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MECHANISM OF ACTION OF THE EXOPOLYSACCHARIDE OF *Mycobacterium cyaneum* ON THE LOCAL CELL REACTION IN EXPERIMENTAL *E. coli* INFECTION

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Among the substances able to activate the reactions of natural immunity an important place belongs to high-molecular-weight polysaccharides (PS) in the bacterial cell wall. The PS of Gram-negative bacteria have been shown to modify the functional state of cells participating in the immune response [2, 9, 10, 14, 15]. PS of Gram-negative bacteria have received much less study as modulators of defensive reactions.

The aim of the present investigation was to study the effect of PS of *Mycobacterium cyaneum* B-646 on the course of the local cell reaction in experimental *Escherichia coli* infection.

EXPERIMENTAL METHOD

The exopolysaccharide of *M. cyaneum* B-646 (supplied by the All-Union Culture Museum) was obtained by growing the culture on synthetic medium [1]. The culture fluid was deproteinized by Sevag's method, dialyzed, and the PS were precipitated by quaternary ammonium salts [2]. After isolation of the PS from the complex it was lyophilized.

Experiments were carried out on 200 noninbred mice weighing 16-18 g, infected intraperitoneally with *E. coli* (strain 173) in a dose of 5×10^8 bacterial cells [6]. Animals of three groups (50 mice in each group) received PS by intraperitoneal injection in doses of 10 μg (group PS-10), 20 μg (group PS-20), and 50 μg (group PS-50) daily for 3 days before infection. The cell response in the peritoneal exudate (PE) was studied 1-96 h after infection, migration of macrophages and neutrophils, phagocytic activity, number of phagocytic cells, and the intensity of phagocytosis (number of phagocytosed microorganisms) were determined by methods described previously [6, 8], and the phagocytic index was calculated as the product of the parameters of phagocytosis per unit weight of the corresponding cells in PE.

Activity of alkaline and acid phosphatases (AlP and AcP, respectively) by Gomori's method and of peroxidase by the Graham–Knoll method was determined in macrophages and neutrophils,

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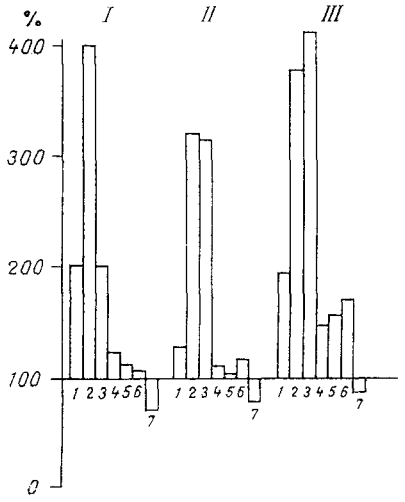

Fig. 1

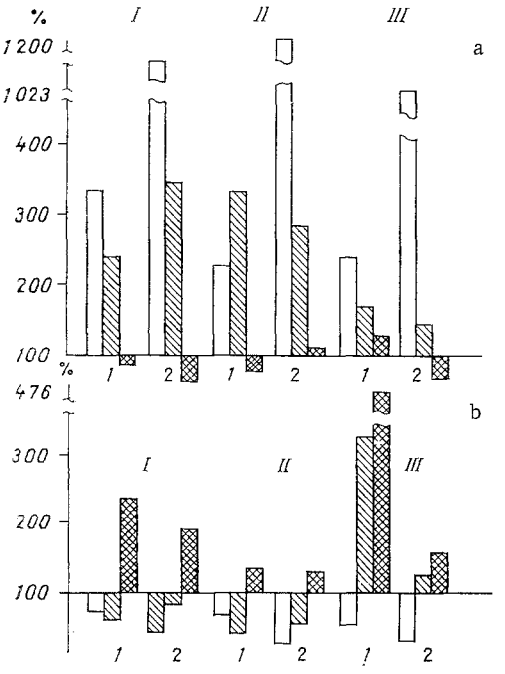

Fig. 2

Fig. 1. Effect of PS on macrophage migration into peritoneal exudate of mice (in % of control taken as 100). I) Group PS-10, II) group PS-20, III) group PS-50. Time after infection: 1) 1 h, 2) 3 h, 3) 6 h, 4) 24 h, 5) 48 h, 6) 72 h, 7) 96 h.

Fig. 2. Effect of PS on activity of enzymes (a) and their diffusion (b) in macrophages (1) and neutrophils (2) of mouse peritoneal exudate (in % of control, taken as 100). Unshaded columns — AlP, obliquely shaded columns — AcP, cross-hatched columns — peroxidase. Remainder of legend as to Fig. 1.

and the distribution of the enzymes in the cells was studied as an indicator of enzyme diffusion. The functional state of the macrophages also was judged by luminescence of lysosomes in a monolayer culture of macrophages from PE of the experimental and control groups [3]. The numerical results were subjected to statistical analysis.

RESULTS

In the experimental mice migration of phagocytic cells was intensified during the first few hours after infection. The number of macrophages and neutrophils exceeded the control level by 151-274% ($P < 0.01$). This was true more especially of macrophages (Fig. 1), whose relative proportion among the cells of PE was higher than in the control ($P < 0.01$-0.001). Meanwhile in the animals of all groups the number of macrophages reached a maximum on the 3rd-4th day, i.e., the kinetics of phagocyte accumulation in the experimental group did not differ from that in the control, as was confirmed by correlation analysis (for macrophages in group PS-10 $r = 0.97 \pm 0.01$, in group PS-20 $r = 0.95 \pm 0.01$, and in group PS-50 $r = 0.82 \pm 0.08$; for neutrophils the values of r were 0.96 ± 0.01, 0.89 ± 0.06, and 0.83 ± 0.07, respectively).

The parameters of phagocytosis in all experimental groups reached their maximal level sooner, and were appreciably higher, than in the control (Table 1). Macrophages and neutrophils of mice of the control group exhibited their highest phagocytic activity after 48 h, but after injection of PS the level of phagocytosis actually exceeded the maximal in the control during the first day, with a high level of correlation (for neutrophils $r = 0.6 \pm 0.2$; for macrophages $r = 0.89 \pm 0.07$) between the number of phagocytic cells and ingestive capacity [15].

The study of intracellular enzyme systems (Fig. 2a) showed that enzyme activity in macrophages and neutrophils was appreciably altered both quantitatively and as regards relations between individual enzymes. The character of the changes depended on the dose of PS, the type of enzyme, and type of cell. For instance, AlP activity increased sharply in neutrophils

TABLE 1. Effect of PS on Phagocytic Activity of Mouse Peritoneal Exudate Cells

Group of animals	Maximal values of phagocytosis parameters			Parameters in experimental groups which exceeded maximal control level		
	time, h	value	% of control	time, h	value	% of control
			Macrophages			
PS-10	48	15,02±0,93	132,45±3,3	24	13,73±0,83	121,1±2,8
PS-20	24	17,47±0,96	154,0±3,5	6	12,05±0,89	106,3±1,6
PS-50	24	18,89±1,21	166,57±3,4	6	14,81±0,1	130,6±3,2
Control	48	11,34				
			Neutrophils			
PS-10	6	9,9±12,70 8,25±0,71	108,4±1,9			
PS-20	24	10,29±0,84	135,2±3,4	3	9,13±0,2	119,9±2,7
PS-50	6	14,53±0,09	190,93±2,1	1	9,1±1,0	119,6±2,7
Control	48	7,61±0,7				

(more than tenfold, group PS-20) and much less in macrophages (especially with an increase in the dose of PS). Since AlP is a marker of secondary specific granules in neutrophils, the number of which rises steadily during maturation of the cells [7, 11], the sharp increase in its activity can be regarded as a manifestation of accelerated maturation of these cells under the influence of PS and the more marked migration of mature cells, with a higher protective potential, into the focus of infection [4, 5, 7, 11, 12].

The increase in AlP activity in neutrophils and macrophages suggests the presence of definite correlation between the change in AlP activity and intensification of cell migration and phagocytosis [4, 8]. In the present experiments this relationship was more marked in mice of the PS-20 group (for macrophages $r = 0.7 \pm 0.21$, for neutrophils $r = 0.57 \pm 0.2$).

Activity of lysosomal enzymes (AcP and peroxidase) was changed differently by the action of PS (Fig. 2a). AcP activity was increased in all groups. The optimal dose of PS for its stimulation was 20 μg per mouse. In the animals of this group activation of AcP, reflecting a general response of the lysosomes [8], was equally strong in macrophages and neutrophils, but in addition, it was higher in the macrophages than in animals of the other groups and it correlated with phagocyte migration ($r = 0.75 \pm 0.2$). Correspondingly, fluorescence of macrophage lysosomes and the number of maximally fluorescent cells in a culture of PE macrophages from mice of the PS-20 group were significantly higher than the corresponding values in the control ($P < 0.001$).

By contrast, peroxidase activity in most cases was lower in the experimental than in the control animals. The exceptions were mice of the PS-50 group, in which the peroxidase level in the macrophages was higher, but AcP activity was lowest. This dissociation in the changes in lysosomal enzyme activity could be the result both of their selective accumulation and of an equally selective change in permeability of the lysosomal membranes, or a change of the enzyme into another form [4, 8, 11, 12]. This hypothesis was confirmed by estimation of diffusion of the enzymes (Fig. 2b).

Diffusion of AlP of macrophages and neutrophils in animals of the experimental groups was below the control level ($P < 0.05$-0.01). Similar results were obtained for AcP, except for the PS-50 group, in which they were higher than in the control (326% for macrophages, 123% for neutrophils). Meanwhile diffusion of peroxidase in all groups was appreciably higher than the control values. This suggests that in the PS-10 and PS-20 groups there was a selective increase in permeability of the lysosomal membranes for peroxidase and increased utilization of this enzyme. In the PS-50 group, there must evidently have been a marked disturbance of membrane permeability, manifested as increased diffusion of both enzymes (AcP and peroxidase).

The results are thus evidence that PS of *M. cyaneum* B-646 stimulates migration of phagocytic cells and facilitates earlier and more active incorporation into the phagocytic process not only of neutrophils, but also of macrophages, i.e., of the effector component which is responsible both for defense against infection at the phagocytic level and for the initial stages

of the immune response, in particular, to thymus-dependent antigen. PS B-646 alters the enzyme balance of macrophages and neutrophils, acting selectively on the accumulation of different enzymes in the cells and on permeability of the cell membranes. The mechanism of action of PS B-646 suggests that its further study with a view to stimulation of cellular defensive reactions would serve a useful purpose.

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THOMSEN ANTIGEN IN NORMAL HUMAN TISSUES AND TUMORS

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The Thomsen–Friedenreich antigen was first discovered in human erythrocytes [8, 9, 13]. The presence of this antigen in human tissues has not been adequately studied. However, it is known not to be present in normal tissues, but to appear in them *de novo* as a result of the action of viral and bacterial neuraminidase on the mucoprotein receptors of the cell, i.e., as a result of removal of N-acetylneuraminic acid from the substrate [2-4, 7, 8, 13, 14]. Thomsen antigen is formed by inactivation of group-specific antigens of the MN system. This antigen likewise has not been found in human tumors by the use of heteroimmune (rabbit) sera [4]. However, the use of sera of human origin, i.e., of isosera, gave different results [10-12]. Springer et al. [10-12], using human sera containing specific antibodies against Thomsen antigen, showed that this antigen is present in carcinomas of the breast and gastrointestinal tract. Meanwhile they could not find this antigen in benign tumors (fibroadenoma of the breast, fibrocystic mastopathy). The authors cited suggest that the appearance of Thomsen antigen in human carcinomas is evidence either of the imperfect biosynthesis of antigens in malignant tumor cells or of increased degradation of normal antigenic components of the cell membrane, which distinguishes cancerous from normal tissues.

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