Ultrastructural Analysis of Interactions of Staphylococci with Mono- and Polynuclear Phagocytes in Nonphlogogenic and Phlogogenic Reaction

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Antibacterial activity of neutrophils and peritoneal macrophages toward massive doses of *Staphylococcus aureus* is studied *in vivo*. Two types of antibacterial response are revealed: nonphlogogenic (physiological) and phlogogenic (inflammatory). Nonphlogogenic reaction is characterized by pronounced antibacterial effect of phagocytes on cocci. Transition to phlogogenic response is accompanied by impaired function of phagocytes involving their self-destruction and disintegration, which decreases their antibacterial activity and promotes inflammation.

Key Words: *neutrophil; staphylococcus; macrophage; phagolysosome*

Despite extensive research, general pathological concept of inflammation has not been formulated. All conclusions concerning the function of phagocytes are based on the investigation of inflammatory reaction [1,3,9,10].

Recent studies deal with nonphlogogenic reactions of neutrophilic leukocytes (NL), i.e., their functioning under physiological conditions [2,6-8]. It was demonstrated that the system of NL effectively reacts under physiological conditions to rather strong bacterial attacks. The functioning of the system in this case is intensive, i.e., a small amount of cells (about 13%) are involved in antibacterial response; phlogogenic reaction is extensive [3,7].

We studied morphological changes of *St. aureus* phagocytized by NL and peritoneal macrophages (PM) and antibacterial function of these cells in nonphlogogenic and phlogogenic responses.

MATERIALS AND METHODS

Suspension of *Staphylococcus aureus* was injected intraperitoneally in outbred albino mice in doses 25×10^6 (series I) and 25×10^8 microbial bodies (series II). The doses were chosen on the basis of the literature data [4]. Experimental conditions were described previously [8]. Peritoneal lavage (PL) and blood were examined. They were centrifuged at 1500 rpm, the supernatant was discanted, and the pellet was used to prepare smears, which were examined by light microscopy. The smears were then fixed with 1% glutaraldehyde and viewed in an electron microscope. The intensity of staphylococcal invasion of the internal organs and blood was estimated by bacteriological methods.

RESULTS

In both series of experiment mice developed purulent peritonitis, as evidenced by the appearance of the peritoneum. High mortality in the second series (about 99%) did not allow us to study the reaction of NL and PM at the late stages.

Phagocytic activity of NL and PM from peritoneal lavage was observed 1 h after administration of *St. aureus* in both doses. These cells formed compact phagosomes containing one coccus. Phagocytized and nonphagocytized cocci were morphologically different. Free cocci were round, had a well outlined

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Fig. 1. Neutrophilic leukocyte in peritoneal lavage after 1 h of experiment. Extracellularly located staphylococcus has a nucleotide, microcapsule; the outer wall and the plasma membrane are well seen; the cytoplasm is homogenous. Phagocytized coccus has no microcapsule, and its nucleotide is lost during the processing of the preparation, ×8900.

Fig. 2. Phagosome in peritoneal macrophage after injection of *St. aureus* in a dose of 25×10^8 microbial bodies. Staphylococci are without the microcapsule, their cytoplasm and nucleotide are clarified, ×39,000.

microcapsule, homogenous outer wall, and thin plasma membrane; the nucleotide was located in the center of the cell. (Fig. 1). In phagocytized cocci the nucleotide had loose structure and no microcapsule.

After administration of *St. aureus* in the higher dose (25×10^8 microbial bodies) phagocytizig activity of NL and PM increased, as evidenced by greater number of cocci in the cytoplasm of these cells, and their antibacterial function was preserved (all phagocytized bacteria had no microcapsule).

The structure of phagosomes in PM after this dose of *St. aureus* did not change: they fused and contained several cocci (Fig. 2). Antibacterial function of NL and PM was preserved: phagocytized cocci had no capsule; in some cocci the nucleole and cytoplasm were lysed. Occasional phagocytes containing phagosomes with deformed cocci were present in peripheral blood.

Phagocytizig activity of PM and NL increased 6 h after administration of *St. aureus* in a dose 25×10^6 microbial bodies (Fig. 3). The signs of destruction were observed in some bacteria, namely, cytoplasm clearing and deformation of the contours. Phagosomes did not fuse. Each phagosome contained one coccus. Lysosomal granules in the phagocyte cytoplasm were empty. The number of phagocytized cocci also increased after their administration in a

Fig. 3. Peritoneal macrophage 6 h after intraperitoneal injection of 25×10^8 *St. aureus*. Compact phagolysosomes contain one coccus, ×3900.

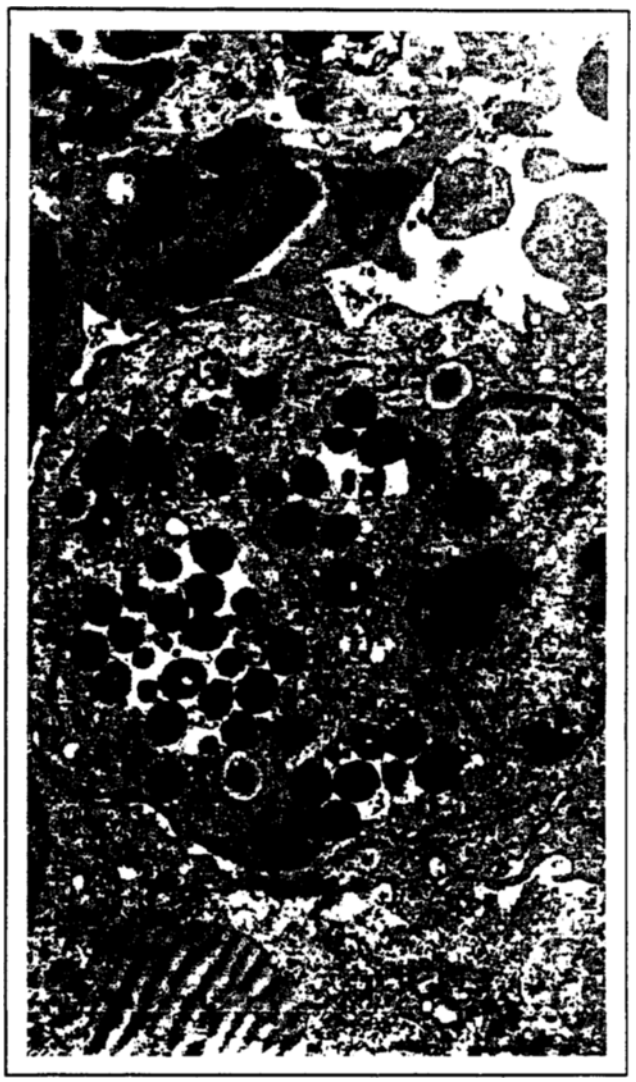

Fig. 4. Peritoneal macrophage 6 h after intraperitoneal injection of 25×10^8 *St. aureus*. Large phagosomes contain destroyed, intact, and dividing cocci, ×3900.

dose of 25×10^8 microbial bodies; however, the phagosomes fused with each other, forming large cavities containing several cocci. In NL, phagosomes contained cocci with signs of destruction (loose nucleole and cleared cytoplasm), while large phagosomes formed in PM (Fig. 4) contained cocci with morphological sings of destruction, intact, and even dividing. Empty lysosomal granules were seen in the cytoplasm of both PM and NL.

The differences in the structure of PM and NL and phagocytized cocci were more pronounced 1 day after their administration of *St. aureus* in different doses. After injection of 25×10^6 *St. aureus*, PM contained individual phagosomes with cocci, while the majority of phagosomes were represented by large cavities containing cocci with deformed contours. Phagosomic cavities were not observed in NL. At the higher dose of *St. aureus*, clarified granules in the cytoplasm, large phagosomes containing cocci with the signs of destruction, and phagosomes without cocci were observed in NL. Loose, homogenous cytoplasm in the majority of PM contained phagosomic cavities with intact and destroyed cocci with preserved contours (Fig. 5). The contours of some PM were indistinct, plasma membrane disintegrated, and the cytoplasm contained numerous empty granules and phagosomes with intact cocci (Fig. 6).

Taken together, these observations indicate that antibacterial response of phagocytes is realized via two pathways: physiological (nonphlogogenic) and phlogogenic (inflammatory).

Fig. 5. Peritoneal macrophage 1 day after intraperitoneal injection of 25×10^6 *St. aureus*. Large phagosomic cavities containing intact and destroyed cocci are seen in unstructured macrophagal cytoplasm, ×2950.

Fig. 6. Peritoneal macrophage 1 day after injection of 25×10^6 *St. aureus*. Macrophagal contours are indistinct; plasma membrane is disintegrated; cytoplasm contains empty granules and phagosomes with intact cocci, ×3900.

The nonphlogogenic pathway (first series of experiments) is characterized by adequacy of intracellular plastic processes, namely, compact phagosomes are formed (they contain bacteria to produce high concentrations of lysosomal enzymes) and the microbes are evacuated by phagocytes from the site of injection to the site of clearance. The intensity of clearance was estimated by bacteriological methods: it reached the maximum on the 6th h after injection of *St. aureus* and was accomplished on the 5th day of the experiment.

The phlogogenic pathway (second series of experiments) is characterized by deterioration of antibacterial reaction: the bacteria are actively phagocytized with formation of large phagosomes which contain or not microbial bodies. The concentration of lysosomal enzymes in these phagosomes is low for destruction of the bacteria. The level of phagocyte degranulation increases, and phagocytes with exhausted antimicrobial potential disintegrate. Viable microbes appear in tissues, and lysosomal enzymes from degraded NL cause secondary tissue damage with formation of clinical and morphological signs typical of purulent inflammation [5].

The physiological (nonphlogogenic) reaction consists in intense adequate functioning of phagocytes: active phagocytosis of bacteria with formation of compact coccus-containing phagosomes, in which the bacteria are destroyed by exocytosis with subsequent evacuation to the sites of excretion.

The phlogogenic (inflammatory) pathway in activated when antibacterial potential of phagocytes is

exhausted. In this case their function deteriorates and the cells disintegrate, which triggers inflammatory process. Antibacterial clearance in the organism is impaired, and rapidly dividing bacteria further exacerbate the response of phagocytizig cells.

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