

Peculiarities of Phagocytosis of Opsonized and Nonopsonized Bacteria *S. aureus* and *E. coli* by Human Neutrophil Granulocytes Studied by Atomic Force Microscopy

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Abstract—Differences in the phagocytosis process of opsonized and nonopsonized strains of *Staphylococcus aureus* 2879 M and *Escherichia coli* 321 were studied. Differences in the character of pseudopodia during phagocytosis by neutrophil granulocytes (NGs) of opsonized and nonopsonized bacteria were detected, and differences in the nature of pseudopodia in reactions to gram-positive and gram-negative microorganisms were not detected. For the first time in dynamic observations at the late stages of phagocytosis, changes in the volume of nuclei and their movement, variations in the intersegment distance of the nuclei, and a slight increase in the volume of NGs were shown. A decrease in the rigidity of the membrane–cytoskeleton NG complex correlating with the intensity of phagocytosis and opsonization of bacteria was shown for the first time. It was established that opsonization does not affect the oxygen-dependent metabolism of NGs and, at the same time, introduces significant adjustments in the implementation of oxygen-independent bactericidal mechanisms of cells.

Keywords: neutrophil granulocytes, phagocytosis, opsonization, *S. aureus*, *E. coli*, pseudopodia, atomic force microscopy, rigidity of the membrane–cytoskeleton complex

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INTRODUCTION

Neutrophil granulocytes (NGs) are one of the main links in a nonspecific resistance system, for which involvement in the development and regulation of adaptive immunity has recently been demonstrated (Yang et al., 2010). Despite the realization of a large number of functions (regulatory, suppressor, pro- and antiinflammatory, pro- and antitumor) (Nesterova et al., 2017), the activity of NGs is most vividly manifested by participation in exudative-destructive inflammation (Mayansky, 2006). From the point of view of development of an inflammatory reaction, an NG is simultaneously an inducer (transendothelial migration, synthesis of cytokines, changes in the receptor spectrum) and an effector cell (phagocytic

killing of microorganisms, a complex of respiratory burst reactions, secretory degranulation, and NETosis).

In the process of phagocytosis, an NG eliminates the foreign agent causes its activation (Brown et al., 2015). There are two variants of phagocytosis. In the course of “nonimmune” phagocytosis (in the absence of opsonins), recognition on the part of an NG is carried out with the involvement of pattern recognition receptors (PRRs), in particular, Toll and Toll-like signaling receptors (Arancibia et al., 2007); as well as mannose and endocytosis receptors (Seimon et al., 2006). These receptors recognize the pathogen-associated molecular pattern (PAMP) of bacteria. Such a system is the case for evolutionarily more ancient ones and, as a rule, is realized during the initial meeting of the phagocyte and pathogen (Møller et al., 2005).

In the process of “immune” phagocytosis (with the participation of opsonins), the mediation function between the phagocyte and the phagocytosis object is performed by opsonins: immunoglobulins (IgG1, IgG3, IgA), complement components (C3b, iC3b, C4b, C1q), acute phase proteins, lipopolysaccharide-binding protein, mannose-binding lectin, and

Abbreviations: AFM—atomic force microscopy, ROSs—reactive oxygen species, ACP—acid phosphatase, LCPs—lysosomal cationic proteins, LCT—lysosomal cation test, MPO—myeloperoxidase, NGs—neutrophil granulocytes, NBT—nitroblue tetrazolium, ACC—average cytochemical coefficient, AP—alkaline phosphatase, PAMPs—pathogen-associated molecular patterns, PBS—phosphate buffered saline, PGRP—peptidoglycan recognition protein.

fibronectin (Belotsky and Avtalion, 2008). Under the most favorable conditions, the absorption of opsonized objects can occur within 10–30 s (Ryter and Chastellier, 1983).

Now, due to the appearance of new high-resolution microscopy methods—atomic force microscopy (AFM)—the process of phagocytosis can be observed in dynamics in living cells with a previously inaccessible resolution. The purpose of this study was to investigate the phagocytosis process of opsonized and non-opsonized *Staphylococcus aureus* 2879 M and *Escherichia coli* 321 strains by the AFM method. A fixation method for samples, optimal for studying the morphological parameters and counting the phagocytic number of NG was selected. We have revealed the peculiarities of formation of NG pseudopodia and lamellopodia during *S. aureus* and *E. coli* phagocytosis and evaluated the dynamics of phagocytosis depending on opsonization using the AFM method. Changes in the basic morphological parameters of NGs in the vital sample during the phagocytosis process were described. In addition, the change in the rigidity of the membrane–cytoskeletal NG complex was determined depending on the phagocytosis object and the morphological NG changes during the phagocytosis of opsonized and nonopsonized bacteria were compared with changes in the main cytochemical tests.

MATERIALS AND METHODS

Isolation of Cells

Venous blood of healthy donors 20 to 40 years of age of both sexes was studied. Blood sampling was performed in the morning, after the donors signed the consent form. Heparinized blood was fractionated on a double ficoll-urographin gradient ($\rho = 1.077$ g/mL, $\rho = 1.116$ g/mL, 200 g, 40 min). The resulting NG fraction was washed twice with phosphate buffered saline (PBS) containing 0.137 M NaCl and 0.0027 M KCl, pH 7.35 (200 g, 3 min), and used at a final concentration of 1×10^6 cells per mL of medium. Cells were used for the experiment immediately after isolation, where viability according to the test with trypan blue was at least 99% (Podosinnikov et al., 1981). Silicone tubes were used for prevention of cell activation by the surface of the glass. In all experiments, PBS was used as the incubation medium.

Bacterial Suspension

The museum *S. aureus* 2879 M and *E. coli* 321 strains of the Department of Nanotechnology and Biotechnology of Alekseev Nizhny Novgorod State Technical University were used in the study. Cultures were grown for 24 h at 37°C on GMR agar (SSC PMB, Obolensk), the bacteria were washed off with sterile PBS three times by centrifugation (2350 g, 10 min), and resuspended in PBS. The optical density was

adjusted using a photocolorimeter (KFK-2MP-UHL, Russia) to 0.75 rel. units for *S. aureus* 2879 M and 0.85 rel. units for *E. coli* 321 ($\lambda = 670$ nm), which corresponds to 1×10^9 cells/mL (initial cell concentration).

For the series of experiments with opsonized bacteria, a serum pool from three or more healthy donors was used. The serum were incubated with a suspension of bacteria (3 : 1 ratio by volume) at 37°C for 60 min, followed by washing with PBS three times, using opsonized bacteria in an initial concentration of 1×10^9 cells/mL. The need to use a pool of serum from several donors was due to the individual variability in the content of opsonins.

AFM in the Study of Fixed Samples

For calculations of the phagocytic index, *S. aureus* 2879 M or *E. coli* 321 bacteria at a concentration of 500×10^6 cells/mL were incubated with NG (1×10^6 cells/mL) in a ratio of 1 : 1 by volume for 5, 10, 15, and 20 min (37°C) for nonopsonized bacteria or for 3, 5, 10, and 15 min (37°C) for opsonized bacteria. After the incubation, samples were transferred to the glass surface. Fixation was performed with either glutaraldehyde (2.5%, 20 min, 24°C) (Panreac, European Union) or methanol (99.8%, 0.05 mL, 5 min, 24°C) (Acros Organic, United States). Smear preparations were washed three times with distilled water and scanned in air using an NTegra Spectra (NT-MDT, Russia) and DNP-A, B, C, and D probes (Bruker, United States) with a rounding radius of 20 nm, an angle of 15°, and a resonant frequency of 18–65 kHz (Pleskova et al., 2005).

AFM in the Study of Phagocytosis Dynamics in Real Time

AFM studies of native NGs were performed as described earlier (Pleskova, 2011) in the vital state in the semicontact mode. A suspension of NGs in PBS (1×10^6 cells/mL) was added into a plastic Petri dish (35 mm, Corning, United States), and, after spontaneous adhesion of NGs (20 min, 37°C), the cells were scanned at least three times (control experiment) using an NTegra Spectra instrument (NT-MDT, Russia) and DNP-A, B, C, and D probes (Bruker, United States) with a rounding radius of 20 nm, an angle of 15°, and a resonant frequency of 18–65 kHz. After the control scans were obtained, bacteria (opsonized or nonopsonized) were added to NGs at a concentration of 500×10^6 cells/mL and the dynamics of the phagocytosis process was studied in real time.

FS Spectroscopy in Determination of the Rigidity of the NG Membrane—Cytoskeleton Complex

For evaluation of the rigidity of the membrane—cytoskeleton complex, the method of force curves with the AFM spectroscopy regime was used. The membrane rigidity was calculated using the Hertz model (Bukharaev et al., 2003). Rigidity studies were performed in PBS by MLCT-C and D probes with a rounding radius of 20 nm, an angle of 35°, a stiffness constant of 0.01 and 0.03 N/m, and a nominal resonance frequency of 7 and 15 kHz (Bruker, United States). In a series of experiments, cells obtained from the blood of healthy donors were studied and the elasticity indices of the NG membrane before and after the addition of bacteria were compared. Nova NT-MDT SPM Software (NT-MDT, Russia) was used to process and visualize the scanned objects. SPMLab Analysis Only (Topometrix, United States) and Gwyddion (Nanometrology Faculty of the Czech Metrology Institute, Czech Republic) were used for further data processing.

Cytochemical Methods in Assessing NG Biochemical Status in the Process of Phagocytosis

For evaluation of the effectiveness of NG oxygen-independent protective reactions, a lysosomal-cation test (LCT) was performed (Pigarevsky, 1988). Dried smears were fixed in an alcoholic solution of formalin for 10–15 s. For selective staining of the cationic protein of granulocytes, a buffered alcohol solution of fast green was used (Sigma, United States). The nuclei of neutrophils were stained with Azur A solution (Sigma, United States). Microscopy was carried out using an Olympus IX71 optical microscope with an immersion objective lens of 100× magnification (Olympus, Japan). Under microscopy, 100 granulocytes were examined. The intracellular content of cationic proteins of granulocytes was estimated on the basis of the average cytochemical coefficient (ACC) and calculated according to the formula

$$ACC = (3a + 2b + 1c + 0d)/100,$$

where *a*, *b*, *c*, and *d* are the number of cells with a certain degree of staining of the granules and the numbers show the degree of staining. In addition to the determination of ACC, a differential evaluation of cytospin samples was carried out, taking into account the percentage of low activity, medium activity, and high activity cells from 100 cells.

Oxygen-dependent NG metabolism was assessed using the Mayansky method (Mayansky and Pikuza, 1993), based on the reduction reaction of nitroblue tetrazolium (NBT) (ThermoFisher Scientific, the United States): accumulates granules of insoluble diformazane accumulate in the cytoplasm during the formation of reactive oxygen species (ROSs). The number of NBT-positive cells was measured before

incubation with bacteria (control) and after incubation for 30 min (experiment). The NBT test was carried out in two versions: monoimpact on NGs, in which the number of diformazane granules in the cytoplasm of cells in the absence of stimulation was determined, or combined impact, after the implementation of a respiratory burst caused by opsonized zymozan.

Studies of alkaline phosphatase activity were carried out by the azo coupling method (Shubich and Nagoyev, 1980), and acid phosphatase was determined according to Burstone's method (Burstone, 1958), using ready-made kits (LLC Gemstandart, Russia) for staining smears fixed in a mixture of ethyl alcohol and formalin (40%) taken in a ratio of 1 : 9 by volume, for 30–40 s at room temperature (LLC El Group, Russia). Under microscopy, 100 granulocytes were examined. The intracellular content of the granulocyte hydrolytic enzymes was assessed based on the ACC value.

The total content of phospholipids was estimated using Ackerman's method in smear preparations fixed by 40% formalin (10 min, 37°C) stained with sudan black B (60 min, 37°C) (Ackerman, 1952). The percentage of lipid-containing granulocytes was determined microscopically with subsequent calculation of ACC.

The stock of NG glycogen was evaluated using the method of Heyhou and Quaglino based on the periodic acid Schiff reaction (Hayhou and Quaglino, 1980). Ready-made sets (RRC Abris +, Russia) were used for staining smear preparations fixed in a 10% alcohol–formalin mixture for 10 min at a room temperature. The intracellular content of glycogen was examined by microscopy of 100 NGs, and quantitative evaluation of carbohydrate stocks was carried out by calculation of ACC.

Statistical Analysis

The boundaries of the normal distribution of the quantitative indices of the samples were determined using the Shapiro–Wilk test. Since all distributions were not normal, a two-sample Wilcoxon test (non-parametric statistics) was used. The differences between the two samples were considered significant at $P < 0.05$. For statistical analysis, the Origin Pro 8 program (Origin Lab Corp., United States) was used.

RESULTS AND DISCUSSION

Two fixation methods were used to study NG morphology: with 99.8% methanol for 5 min followed by “hard” washing with distilled water or with 2.5% glutaraldehyde for 20 min with a “soft” step washing in distilled water by full immersion in Petri dishes.

Calculation of the phagocytic index is practically impossible when glutaraldehyde is used as a fixing agent (Fig. 1a), because with this fixation method the cytoplasmic content is concentrated around the

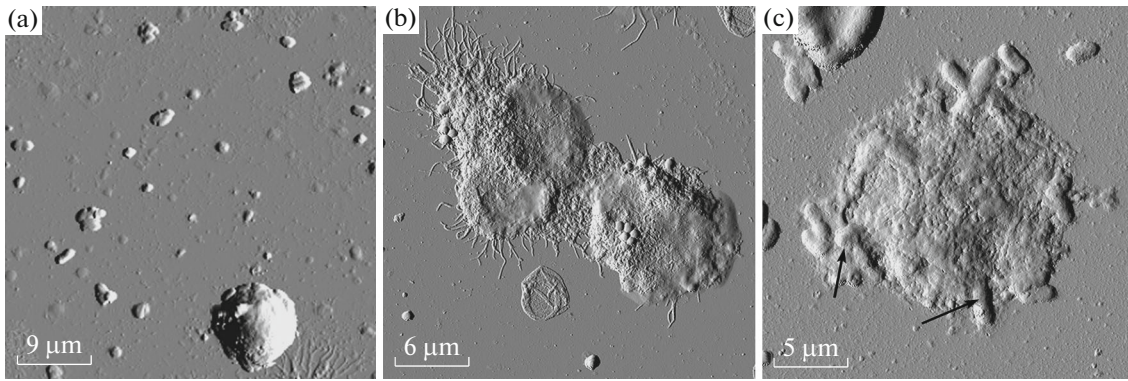


Fig. 1. AFM images of fixed neutrophil granulocytes (a, c) after incubation with *Escherichia coli* 321 and fixation with 2.5% glutaraldehyde and (b) after incubation with *Staphylococcus aureus* 2879 M and fixation with methanol. (c) The arrows indicate *E. coli* 321 bacteria absorbed by the neutrophil granulocyte, visible after fixation with 2.5% glutaraldehyde (an exceptional case).

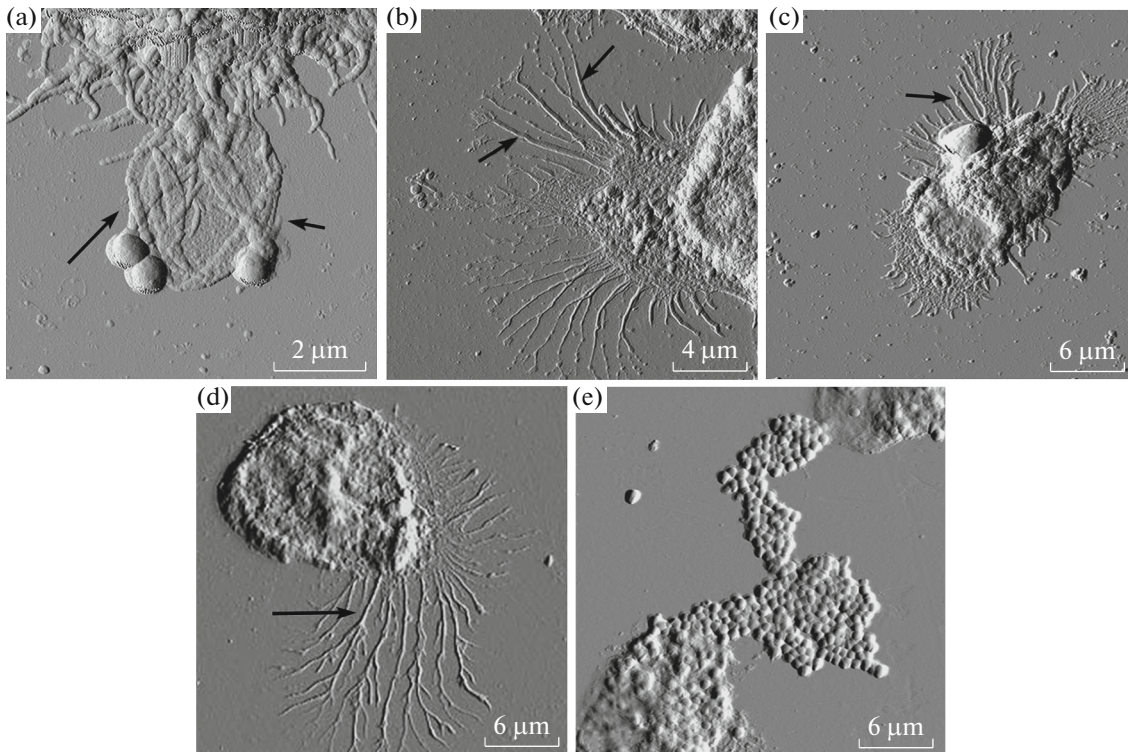


Fig. 2. Formation of extracellular structures during bacterial phagocytosis. The arrows indicate (a) lamellopodia and (b) pseudopodia under phagocytosis of nonopsonized *S. aureus* 2879 M, (c) pseudopodia at phagocytosis of nonopsonized *E. coli* 321, and (d) well-developed pseudopodia 5 min after the addition of opsonized *S. aureus* 2879 M. (e) Screening of pseudopods with opsonized *S. aureus* 2879 M after 10 min of coincubation with neutrophil granulocytes.

nucleus and (with rare exceptions; Fig. 2c) it is impossible to determine the intracellular localization of bacteria. Since glutaraldehyde, as a fixing agent, acts rather slowly, it is more difficult to evaluate the morphology of pseudopodia than after fixation with methanol (Figs. 1a, 1b). Under fixation with methanol, the cell is virtually instantaneously deprived of water and, therefore, the structures formed at the time of the

action of fixing agent remain bound to the substrate and well visualized.

NGs are completely spread out over the surface of the substrate. Therefore, the topography of the internal structure becomes clearly pronounced. This allows not only to determine the presence of bacteria inside an NG, but in some cases even to calculate the phagocytic index. The main morphological parameters of an

NG (diameter and height of cells) vary considerably when using different fixation variants (Pleskova et al., 2005), but, since methanol allows good visualization of pseudopodia and counting of the phagocytic index, preference in studies with fixed samples should be given to it.

Thin flat protuberances formed by strongly adherent leukocytes are called “lamellopodia.” The motion of the lamellopodia of the leading edge of the cell is due to the growth of branched actin networks crossing through the Arp2/3 complex (Fritz-Laylin et al., 2017). The more widespread form of cell movement requires only a weak nonspecific interaction with the extracellular environment, and the speed of movement thus exceeds the adhesion-based movement by several orders of magnitude (Loomis et al., 2012). This method of cell migration is associated with the formation of complex three-dimensional pseudopodia formed by branched networks of actin filaments. Complex pseudopodia are probably not as important for directed chemotaxis as for a study of the environment (Leithner et al., 2016; Vargas et al., 2016). In particular, they are responsible for the process of phagocytosis. In experiments on the study of phagocytosis on fixed samples, it is obvious that both pseudopodia and lamellopodia are able to take a most active part in the process of phagocytosis (Fig. 2). The formed pseudopodia and lamellopodia are quite universal and do not differ in morphological features during the phagocytosis of *S. aureus* 2879 M and *E. coli* 321 (Figs. 2b, 2c). At the same time, opsonization can significantly affect the nature of the pseudopodia being formed. In particular, NGs formed long, pronounced, and well-branched pseudopodia (Fig. 2d) after 5 min of interaction with opsonized *S. aureus* 2879 M, and, after 10 min, pseudopodia already were not detected due to the staphylococci actively bound to them (Fig. 2e).

After fixation with methanol, smear preparations were examined by AFM, determining the number of NGs phagocytizing one or more bacteria from 100 cells (phagocytic index). To study the dynamics of phagocytosis, smear preparations were fixed at 3, 5, 10, and 15 min after the addition of opsonized *S. aureus* 2879 M or *E. coli* 321 and 5, 10, 15, and 20 min after the addition of nonopsonized strains (Fig. 3).

For both opsonized and nonopsonized bacteria, a similar trend was observed: *S. aureus* 2879 M was more actively absorbed by NGs than *E. coli* 321. The more active absorption of nonopsonized staphylococci can be explained by differences in the PAMP of gram-positive and gram-negative bacteria. It was found, for example, that the peptidoglycan recognition protein (PGRP) binds to peptidoglycan with higher affinity than to lipopolysaccharide (Yang et al., 2017). In addition to the different specificity and binding affinity, the possible screening function of such cellular struc-

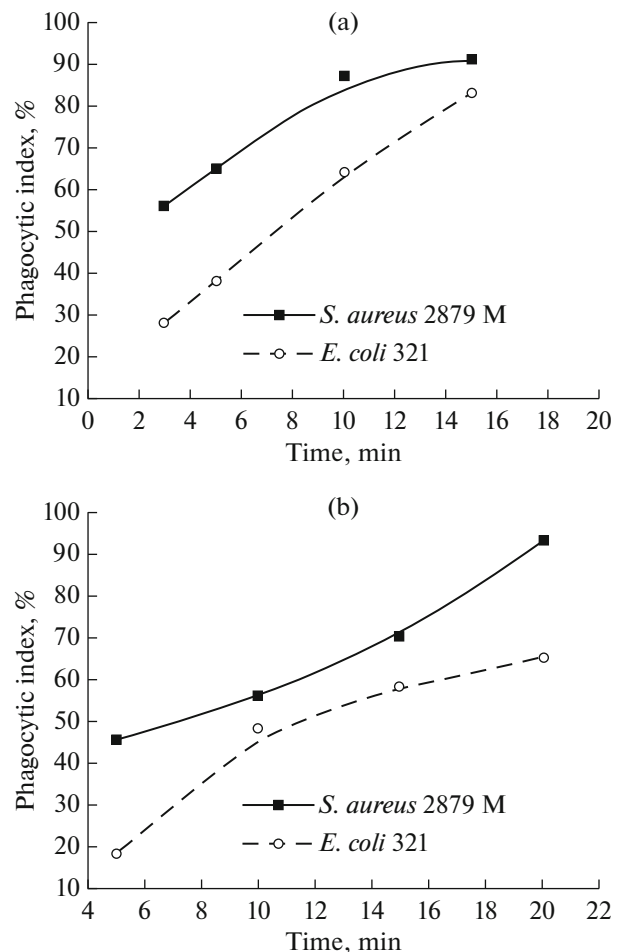


Fig. 3. Change in the phagocytic index of (a) opsonized and (b) nonopsonized *S. aureus* 2879 M and *E. coli* 321 in dynamics.

tures as, for example, peritrichia and pili, in *E. coli* cannot be completely ruled out.

In the case of opsonized *S. aureus* 2879 M, all major groups of opsonins (IgG, C3b, and iC3b) bind on the staphylococci surface, determining exclusively the opsonic function and absorption of bacteria by NGs during phagocytosis. In gram-negative bacteria, IgG can trigger a classic complement activation way, while iC3b can trigger an alternative complement way. The complement system, in turn, depending on the specific features of the cell wall lipopolysaccharides of gram-negative bacteria, can also activate the non-phagocytic system of killing bacteria and destroy of *E. coli* 321 by the formation of a membrane-attack complex (Pleskova et al., 2004).

At the same time, the overall change in the dynamics of phagocytosis of opsonized bacteria was the same: absorption was more rapid (for opsonized *S. aureus* 2879 M, 50% phagocytosis was observed after 2 min, and, for opsonized *E. coli* 321, after

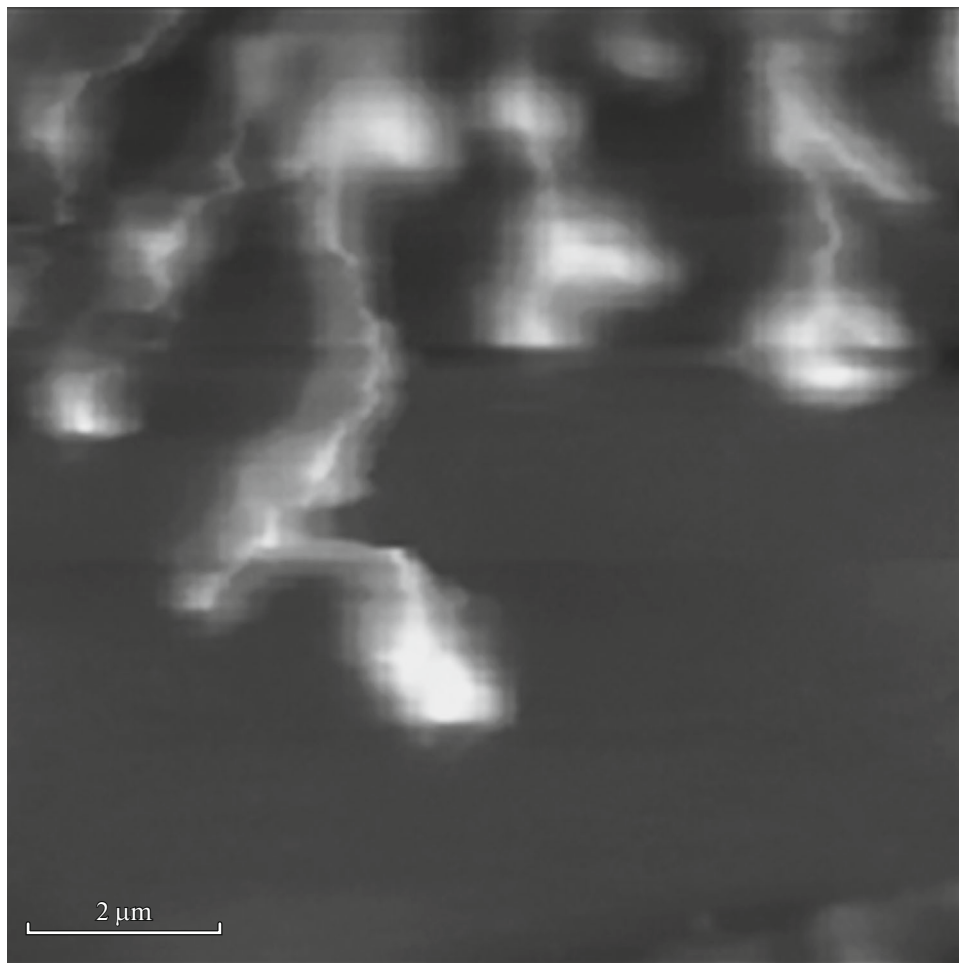


Fig. 4. Formation of well-developed pseudopods by live neutrophil granulocyte in PBS 10 min after the addition of opsonized *S. aureus* 2879 M.

7 min). For nonopsonized bacteria, this time was 7 and 11 min, respectively. Moreover, the phagocytic index (the number of NG phagocytized bacteria) was higher for opsonized strains. In particular, after 15 min of coincubation with opsonized *S. aureus* 2879 M, the phagocytic index was 90%, whereas for the nonopsonized strain for the same time the phagocytic index was only 70%.

A similar trend was observed for *E. coli* 321: after 15 min of coincubation with opsonized *E. coli* strain 321, the number of phagocytized NGs was 80%, while with nonopsonized the number was 60%. Such significant differences were due to the same experimental conditions for the study of phagocytosis. At the same time, the fact that phagocytosis of bacteria by suspended NGs depends on opsonization was recently revealed, whereas adherent phagocytes absorb predominantly nonopsonized microorganisms (Lu et al., 2014; van Kessel et al., 2014).

The results of morphological changes of NGs in dynamics after phagocytosis of opsonized *S. aureus* 2879 M are presented in Figs. 4 and 5. It is clearly seen

that NGs form pronounced pseudopodia after 10 min the introduction of opsonized *S. aureus* 2879 M in the study of vital NGs (Fig. 4). The determination of the main morphological parameters of NGs was carried out 140 min after the introduction of microorganisms to the cells with a 10-min interval (Fig. 5).

It is obvious that, after the end of the phagocytosis process, active movement, probably associated with the processes of intracellular microbial killing, continues in NGs. It was possible to reveal some structural and morphological rearrangements of the cell: constant movement of cytoskeleton elements, not associated with the movement of cells, but only with the movement of granular-cytoplasmic contents (Fig. 5c); constantly varying distance between the nucleus segments; the vibrations of the nucleus volume (Figs. 5a, 5b), respectively, with increasing volume of the nuclear segments; decrease in the intersegmental distance; and constant fluctuations in the volume of the cell.

Studies of the dynamics of phagocytosis with high resolution in real time were conducted for the first time. Based on the structural and morphological

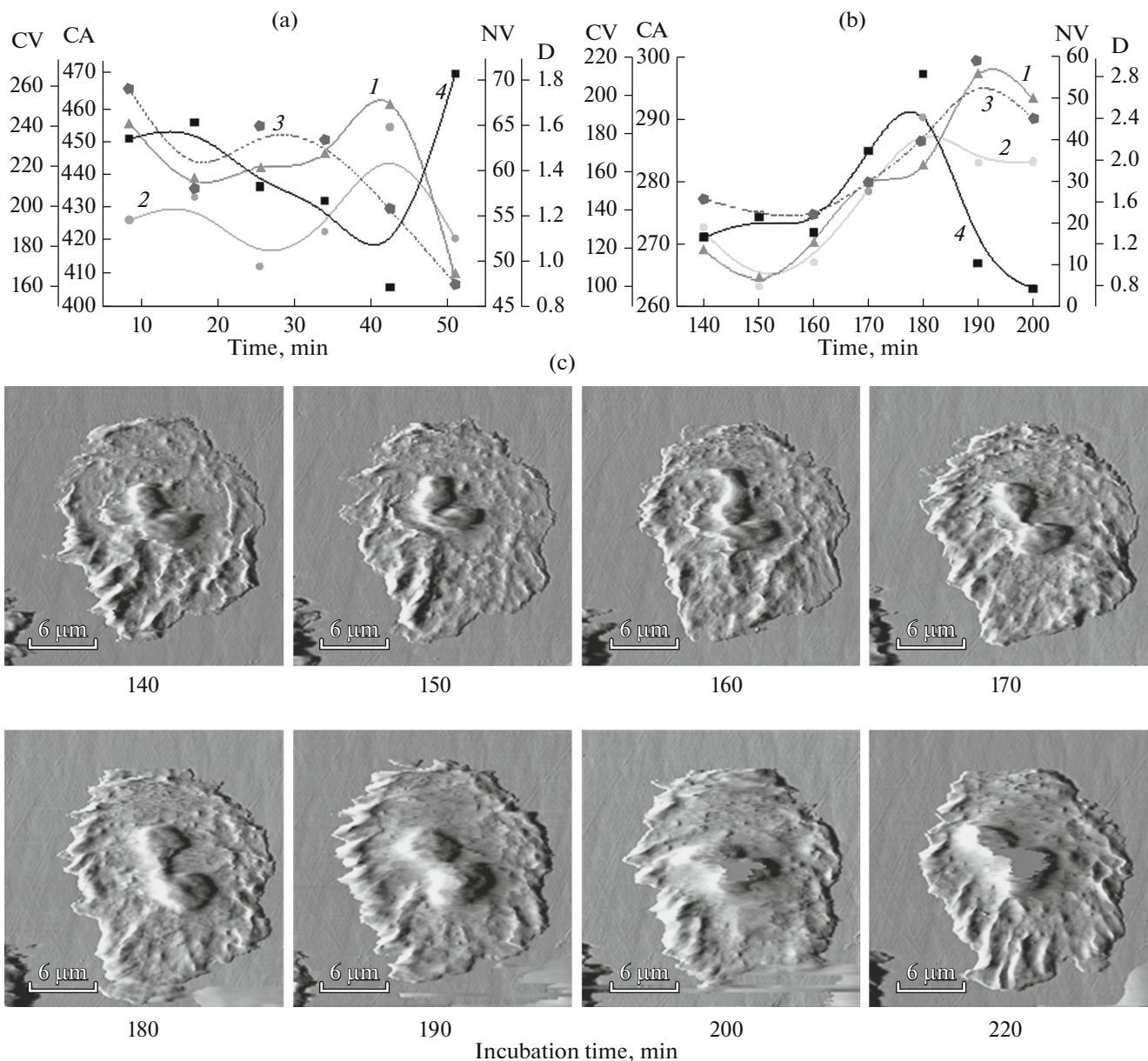


Fig. 5. Changes in the basic morphometric parameters of neutrophilic granulocytes in the process of phagocytosis of nonopsonized (a) *S. aureus* 2879 M and (b) *E. coli* 321 strains and (c) AFM images of living neutrophil granulocyte in dynamics after incubation with opsonized *S. aureus* 2879 M. On the ordinate axis: CV, cell volume, μm^3 (curve 1); CA, cell area, μm^2 (curve 2); NV, nucleus volume, μm^3 (curve 3); D, distance, μm^3 (curve 4). The scale segment is 6 μm .

changes of NGs, it was established that not only cytoskeleton elements of the cell, but also the nucleus, were involved in the process of killing of phagocytized bacteria. In addition, it should be noted that, in the process of phagocytosis, the nucleus volume increased (by an average of 31%), while the area of adhesion surface did not change.

For NGs, which, in the diapedesis process, experience resistance force of 100 pN from endothelial cells

(Edmondson et al., 2005), the rigidity of the membrane–cytoskeleton complex is important. The AFM method allows one not only to carry out dynamic observations in a physiological environment, but also to establish viscoelastic characteristics of cell membranes. Using the FS spectroscopy method and the Hertz model, we determined the Young's modulus. The results of its change during the phagocytosis of opsonized and nonopsonized *S. aureus* 2879 M and *E. coli* 321 are shown in Table 1.

Table 1. A change in the rigidity of an NG membrane–cytoskeleton complex (Young’s modulus) in the process of phagocytosis of opsonized and nonopsonized bacteria

Young’s modulus, kPa				
control group without phagocytosis	phagocytosis			
	<i>S. aureus</i> 2879 M		<i>E. coli</i> 321	
	OPS ⁺	OPS ⁻	OPS ⁺	OPS ⁻
28.63 ± 3.33	13.67 ± 3.27 ^{a, b}	24.16 ± 7.37 ^b	16.55 ± 7.77 ^{a, b}	29.54 ± 3.21 ^b

^a Difference with the control; ^b differences between the phagocytosis groups of opsonized and nonopsonized bacteria according to 12 experiments, *P* < 0.05; OPS⁺ and OPS⁻, opsonized and nonopsonized strains, respectively.

Table 2. Changes in NG biochemical profile as a result of phagocytosis of opsonized/nonopsonized bacteria

Enzymes/ storage compounds	Control	Nonopsonized bacteria		Opsonized bacteria	
		<i>S. aureus</i> 2879 M	<i>E. coli</i> 321	<i>S. aureus</i> 2879 M	<i>E. coli</i> 321
NADPH ^{MI}	25.3 ± 1.5 (<i>n</i> = 9)	61.2 ± 5.31 ^a (<i>n</i> = 9)	62.78 ± 5.17 ^a (<i>n</i> = 9)	61.8 ± 3.76 ^a (<i>n</i> = 12)	64.0 ± 3.25 ^a (<i>n</i> = 12)
NADPH ^{CI}	41.2 ± 2.7 (<i>n</i> = 9)	66.1 ± 5.21 ^a (<i>n</i> = 9)	68.7 ± 4.36 ^a (<i>n</i> = 9)	68.7 ± 3.4 ^a (<i>n</i> = 12)	69.8 ± 3.01 ^a (<i>n</i> = 12)
MPO	1.05 ± 0.05 (<i>n</i> = 10)	1.3 ± 0.11 ^a (<i>n</i> = 10)	1.29 ± 0.18 ^a (<i>n</i> = 10)	1.44 ± 0.18 ^a (<i>n</i> = 10)	1.34 ± 0.11 ^a (<i>n</i> = 10)
ACP	1.61 ± 0.05 (<i>n</i> = 12)	1.63 ± 0.16 (<i>n</i> = 12)	1.63 ± 0.18 (<i>n</i> = 12)	1.79 ± 0.11 ^{a, b} (<i>n</i> = 12)	1.76 ± 0.09 ^{a, b} (<i>n</i> = 12)
AP	0.75 ± 0.07 (<i>n</i> = 12)	0.55 ± 0.16 ^a (<i>n</i> = 12)	0.54 ± 0.08 ^a (<i>n</i> = 12)	1.32 ± 0.27 ^{a, b} (<i>n</i> = 12)	1.41 ± 0.26 ^{a, b} (<i>n</i> = 12)
CP	1.54 ± 0.1 (<i>n</i> = 12)	1.1 ± 0.18 ^a (<i>n</i> = 12)	1.04 ± 0.17 ^a (<i>n</i> = 12)	1.31 ± 0.17 ^{a, b} (<i>n</i> = 12)	1.34 ± 0.15 ^{a, b} (<i>n</i> = 12)
Glycogen	1.77 ± 0.18 (<i>n</i> = 12)	1.76 ± 0.45 (<i>n</i> = 12)	1.57 ± 0.37 (<i>n</i> = 12)	2.13 ± 0.38 ^a (<i>n</i> = 12)	2.31 ± 0.35 ^{a, b} (<i>n</i> = 12)
PL	2.72 ± 0.05 (<i>n</i> = 12)	2.68 ± 0.19 (<i>n</i> = 12)	2.7 ± 0.21 (<i>n</i> = 12)	2.69 ± 0.22 (<i>n</i> = 12)	2.79 ± 0.18 (<i>n</i> = 12)

^a Differences with control, *P* < 0.05; ^b differences between phagocytosis groups of opsonized and nonopsonized bacteria, *P* < 0.05; NADPH^{MI}, NADPH oxidase under monoimpact; NADPH^{CI}, NADPH oxidase under combined impact; MPO, myeloperoxidase; ACP, acid phosphatase; AP, alkaline phosphatase; CP, cationic proteins; PL, phospholipids; *n*, number of experiments.

It can be seen that, in the process of phagocytosis, a significant decrease in the rigidity of the membrane–cytoskeleton NG complex occurred and, the more intense the process of phagocytosis (in the case of opsonized bacteria), the lower the rigidity of the membrane.

A study of the main biochemical parameters of NGs in the process of phagocytosis of opsonized and nonopsonized strains (Table 2) showed that the oxygen-dependent NG metabolism does not change depending on the presence or absence of opsonins on the surface of bacterial cells, since both NADPH-oxidase and myeloperoxidase (MPO) changed significantly in comparison with the control (not phagocytosed NG), whereas differences between groups of

opsonized and nonopsonized bacteria were not detected. Interestingly, these results are consistent with the studies of Vandenbroucke-Grauls et al., who showed for the first time that the release of lysosomal enzymes, MPO level, and the formation of superoxide anion are the same during phagocytosis of opsonized or nonopsonized staphylococci (Vandenbroucke-Grauls et al., 1987).

At the same time, the opsonization process had a significant effect on the oxygen-independent metabolism of NGs. In particular, we detected the activation of acid phosphatase (ACP) in the phagocytosis of opsonized bacteria, whereas, in reactions with nonopsonized bacteria, no differences with the control were observed. Even more pronounced changes were found

for alkaline phosphatase (AP), since the enzyme activity is suppressed at the phagocytosis of nonopsonized bacteria in comparison with opsonized strains and in comparison with the control. At the same time, the activity of AP increased statistically significantly at phagocytosis of opsonized microorganisms.

The ratio of bacterial cells to NGs used in our experiments (500 : 1) caused a suppression of the synthesis of lysosomal cation proteins in the process of phagocytosis of all investigated bacterial variants, but opsonization of *S. aureus* 2879 M and *E. coli* 321 reduced the consumption of nonenzyme proteins (Table 2).

The process of phagocytosis did not affect the phospholipid level of the cell, while the glycogen level significantly increased in phagocytosis of opsonized bacteria.

Thus, a comparison of the phagocytosis of opsonized and nonopsonized bacteria revealed a difference in the morphology of the pseudopodia, the dynamics of the phagocytosis process, the rigidity of the membrane–cytoskeleton complex, and the realization of oxygen-dependent metabolism of NGs. *S. aureus* was absorbed by NG more actively than *E. coli*, this did not affect the biochemical profile of NGs, but affected the rigidity of the membrane–cytoskeleton complex of the cell.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. This article does not contain any studies with animals performed by any of the authors.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Blood sampling was performed in the morning, after the donors signed the consent form.

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