On the Mechanisms of Bacteriopyknosis

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Morphological examination of primary foci several hours after intramuscular infection of rats with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Staphylococcus epidermidis* showed that bacteriopyknosis is associated with deficiency of compounds necessary for the microorganisms or excess of secreted microbial metabolites. Motility is a factor of virulence, since it inhibits bacteriopyknosis. The biological significance of divalent antibodies and agglutination provided by them probably lies in considerable stimulation of bacteriopyknosis in agglutinates. Formation of IgM also enhances bacteriopyknosis.

Key Words: *antibodies; infectious immunity; nonspecific resistance of the organism; bacterial motility*

Bacteriopyknosis — death of bacteria in the intracellular space followed by formation of detritus — has been recently described [1,2]. The mechanisms of this phenomenon have not been investigated. Bacteriopyknosis is a manifestation of nonspecific resistance of nonimmune organism. In immune organism, bacteriopyknosis is more intense, i.e., specific immunity potentiates nonspecific resistance, and this process involves antibodies. Enhanced bacteriopyknosis has been observed after passive immunization and inoculation of pathogenic microorganisms in 5% immune serum.

Bacteriopyknosis is a complement-independent process, as evidenced by morphological differences between complement-dependent lysis and formation of pyknomorphous detritus and the observation that complement-resistant strains of Gram-negative bacteria and complement-insensitive strains of Gram-positive bacteria are turned into pyknomorphous detritus. Bacteriopyknosis is not associated with phagocytosis. Death of microorganisms and formation of detritus occur in the intracellular space. In the immune organism with considerably increased bacteriopyknosis, detritus is often formed prior to mobilization of phagocytes into the focus of infection.

It should be remembered that most bacteria are not pathogenic, i.e., they cannot live in mammals. Although pathogenic microbes have adapted to life in other organisms, the adaptation is not perfect, judging from the facts that even in acute lethal infections the rate of microbial reproduction in the organism is lower than that in growth medium. Consequently, some factors prevent bacterial growth in all infections. In general terms, this prevention results from the inconsistency between bacterial metabolic requirements and growth conditions provided by infected organism. Presumably, bacterial growth is inhibited by the deficiency of some compounds, for example, iron [3,5-7]. The results of the present study are consistent with the hypothesis that inhibition of bacterial growth and bacteriopyknosis results from the deficiency of some compounds vital for bacteria or from the excess of bacterial metabolites.

MATERIALS AND METHODS

Experiments were performed on outbred rats weighing 170-300 g. The animals were infected by lavages of 24-h cultures of *Pseudomonas aeruginosa* (strain 453),

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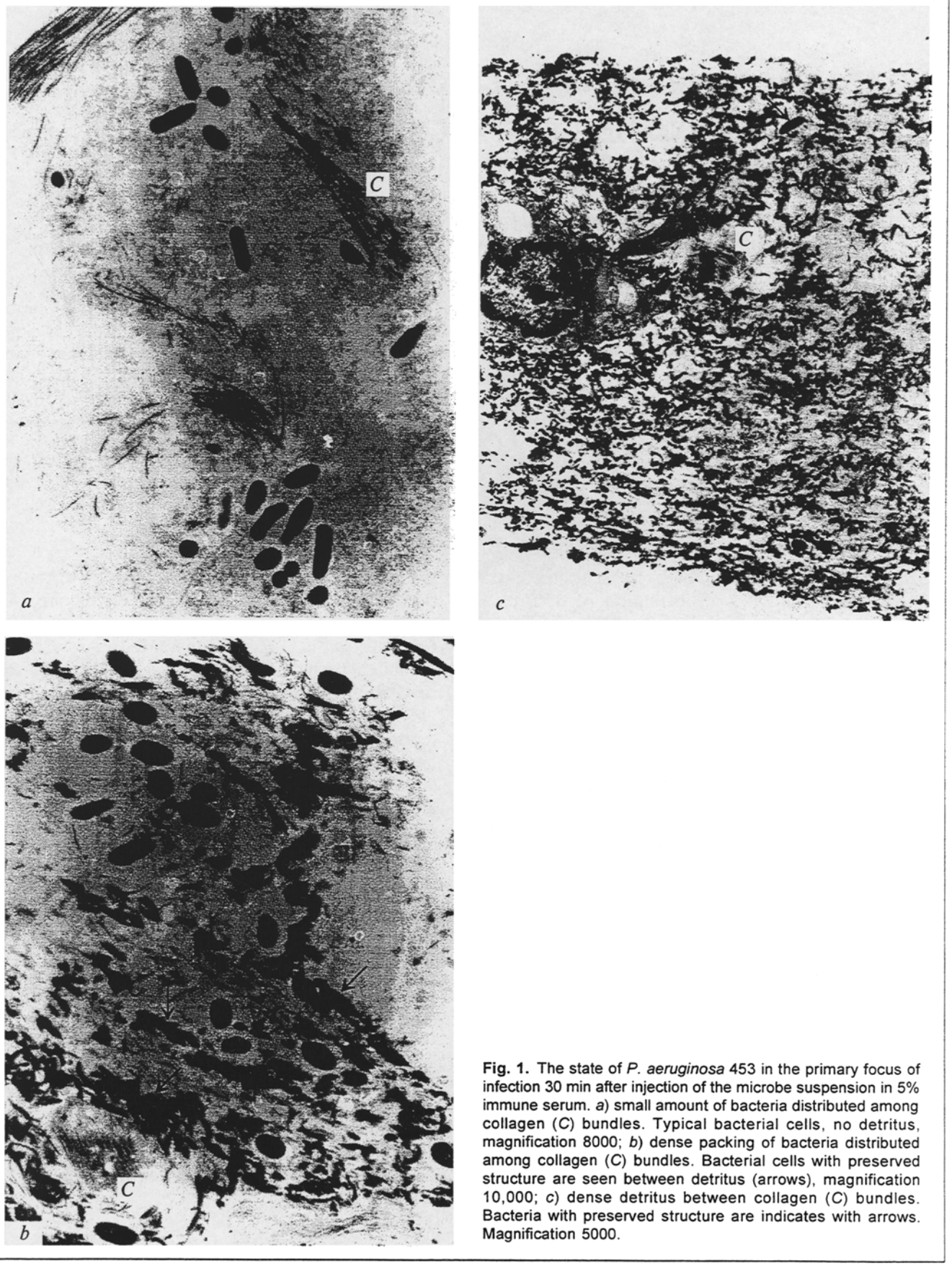

Fig. 1. The state of *P. aeruginosa* 453 in the primary focus of infection 30 min after injection of the microbe suspension in 5% immune serum. *a*) small amount of bacteria distributed among collagen (*C*) bundles. Typical bacterial cells, no detritus, magnification 8000; *b*) dense packing of bacteria distributed among collagen (*C*) bundles. Bacterial cells with preserved structure are seen between detritus (arrows), magnification 10,000; *c*) dense detritus between collagen (*C*) bundles. Bacteria with preserved structure are indicates with arrows. Magnification 5000.

TABLE 1. Body Mass and Mass of Exudate in the Infection Foci After Inoculation of Undiluted and Diluted Bacterial Suspensions

Suspension volume, ml	Microorganism	Number of bacteria in inoculate, $\times 10^8$	Mean body mass loss for 7 days, g	Mean mass of exudate, mg
P. aeruginosa 453 (n=10)	0.5	1.6	9.2	339
	0.05	1.6	12.4	364
P. aeruginosa 103 (n=15)	0.5	5	15	941
	0.05	5	8.5	596*
S. aureus 3377 (n=10)	0.5	5	9.3	309
	0.05	5	0.3*	154**

Note. Significance of differences (*p<0.01, **p<0.05) after infection with undiluted and diluted suspension.

Staphylococcus aureus (strains 3377 and 3384), or *Staphylococcus epidermidis* (strain 4017). The microorganisms were isolated from human burns and grown on agar. The lavage (0.3 ml) was injected in the gastrocnemius muscle. In the first experimental series, rats were sacrificed under ketamine anesthesia 30 min—22 h after infection. In the second series, the animals were infected with *P. aeruginosa* (strains 453 and 103) or *S. aureus* (strain 3377). Two groups of rats were infected with each strain: group 1 received 0.05 ml of undiluted suspension, while group 2 was injected with 0.5 ml of 10-fold diluted suspension. The disease severity was assessed by the body weight loss on days 3 and 7 after infection. The intensity of bacteriopyknosis was estimated by the mass of purulent-necrotic exudate formed in the site of injection 7 days after injection of microbial suspension. Previously, it was demonstrated that bacteriopyknosis inhibits phagocytosis and reduces the size of abscess [1,2].

The significance of differences was evaluated by Wilcoxon's test.

RESULTS

Electron and light microscopy revealed considerable amounts of detritus in the sites were the bacteria were packed and numerous bacteria with preserved structures at the sites were they were loosely distributed in the muscle (Fig. 1). This phenomenon can be explained as follows: in the organism the microbes lack some necessary compounds. Dense packing results in severe competition for these compounds; as a result, their concentration drops to the levels incompatible with microbes' life. The life span of loosely packed bacteria is longer due to less intense competition. There is another explanation: microbial growth is inhibited by microbial metabolites, the inhibition being more pronounced upon dense packing.

It should be emphasized that this state of bacteria was observed in most rats. Sometimes, microbes with preserved structure were found in densely packed clusters. Since objectivization of morphological observations is difficult, in the second series of experiments different densities of bacteria in the muscle were attained by infecting suspensions with different contents of microbial bodies. The results of these experiments are summarized in Table 1.

Statistical analysis showed that injection of undiluted or diluted suspension of the mobile microbe *P. aeruginosa* 453 caused a disease of a similar severity with a similar development of abscesses in the site of injection. The immobile microbes *P. aeruginosa* 103 and *S. aureus* 3377 induced a less severe process with lesser weight loss and smaller abscesses. Thus, the second series experiments confirmed the findings that dense packing resulting from injection of undiluted suspension suppresses the microbes and promotes bacteriopyknosis. Mobile microorganisms rapidly spread over the intracellular space. Therefore, the severity of infection is practically the same after injection of undiluted or diluted suspension. Thus, a reduction in bacteriopyknosis may at least partially account for the higher virulence of mobile bacteria [8].

Pyknosis of densely packed bacteria sheds more light on divalence of antibodies which lies in the basis of agglutination. The significance of this phenomenon remains unclear. It has been suggested that the ability of antibodies to agglutinate cells and bacteria *in vitro* plays no role in the immunity *in vivo* [4]. Our results argue with this suggestion. Agglutination *in vivo* provides dense packing of both mobile and immobile microbes, which serves as a potent protective factor and facilitates bacteriopyknosis (Fig. 1). Our hypothesis concerning the significance and mechanisms of bacteriopyknosis is supported by the fact that the immune response starts from the production of IgM. These antibodies do not bind to phagocytes but act as a potent agglutinating factor. Consequently, the earliest immune reaction is not aimed at stimulating phagocytes but at enhancing bacteriopyknosis.

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Effect of Direct Cardiac Massage on Peripheral Blood Erythrocytes

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The effect of direct cardiac massage on peripheral blood erythrocytes is examined. A significant increase in the count of transitory reversible forms of erythrocytes is observed after 1 h of direct cardiac massage without no significant changes in the hemoglobin content, hematocrit, and erythrocyte and reticulocyte counts. It is concluded that direct cardiac massage produces less severe damage to erythrocytes than other means of assisted circulation.

Key Words: *direct cardiac massage; erythrocyte morphology; scanning electron microscopy*

A relationship between erythrocyte damage and progressive microcirculatory disorders upon artificial [1,5,7] and assisted circulation [6] has been established. However, the information regarding the effect of direct cardiac massage (DCM) on peripheral blood erythrocytes is scanty.

Our goal was to examine the state of erythrocytes and their count upon DCM.

MATERIALS AND METHODS

Experiments were performed on 10 mongrel dogs (body weight 8-10 kg) maintained under the standard vivarium conditions.

After droperidol premedication, pentobarbital (30-40 mg/kg) and intubation anesthesia were provided, and neuroleptic analgesia was used during experiment.

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Routine thoraco- and pericardiotomy were performed, and the heat was exposed. Direct cardiac massage with an appropriate assistor was started after a 10-min heart arrest. The massage lasted 4 h. Hemodynamic parameters were monitored throughout the entire experimental period.

Blood was collected from the femoral vein immediately before, 1, 2, and 4 h after the start of DCM. Hematocrit, hemoglobin content, and erythrocyte and reticulocyte counts were determined by conventional methods. For identification of the lipoprotein complex blood smears were stained by the method of Barenbaum (1956), and light absorbance was measured in a LYuMAM-I2 microscope by one-beam photometry at 590 nm. Preparations for electron microscopy were processed by routine methods and studied in a REM-200 electron microscope [3,4].

The results were analyzed using Student's *t* test.

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