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Investigation of the influence of complement system on the various strains of *Proteus* by methods of atomic force microscopy and luminol-dependent chemiluminescence.

S. N. Pleskova[1], Yu. Yu. Guschina[2], M. B. Zvonkova[3]

[1] Microbiology and Immunology Department, Nizhny Novgorod Medical Academy, Minin sq, 10/1

[2] Research and Educational Center for Scanning Probe Microscopy, University of Nizhny Novgorod, Gagarin Ave, 23

[3] Physiology and biochemistry of Human and Animal Department, University of Nizhny Novgorod, Gagarin Ave, 23

E-mail: pleskova@mail.ru

Abstract

Activity of proteins of complement system (serum proteins) with the reference to the different strains of bacteria of genus *Proteus* (*P. vulgaris* and *P.mirabilis*) has been investigated by methods of atomic force microscopy (AFM) and luminol-dependent chemiluminescence. The results have shown the different activity of serum proteins. They can cause lysis of bacterial cells (*P. vulgaris 296, P.mirabilis 120*) or opsonization them only (*P. vulgaris 856, P.mirabilis 210*). If bacterial cells were lysed they swelled and then collapsed, which was visualized by a method of AFM. In the cause of opsonization proteins surrounded the bacteria. The same results were achieved by a method of luminol-dependent chemiluminescence.

The difference in bacteria' sensitivity to serum proteins was determined by strains, but not specious peculiarity of bacteria.

Introduction

Polymorphonuclear neutrophils (PMN) and protein of complement system (serum proteins) play an important role in the first-line (or nonspecific) defense of the organism [1]. Serum protein can cause lysis of bacteria by forming membrane attack complex or increas phagocytosis by opsonization of bacteria. It is well known that the first mechanism is realized with reference to some strains of *E.coli* and *N.gonorrhoea* [3]. In this work we has investigated activity of serum proteins with the reference to the different strains of bacteria of genus *Proteus* (*P. vulgaris* and *P.mirabilis*) by methods of atomic force microscopy (AFM) and luminol-dependent chemiluminescence (LDCL).

Experiment

The *Proteus vulgaris* (strains 856 and 296) and *Proteus mirabilis* (strains 120 and 210) were incubated with pool of serum of healthy volunteers (at 0,5ml) or PBS (control) at 60 min, 37°C, washed by PBS (1000g, 10 min) and used in concentration 10^6 cell/ml in PBS. Bacterial suspension (0.05 ml) was placed on slides. After cell deposition and adhesion on the slide (40 min, 20°C) their were fixed with methanol (0.05 ml, 10 min). Smears were washed by distilled water and dried in the air. At last, bacterial cells were investigated by AFM (SOLVER BIO NT-MDT, Russia) using a S probe in noncontact mode with the tip having 20 nm curving radius.

Human PMN were isolated from venous blood of healthy volunteers by centrifugation through ficoll-verografin density gradient (Pharmacia, Sweden) using densities of 1.077 and 1.116 g/ml. The granulocytes were washed with PBS and resuspended in HBSS at a concentration of $5 \cdot 10^5$ cells/ml. Neutrophil suspension, containing luminol (10^{-5} M, Serwa, USA) was mixed with 0,2 ml the suspension of microorganism ($2 \cdot 10^4$ cell/ml), which had been incubating with serum at different time (15, 30, 60 min) at 37°C and washing (1000g, 10 min) or HBSS (negative control) in total volume 1 ml. Kinetic of LDCL has been registered during 60 min by chemiluminescence counter "Beta-1" (Medapparatura, Ukraine) [2].

Results and Discussion

The result of PMN stimulation by bacteria was the formation of phagolysosome keeping active forms of oxygen for killing microbes (respiratory burst). We have measured respiratory burst during 60 min. Data (integral factors of total sumlight) are shown in table 1.

Table 1

Luminol-dependent chemiluminescence of polymorphonuclear neutrophils, which was stimulated with strains of *Proteus*

Strain	Control	Strain, which was incubated with serum		
	S ($x10^5$)	15 min	30 min	60 min
		S ($x10^5$)	S ($x10^5$)	S ($x10^5$)
P.mirabilis 120	5,5±3,7	87,4±0,8*	70,6±4,9*	5,6±0,6
P.mirabilis 210	2,0±1,0	34,3±12,9*	46,5±7,9*	60,5±22,5*
P.vulgaris 296	3,3±0,2	26,3±9,1*	16,4±4,7*	10,9±5,0
P.vulgaris 856	3,7±1,5	110,4±0,8*	110,4±1,8*	182,4±34,5*

* At the 0,05 level, the means are significantly different.

The results have shown different dynamics of PMN stimulation. Two strains of *Proteus* (*P.mirabilis 120* and *P.vulgaris 296*) after 60 min incubation with serum protein did not cause stimulation of PMN (level of respiratory burst the same as the control), but two other strains

(*P.mirabilis 210* and *P.vulgaris 856*) caused. Their ability of PMN stimulation after 60 min incubation with serum increased by 30- 49 times. We have expected that the serum may have different activity with reference to some strains of *Proteus*: were lysed or opsonized by serum protein. In the first variant the bacteria were killed and cannot cause of PMN stimulation. If bacteria were opsonized by serum protein (especially by C3b component of complement), the pagocytosis was facilitated and the respiratory burst increased. To confirm this suggestion we used scanning probe microscopy method. The results are presented on the fig. 1, 2 (for the strains *P.mirabilis 120* and *P.vulgaris 856*). The results are the same for strains *P.vulgaris 296* and *P.mirabilis 210.*

In the first case bacterial cells were lysed; they swelled and then collapsed, which was visualized by a method of AFM (fig.1).

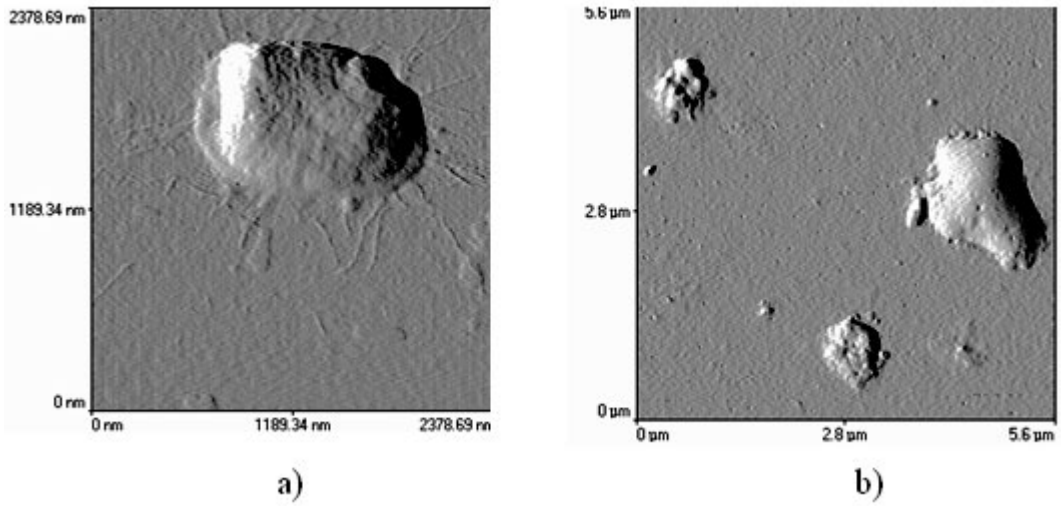
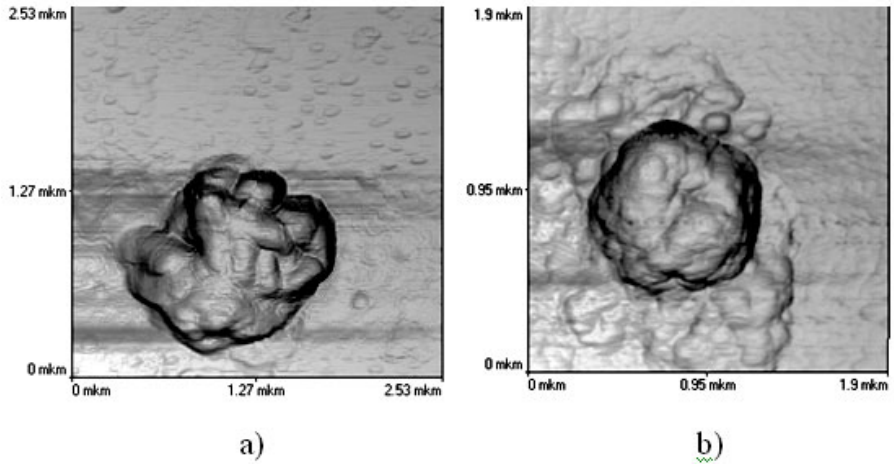


Fig. 1. Pleskova S.N. *et al* Investigation of the influence of complement system on the various strains of *Proteu*

In the cause of opsonization proteins surrounded the bacteria *P. vulgaris 856* and making it more suspectible to phagocytosis and subsequent elimination (fig.2).



As possible see from result, the difference in bacteria's sensitivity to serum proteins was determined by strains, but not specious peculiarity of bacteria.

Conclusion.

Result of investigation shown that bacteria genus *Proteus* can were only opsonization or were opsonization and after some time were lysed (these results were received by two different independent methods: luminol-dependent chemiluminescence and atomic force microscopy). Probably the ability of bacteria to be lysed or only to be opsonization dependent on features of cell wall, but not specious peculiarity of bacteria.

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