomal membranes, and a decrease in alkaline phosphatase activity, localized in granules of a different type, in connection with its utilization during incorporation into phagosomes, are observed. Correlation analysis showed that the digestive power of the microphages correlates with their phosphatase activity (correlation is direct for acid phosphatase and inverse for alkaline). Control investigations showed no change in the concentration of cationic protein or enzyme activity.

Investigations have demonstrated the important role of lysosomes and other granules of microphages in the phagocytic response [10, 11].

The purpose of this experimental investigation was to study changes in the activity of some cytochemical parameters of rabbit peripheral blood microphages (pseudoeosinophilic leukocytes) during phagocytosis.

EXPERIMENTAL METHOD

Ten rabbits were used. Blood for the cytochemical study of the concentration of cationic protein (CP) and enzyme activity of the microphages and for determination of their phagocytic activity against Escherichia coli (strain K 12 S) was taken from the marginal viein of the ear. The degree of completeness of phagocytosis was studied by the methods of Berman and Slavskaya [2] and Ivanov and Chukhlovin [5]. To determine the phagocytic power of the microphages the index of Hamburger and Wright was calculated, and to estimate their digestive power the index of digestion [1] and the percentage of leukocytes engaged in phagocytosis were calculated. Alkaline phosphatase (ALP) and acid phosphatase (ACP) activity was determined by the azo-coupling reaction [4, 7] and myeloperoxidase (MP) activity of Sato's method [16]. CP were estimated after fixation by Sannomiya's method [6] and staining as described by Ringhertz and Zetterberg [15]. The levels of the cytochemical parameters were estimated visually in accordance with Kaplow's principle [13].

In the control investigations the activity of the enzymes and the CB concentration were determined under corresponding conditions but without the addition of bacteria. The experiments to study the phagocytic activity of the microphages were carried out with observance of all the fundamental requirements [8].

EXPERIMENTAL RESULTS

The results of determination of the phagocytic activity and the study of the cytochemical parameters are summarized in Tables 1 and 2. Table 2 shows that cultivation of the microphages for 1 h in agar in the control test had no effect on the activity of the enzymes studied or on the CP concentration.

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TABLE 1. Phagocytic Activity of Rabbit Blood Microphages (M ± m)

Experimental conditions	Hamburger's index	Wright's index	Index of digestion	% of leukocytes engaged on phagocytosis
Before exposure on agar	72,0±2,3	1,2±0,2	0,4±0,04	31,0±2,8
After " " "	70,0±2,4	2,6±0,3	1,4±0,1	58,0±3,9
	>0,05	<0,05	<0,05	<0,05

Note: Significance of differences between values before and after exposure on agar are given.

TABLE 2. Enzymic Activity and Cationic Protein of Rabbit Blood Microphages (M ± m)

Cytochemical parameters	Index of activity in control		Index of activity in experiments	
	before exposure on agar	after exposure on agar	before exposure on agar	after exposure on agar
CP	300±1	298±2	238±9	217±10
		P_1>0,05	P_2<0,05	P_3>0,05
MP	208±12	200±10	156±10	191±4
		P_1>0,05	P_2<0,05	P_3<0,05
AcP	178±10	174±6	184±8	207±6
		P_1>0,05	P_2>0,05	P_3<0,05
AlP	216±7	210±5	222±9	146±13
		P_1>0,05	P_2>0,05	P_3<0,05

Legend: P_1) significance of differences between values before and after exposure on agar in control; P_2) significance of differences between values in control and experiment; P_3) significance of difference between values before and after exposure on agar in experiments.

A study of the control films showed that CP and MP activity were found in all microphages. CP granules were oval in shape and were distributed uniformly in the cytoplasm; the intensity of staining and distribution of the granules were identical in the various microphages (Fig. 1).

In the initial phase of phagocytosis the CP concentration and MP activity decreased. After ingestion of the bacteria, the granules of CP became indistinct and the intensity of staining and distribution of the granules were identical in the various microphages (Fig. 1).

In the initial phase of phagocytosis the CP concentration and MP activity decreased. After ingestion of the bacteria, the granules of CP became indistinct and the intensity of their staining was reduced. Sometimes (empty) granules appeared in the microphages engaged on phagocytosis. Often nonviable bacteria lying inside the digestive vacuoles were blue in color probably through adsorption of CP from the microphages. Viable bacteria did not stain in the tests for CP. Microphages which have lost their CP appeared in the films. Nonviable bacteria were found more often in such microphages (Fig. 2). In the course of digestion of the bacteria no further change in the CP content was observed (Table 2).

After ingestion of the bacteria the MP activity in the microphages decreased (Table 2) and this was accompanied by a decrease in the number of granules and in the intensity of their staining. Degranulation was observed in some microphages. With an increase in the degree of fragmentation of the bacteria the MP activity was restored, the number of granules increased, and their staining was intensified.

In the final stage of phagocytosis the ACP activity increased, and many darkly stained oval granules appeared in the microphages. Attraction of bacteria was observed, to microphages with moderate enzyme activity, while nonviable bacteria were found more often in microphages with high ACP activity. Some bacteria located in the digestive granules became reddish-brown in color, indicating ACP activity. The reason for this was evidently deposition of enzyme protein on their surface.

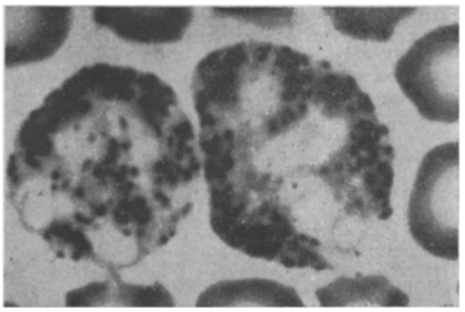

Fig. 1

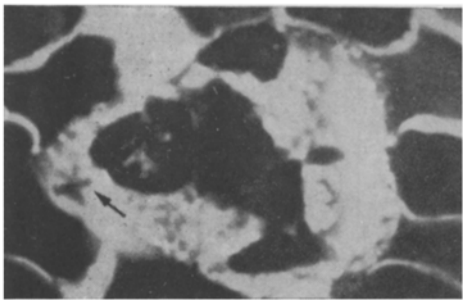

Fig. 2

Fig. 1. CP granules in cytoplasm of microphages (control tests before exposure on agar). CP stained by the method of Ringhertz and Zetterberg, 2500×.

Fig. 2. Sharp decrease in CP content in cytoplasm of microphages in initial stage of phagocytosis (ingested bacteria indicated by arrow). Nuclei stained with basic fuchsin, 2500×.

In the course of digestion of the bacteria the ALP activity decreased (Table 2). This matter is described in more detail elsewhere [3].

Correlation analysis showed that the digestive power of the microphages correlates with their phosphatase activity. Correlation for ACP was direct ($r = +0.56$) and for ALP it was inverse ($r = -0.67$).

In view of evidence [18, 19] of interaction of CP extracted from leukocytes with bacteria, the decrease in the CP content in the early stages of the phagocytic response can be presumed to have taken place as a result of interaction with the anionic component of the bacterial surface, leading to a bactericidal effect.

The decrease in MP activity in the macrophages during phagocytosis is evidently linked with its bactericidal action [14, 17].

The writers have shown previously [3] that microphages with high ALP activity which, like the other microphages, are also characterized by moderate ACP activity, ingest bacteria most actively. The observed increase in ACP activity during phagocytosis is probably attributable to increased permeability of the lysosomal membranes and is independent of any increase in the content of the enzyme. The increase in ALP activity located in granules of another type [9] is possibly explained by its partial utilization of incorporation into phagosomes [12].

The initial and final stages of the phagocytic response are thus characterized by regular changes in the CP content and in the enzyme activity of the lysosomes and other granules of the microphages.

LITERATURE CITED

1. O. G. Alekseeva and A. P. Volkova, Gig. i San., No. 8, 70 (1966).
2. V. M. Berman and E. I. Slavskaya, Zh. Mikrobiol., No. 3, 8 (1958).
3. E. A. Venglinskaya and M. G. Shubich, Byull. Éksperim. Biol. i Med., No. 11, 61 (1972).
4. V. I. Dudetskii, in: Proceedings of a Scientific Conference of Junior Research Workers of the Kuban Medical Institute [in Russian], Krasnodar (1971), p. 33.
5. A. I. Ivanov and B. A. Chukhlovin, Lab. Delo, No. 10, 610 (1967).
6. B. Romeis, Microscopic Techniques [Russian translation], Moscow (1953).
7. M. G. Shubich, Lab. Delo, No. 1, 10 (1965).
8. F. Allison, Jr., M. G. Lancaster, and M. D. Crosthwaite, Am. J. Path., 43, 775 (1964).
9. D. Bainton and M. Farquhar, J. Cell Biol., 39, 299 (1968).
10. L. Cohn and J. Hirsch, J. Exp. Med., 112, 1015 (1960).
11. C. de Duve, Triangle, 9, 200 (1970).
12. R. Horn, S. Spicer, and B. Wetzel, Am. J. Path., 45, 327 (1964).
13. L. Kaplow, Blood, 10, 1023 (1955).
14. S. Klebanoff, Science, 169, 1095 (1970).

15. R. R. Ringhertz and A. Zetterberg, Exp. Cell Res., 42, 243 (1966).
16. A. Sato and S. Yashimato, Am. J. Dis. Child., 29, 301 (1925).
17. R. R. Strauss, B. B. Paul, A. A. Jacobs, et al., Infect. Immun., 5, 120 (1972).
18. J. K. Spitznagel and H. Chi, Am. J. Path., 43, 697 (1963).
19. H. J. Zeya and J. K. Spitznagel, Lab. Invest., 24, 237 (1971).