MORPHOLOGY AND PATHOMORPHOLOGY

Function of Neutrophils in Nonphlogogenetic Reaction

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Clinically nonphlogogenetic phagocyte reaction under conditions of bacterial challenge was studied *in vivo*. The "mission" of phagocytes under such conditions is completed by evacuation of phagocytized bacteria from the site of capture into the blood and then into the intestine. The purulent process induced by massive doses of *Staphylococcus aureus* (25×10^6 and 25×10^8 bacteria), without any concomitant injury to the peritoneum does not lead to the development of inflammation.

Key Words: *inflammation; neutrophilic leukocyte; noninflammatory reaction*

The phagocytic theory of inflammation [4] provides the basis for the concept that inflammation is the unique form of defense and adaptive reaction of phagocytes to bacterial invasion and that phagocytosis is the basic defense mechanism in inflammation. I. I. Metchnikoff's theory of comparative pathology has been predominating for many decades, and therefore the mechanisms of antibacterial resistance in the absence of inflammation have been neglected. The level of antibacterial resistance in health was never assessed, and there were no purposeful studies in this field. The inflammation theory does not cope with the requirements of practical medicine: the practitioners do their best to control the inflammatory reaction [6,10,12] which theoretically represents the only form of antibacterial defense [1,7,9].

The basic contradiction of I. I. Metchnikoff's theory is the clinical formula: inflammatory processes develop in weak patients because their antibacterial resistance is attenuated. Therefore, mechanisms suppressing a bacterial agent by means **other than inflammation** exist in a normal organism. This contradiction was noted by advocates of Metchnikoff's theory; although with some amendments, they admit that **inflammation** and **phagocytosis** are not equivalent notions [1,7,9,11].

We studied the function of phagocytes *in vivo* under conditions of bacterial loading, which caused no pyoinflammatory process, in order to detect the mechanisms of antibacterial resistance of an organism in health and other than inflammatory mechanisms of suppression of bacterial aggression.

MATERIALS AND METHODS

Two series of experiments were carried out on outbred albino mice. In the first series, corpuscles of *Staphylococcus aureus* (25×10^6 cells/ml normal saline) was injected intraperitoneally to 40 animals. In the second series, the bacterial dose was 100 times higher (25×10^8). Control mice were injected with 1 ml of sterile normal saline intraperitoneally.

The animals were sacrificed under ether narcosis after 1 and 6 h and 1 and 5 days. The state of the peritoneum was assessed from microphotographs. Bacteriological investigation of the organs and blood, histological studies of visceral tissues, cytological analysis of blood smears, and electron-microscopic

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Fig. 1. Mouse peritoneal organs 1 day after intraperitoneal challenge with *Staphylococcus aureus*. Magnification 4.

examination of lavage from the abdominal cavity, visceral tissues, and blood were carried out.

RESULTS

None animal in both series developed peritonitis. The behavior and status of animals in group 1 did not differ from those of normal controls, there were no lethal outcomes. The peritoneum was smooth and glossy, there were no fibrin depositions or inflammatory exudation (Fig. 1).

Electron microscopy showed staphylococci in the lavage fluid as well as on the surface and inside neutrophilic leukocytes (NL) and peritoneal macrophages (Fig. 2). Staphylococci were detected in phagocytes and in blood vessels (Fig. 3, *a*, *b*), inside NL

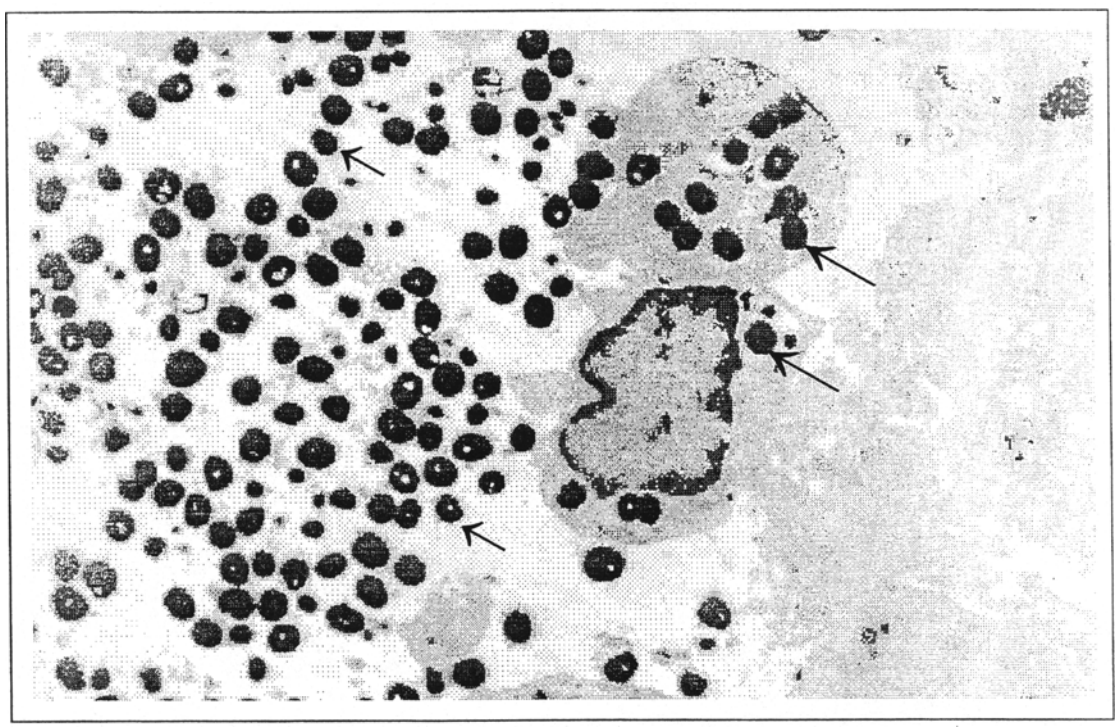

Fig. 2. Lavage from mouse abdominal cavity 1 h after intraperitoneal injection of *Staphylococcus aureus*. The cocci float free in the liquid (short arrows) and in phagosomes of phagocyte cytoplasm (long arrows). Magnification 2950.

migrating towards the intestinal lumen, and in the intestinal lumen (Fig. 4).

The animals of this group left alive behaved normally, there were no symptoms of any disease. Therefore, the dose of staphylococcus (25×10^6 bacterial corpuscles), to which the animals were never exposed under natural conditions, was insufficient for inducing inflammation upon intraperitoneal administration. Saturation of a healthy animal with massive dose of bacteria triggers generalized nonphlogogenetic mechanisms of their liquidation.

Despite a sharp increase in bacterial dose in the second series of experiment, we failed to induce peritonitis in any of the animals. The behavior and

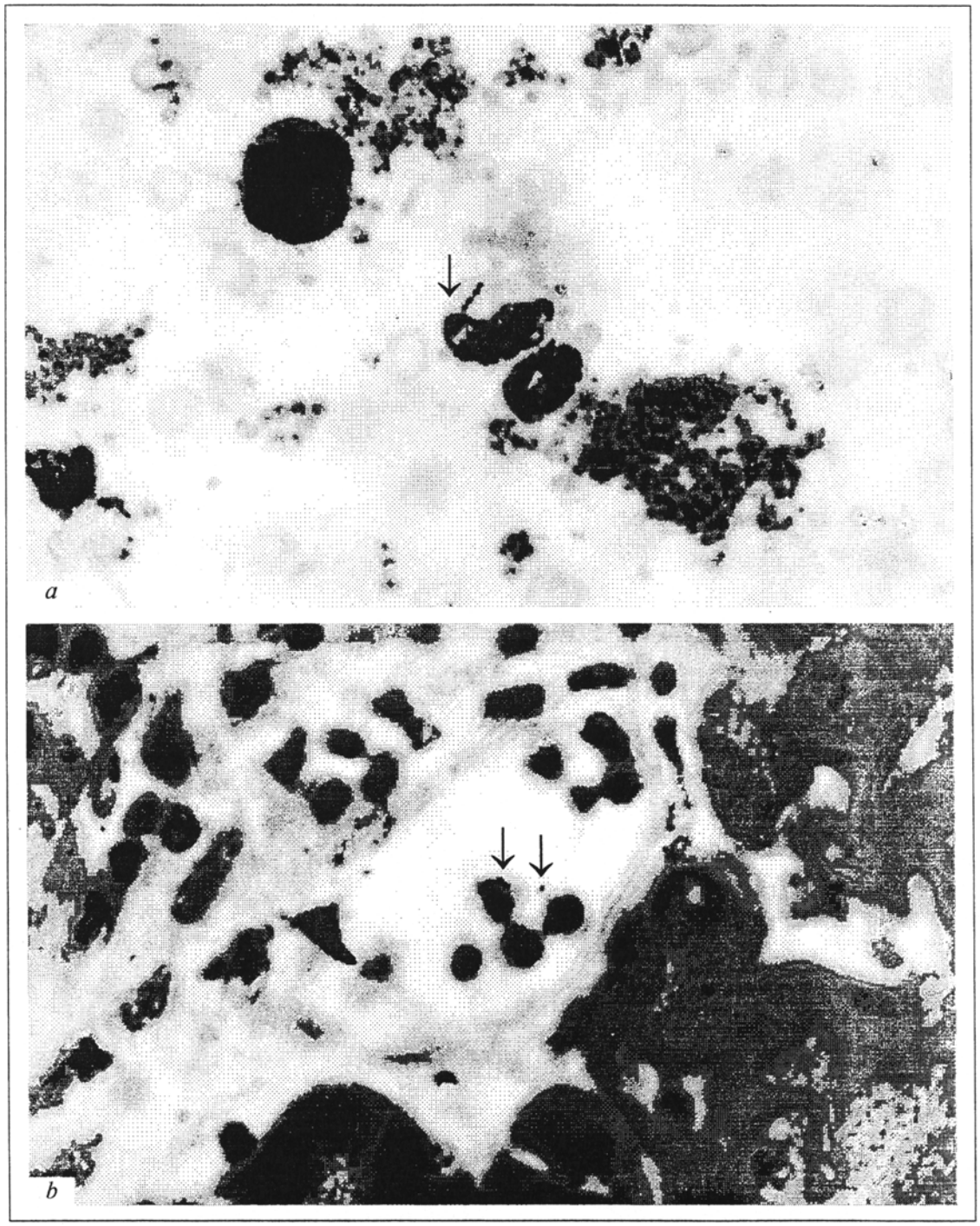

Fig. 3. Mouse blood smear (*a*) and pancreas (*b*) 6 h after intraperitoneal injection of *Staphylococcus aureus*. Magnification 900. *a*) phagocyte with phagocytized cocci (arrow). Giemsa staining. *b*) phagocyte with phagocytized cocci (short arrow) in the interlobular connective tissue in the lumen of a blood capillary and coccus floating in the blood plasma (long arrow). Gram staining.

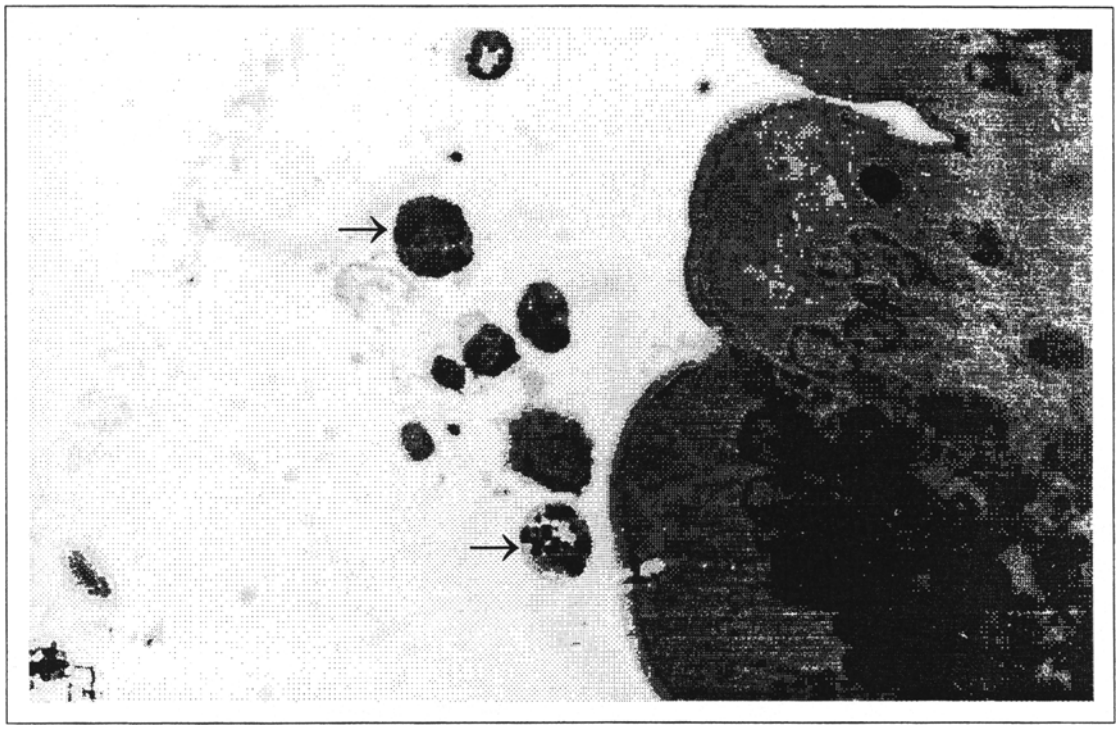

Fig. 4. Mouse intestine 6 h after intraperitoneal injection of *Staphylococcus aureus*. Phagocytes containing phagosomes with cocci in their cytoplasm (arrow) in the lumen of the intestine. Semithin section; toluidine blue staining. Magnification 900.

status of mice changed: the animals were anxious during the first 0.5-1.5 h postinjection, and motor excitation was observed. Starting from the second hour after challenge, motor excitation was replaced by stiffness: the mice sat in groups, with their fur disheveled and respiration rate sharply increased. Approximately half of the animals excreted dark urine and dark scybalum. By the end of day 1 the animals died (mortality 98.8%).

Autopsy showed a smooth and glossy peritoneum in all animals, both dead and sacrificed. There were no fibrin deposits on its surface or accumulation of inflammatory infiltrate. The intestine was swollen and atonic. These changes were the more expressed the longer was the disease.

Electron microscopic examination showed a picture similar to that in the first series of experiment. It is noteworthy that the number of staphylococci detected in the second series was higher than in the first series of experiment.

The main conclusion from the second series of experiment is as follows: a 100-fold increase (*vs.* the first series) in the intraperitoneal dose of staphylococci was also insufficient to induce inflammation, although overall deaths were observed caused by bacterial toxicosis.

Analysis of these results suggests that besides the inflammatory forms of resistance to pathogenic microorganisms, higher animals possess rather effective mechanisms other than inflammatory, determining normally high antibacterial resistance.

What do we mean speaking about noninflammatory mechanisms of resistance to pathogenic microorganisms? First of all, the capture and evacuation of bacteria by phagocytes into the blood and then into the intestine.

It is noteworthy that all phagocytes in abdominal lavage were highly reactive. The phagosomal cavity of the majority of these cells were compact and corresponded to the size of staphylococci. We saw no fusion of phagosomes with the formation of large cavity, reported by some scientists [2]. In abdominal lavage, NL were represented mainly by moderately active forms characterized by lobo- and pseudopodia on the surface, intact nuclear and cell membranes, phagosomes in the cytoplasm, more or less intact cytoplasm granules, some of them being clarified, and the absence of massive degranulation. Ultrastructural analysis of blood NL showed mostly passive or moderately active forms. Such a structure of NL means that during noninflammatory reaction these cells do not attain a high degree of activation not only in the blood, but even at the site of bacterial reaction (in the abdominal cavity) and thus retain their bactericidal and energy potential and do not phagocytize bacteria at the site of capture. The presence of bacteria in the intestinal lumen suggests that the main activity of NL is the capture and trans-

portation of foreign agent to channels of evacuation from the organism (the intestine).

We realize that this suggestion can be disputed by our readers, and therefore should like to cite a paper by I. I. Metchnikoff [5]. Discussing the fate of goose erythrocytes injected into the abdominal cavity of a guinea pig, he noted that phagocytes together with phagocytized erythrocytes leave the peritoneal cavity and enter the blood. Unfortunately, the great scientist only stated that "phagocytes coming from the blood, ... when their function is over, ... return into the blood," and since then, never commented this fact in his reports. And we believe that it is this very phenomenon which would not allow him equalize the phagocytosis and inflammation and discriminate between the phagocyte functions in health and inflammatory reaction.

We believe that this fact is of crucial importance, because it indicates that the phagocyte function under conditions of noninflammatory reaction differs in principle from that in inflammation. In this connection, we should like to remind our readers that the return of phagocytes into circulation is due to the Theseus phenomenon [3].

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