MEMBRANE CHARACTERISTICS AND FUNCTIONAL ACTIVITY OF PHAGOCYTIC ALVEOLAR MACROPHAGES

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It was shown previously that stimulation of the mononuclear phagocyte system by zymosan granules (ZG) changes the functional state of the alveolar macrophages (AM) [5]. This is manifested as an increase in their phagocytic and bactericidal activity and a decrease in viscosity of the membrane lipid bilayer. The AM population studied was obtained 5 days after intravenous injection of ZG. By this time it had been significantly renewed due to the inflow of monocyte-like macrophages, whose functional activity and membrane properties are modified by some of the factors secreted in the body after phagocytosis of ZG. One of the main signs of macrophagal activation is the intensity of the "respiratory burst" [8]. It has been shown [6] that release of active forms of oxygen by AM in vitro in response to a stimulus is proceded by depolarization of membranes and a decrease in their resistance. It was considered interesting to study how the properties of membranes characteristic of previous stimulated AM change during phagocytosis of ZG.

The aim of this investigation was to compare changes in microviscosity and area of AM membranes and the functional state of the cells during phagocytosis.

EXPERIMENTAL METHOD

Experiments were carried out on male and female Wistar rats weighing 200-250 g. For stimulation, ZG (100 mg/kg) in 1 ml of 0.85% NaCl was injected intravenously 5 days before the investigation. Control animals were given an injection of 1 ml NaCl. AM were obtained by the method described previously [5]. The cell concentration was adjusted to 2×10^6 cells/ml with Hanks' medium without phenol red. A suspension of ZG equivalent to 50 particles were added to it and the mixture was incubated in Teflon bottles at 37°C. The microviscosity of the lipids and total area of the cell membranes were determined after 0.5-4 h by Dobretsov's method [1, 2], as described previously [5], using pyrene and OSP-14 (pyridine derivative) and a Hitachi MPF-4 spectrofluorometer (Japan). Lipid peroxidation (LPO) products, namely diene conjugates, were determined on an SF-26 spectrophotometer, by recording UV spectra of lipids [4] extracted from 10^6 cells. To investigate the adhesive properties of AM, 0.5×10^6 cells were deposited on slides in special chambers in the course of 1 h at 37°C. Specimens were then prepared; after rinsing the number of adherent cells was counted in 10 fields of vision with a total area of 24×10^4 μ^2. A viable monolayer of AM [5] was obtained on the slides in the chambers, ZG were added to it, and the specimens were incubated at 37°C for 0.5, 1, and 2 h. Two identical AM monolayers phagocytosing ZG were prepared in each case. One of them was stained by Pappenheim's method and the number of ZG bound with 100 AM in the monolayer was counted (Fig. 1). The second monolayer was washed off and 0.2 ml of a 0.02% solution of nitro-blue tetrazolium (nitro-BT) in Hanks' medium was added to the chamber, and the material was incubated for 30 min at 37°C. The cell nuclei were counterstained with carmine and the number of ZG around which diformazan was deposited was counted (the number per 100 AM in the monolayer). The results were subjected to statistical analysis by Student's t test.

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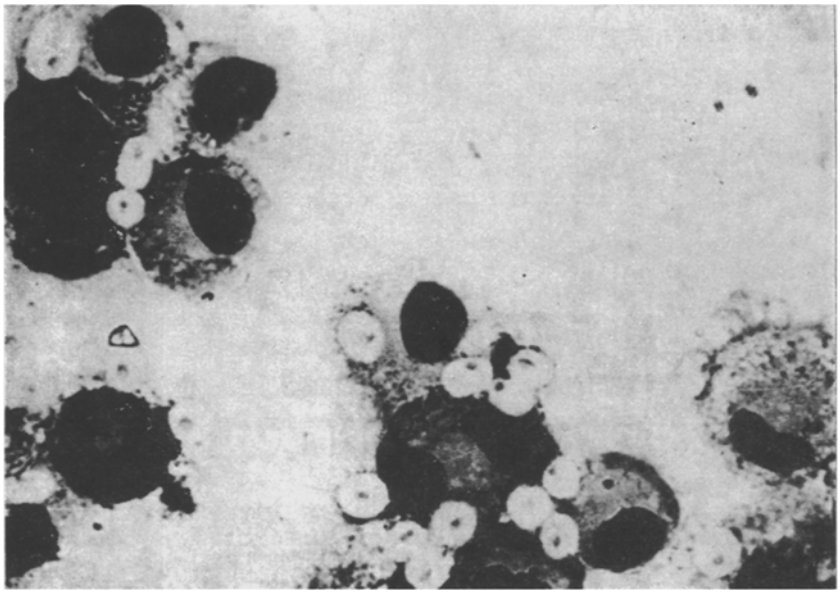

Fig. 1. Phagocytosis of ZG by AM.

TABLE 1. Number of Phagocytosed ZG (I) and Number of ZG around Which Nitro-BT Was Reduced (II)

Parameter	Incubation time of AM monolayer with ZG		
	0,5 h	1 h	2 h
I	19±1,3* / 31±2,0	65±4,6 / 60±4,3	125±9,8* / 101±7,6
II	19±1,4 / 22±1,6	37±2,4* / 31±2,0	46±2,6* / 40±1,4

Legend. Numerator — experiment (n = 7); denominator — control (n = 8). *p<0.05.

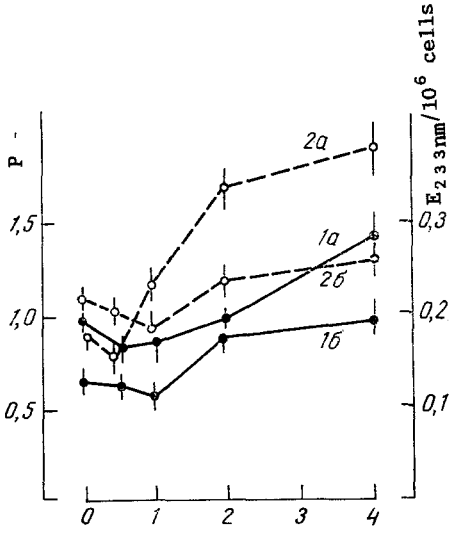

Fig. 2. Changes in microviscosity of membrane lipids (1) and content of LPO products (2) during phagocytosis of ZG by control (a) and previously stimulated (b) AM. Abscissa, incubation time (in h); ordinate: on left, microviscosity (in P), on right — diene conjugates, $E_{233nm}/10^6$ cells.

EXPERIMENTAL RESULTS

Phagocytosis of ZG by AM induced a "respiratory burst" in them (Table 1), the degree of which increased with an increase in the number of ZG ingested. After incubation for 2 h the AM ingested 3 times more ZG than after 0.5 h. The intensity of secretion of active forms of oxygen, recorded as the number of ZG around which nitro-BT was reduced, rose by 80%. Previously stimulated AM phagocytosed 6 times more ZG after incubation for 2 h than for 0.5 h. During this time the increase in the amount of reduced nitro-BT was 140% (Table 1). On comparison of the experimental and control values it is clear that ingestion of ZG by previously stimulated AM was significantly less during the first 2 h of incubation than in the control. However, after incubation for 2 h the number of ZG phagocytosed in the population of activated AM was 25% greater, and production of active forms of oxygen during phagocytosis was 15% greater than in the control.

Stimulation of production of biological oxidizing agents may be the cause of activation of LPO. Figure 2 shows that during incubation with ZG the content of diene conjugates in AM rises. Whereas initially the content of these LPO products was a little higher in the experimental cells, after 2 h and, in particular, after 4 h their content was significantly higher in the control cells. This increase amounted to 25% for the experimental and 250% for the control AM after 4 h. Since mainly unsaturated fatty acid residues of phospholipids are utilized in LPO, its activation leads to an increase in viscosity of the membrane lipids [2]. Meanwhile, accumulation of small quantities of LPO products, not exceeding certain threshold values, may lead to an increase in the lateral mobility of the lipids and the flowability of the membranes [3]. This rule, in our view, was reflected in the fact that, with an initially higher concentration of LPO products, the stimulated cells differed in having lower values of membrane lipid viscosity. During the first hour of incubation of AM with ZG a tendency could be noted for the viscosity of membrane lipids of the control and stimulated AM to fall (Fig. 2). After 2 h the viscosity of the membrane lipids of the stimulated AM only reached the initial level for the control cells.

Activation of LPO and oxidation of membrane lipids could be one cause of the change in total area of the membranes of AM. The initial area was the same for control and experimental cells [5]. During incubation it gradually decreased, and after 4 h it was 14% less in both the control and experimental cells ($p<0.01$).

Changes in viscosity of the lipid membranes and in their area can be regarded as manifestations of changes in the surface properties of the cells, which also were revealed during a study of the adhesive properties of AM during incubation. In the control the number of cells which adhered to the slide before incubation with ZG was 250 ± 12.5 per $24 \cdot 10^4$ μ^2. After incubation for 0.5 h the value of this parameter fell to 38 ± 2.4, and after 2 h, to 9 ± 1.7. In the monolayer of previously stimulated AM, there were 430 ± 23.1 cells in the same area. After incubation for 0.5 h the number of cells adherent to the slide fell to 54 ± 4.8, and after 2 h to 19 ± 1.3. It must be emphasized that the number of cells adherent to the slide, under identical conditions of incubation, was twice as great in the monolayer of activated AM than in the controls, in all cases studied.

Preliminary stimulation of AM thus prevents considerable accumulation of LPO products normally taking place in control cells, and changes connected with its activation in the membrane characteristics. This phenomenon can be explained on the grounds that in the experimental cells the "respiratory burst" takes place aginst the background of increased activity of antioxidative systems [7], which enable AM to maintain a higher level of functional activity compared with the controls during incubation for 2 h.

Ability to phagocytose ZG and to secrete active forms of oxygen was greater in the monolayer of stimulated AM. This is shown by the greater number of ZG adherent to AM and reducing nitro-BT in the experiment after incubation for 2 h than in the control. However, after 0.5 h the first parameter in the control was higher. This may perhaps be explained on the grounds that in stimulated AM more receptors, able to bind ZG (lectin-like, receptors for fibronectin, C3B, etc.), were involved in the realization of their increased adhesiveness, being linked with serum factors passively adsorbed on the surface of the glass. Accordingly, by the time of the reaction with ZG, there were fewer receptors for ZG on the free part of the membrane of AM in the experiment than in the control. Contact between ZG and the membrane of the activated AM mobilized their ingestive powers. This may have taken place through redistribution or expression of receptors. After incubation for 2 h with ZG, the phagocytic index of the activated AM therefore exceeded the control value already. It is quite probable that the lower value of

membrane viscosity of the stimulated AM compared with the control favors realization of their higher phagocytic and bactericidal potential.

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PANEL OF MONOCLONAL ANTIBODIES TO ANTIGEN CD38

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Monoclonal antibodies (McAb), an important tool in immunologic methods, have found wide application in the study of the structure and function of cell surface antigens and receptors. Research into differential antigens of human hematopoietic cells is particularly important because it enables the mechanisms of blood cell differentiation and of the generation and regulation of immunity to be understood.

An important marker of proliferating cells of the human lymphoid system is an antigen which was classed at the International Working Conference on differential antigens of human leukocytes with the CD38 cluster [3]. Antigen CD38 is very similar in its structural organization to antigens of the class I human major histocompatibility complex [2], but differs in the character of its distribution on hematopoietic cells [1, 4]. Normally antigen CD38 is represented on all thymocytes, myelocytes, myeloblasts, promyelocytes, and medullary B lymphocytes. In the peripheral blood the antigen is expressed on the majority of null cells, NK cells, and 5% of T cells and weakly on monocytes. Mature peripheral T and B lymphocytes and erythrocytes do not contain this antigen. During activation of T cells by mitogens or alloantigens, expression of CD38 rises sharply [5].

As a result of somatic hybridization of mouse myeloma P3X63A 8.653 cells and spleen cells of BALB/c mice, repeatedly immunized with human thymocytes, the following strains of hybrid cells in culture were obtained in the Clinical-Radioimmunologic Laboratory of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR: ICO-16, ICO-17, ICO-18, ICO-19, ICO-20, ICO-27, and ICO-28.

The aim of this investigation was to characterize antigen CD38, revealed by the McAb panel thus obtained.

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