

The Role of the Functional State of Polymorphonuclear Leukocytes in the Pathogenesis of Experimental Sepsis

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One of the key problems in the pathogenesis of sepsis concerns the correlation between septicemia and pyosepticemia and the factors which determine the form of the infection process [4]. Disturbances of migration and of phagocytic and cytotoxic activity of the neutrophilic leukocytes are involved in the development of sepsis in surgical patients [1-3,8]. Bacterial pathogenic factors are able to block some kinds of polymorphonuclear leukocyte activity depending on the biological properties of the microorganism [6,7], indicating a possible role of specific features of the interaction between infection agent and phagocytic cells of the macroorganism in determining the form of the infection process. The present investigation aimed to reveal this role using a previously developed model of experimental sepsis [5].

MATERIALS AND METHODS

Experiments were carried out on 112 pubertal albino male rats weighing 180-200 g. Two sets of experiments were performed using 2 strains of *Pseudomonas aeruginosa* № 453 and № 103A from the culture museum of the L.A.Taresevich Research Institute for

the Standardization and Control of Medical and Biological Preparations. The infection process was induced by a single i.m. injection of 0.3 ml of a one-day culture suspension, containing 8×10^9 microbial bodies per ml in 10% CaCl_2 solution.

For the visual and histological study the animals were killed 2-14 days after infection; animals which had died were also examined. The histological sections of the internal organs were stained with hematoxylin and eosin and then with Azure II. Neutrophil mobility in donor human blood was registered in an experimental chamber on a "Jena-Luar" microscope thermostable. This chamber made it possible to record the free wandering movements of neutrophils under normal conditions and in the case of bacterial filtrate supply. The recording was performed in the dark field, $\times 6.3$. Slow shooting was performed with an interval of 1 min; the image was stored in a "Magiscan 2A" complex. The program followed the movement of the mass center of each neutrophil in the visual field. Information on the average rate of movement of the cell population was obtained using mathematical processing. Neutrophil cytotoxic activity in the primary infection focus was analyzed by the chemiluminescence method using an LKB-1252 luminometer. The results were expressed in mV/sec. The true results were the values at the homogenate reaction peak in the luminol buffered solution (pH 7.2-7.4) converted to 1 g of tissue.

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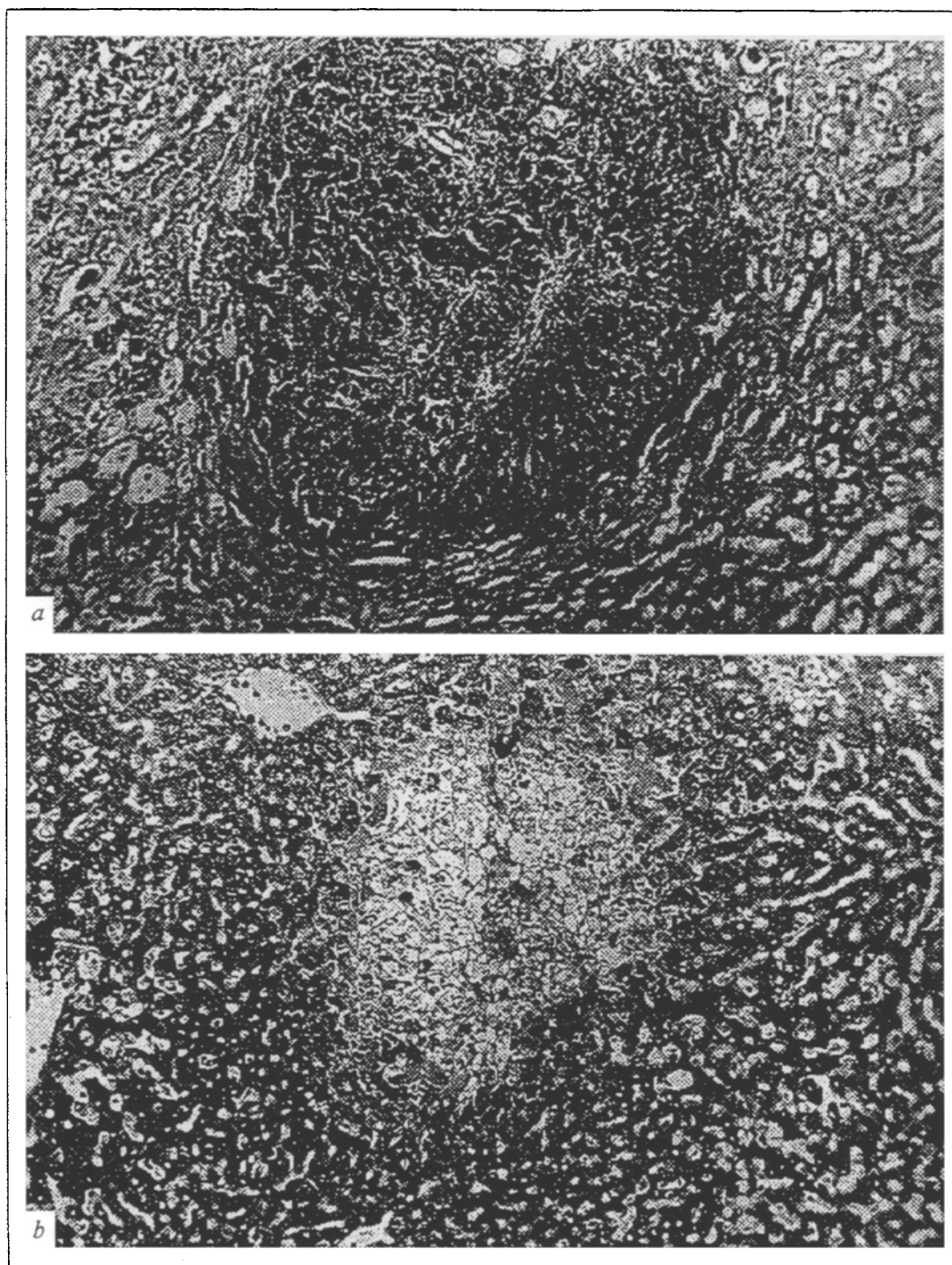


Fig. 1. Features of secondary septic foci in infection induced by different strains of *Pseudomonas aeruginosa*. a) necrotic focus without direct features of leukocyte infiltration in infection induced by strain № 103A; b) septic thrombovasculitis and formation of perivascular abscess (large number of neutrophilic leukocytes) in infection induced by strain № 453, stained with hematoxylin – eosin and Azure II. $\times 400$.

RESULTS

Clinicomorphological differences were established in the development of the septic processes induced by strain № 453 and by № 103A. There was 77% lethality in the first experimental set with strain № 453 as the infection agent. Foci of pyemic metastasis

were found in the kidneys of 60% of the animals (in 50% of those which died and in 100% of survivors). Kidney abscesses appeared as small necrotic foci infiltrated mainly with neutrophilic leukocytes, displaying septic vasculites with Gram-negative rod-shaped flora in the center (Fig. 1, a). A specific feature of this experimental set was the formation of

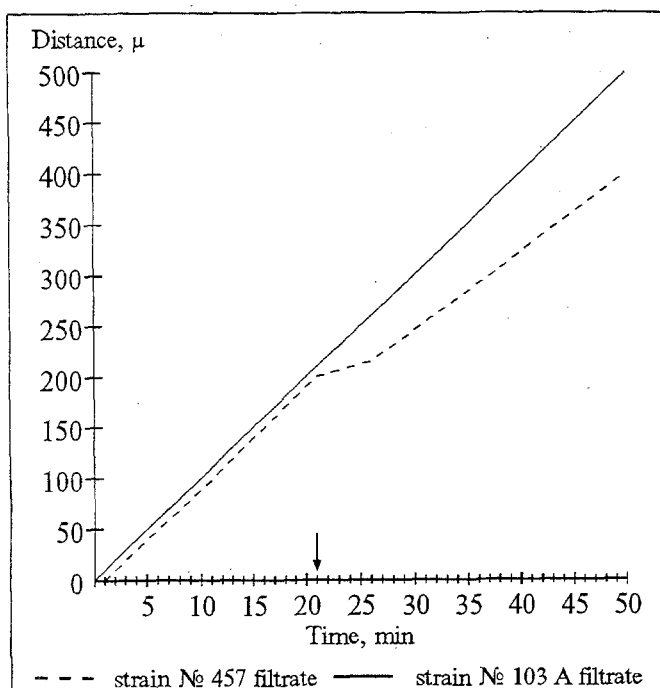


Fig. 2. Distance traveled by neutrophils as a function of time. Abscissa: time, min; ordinate: distance, μ . Curve 1) strain № 103 A filtrate; 2) strain № 453 filtrate. The arrow points to the moment of filtrate feed into the chamber.

a granular barrier with a pyogenic capsule around the pyemic foci, which reached 0.3-0.5 cm in diameter in 10-14 days. In the second experimental set (inoculation with strain № 103A) 92% of the sepsis exhibited no suppuration. There was significant impairment of the microcirculation in the parenchymatous organs, granular degeneration of cells, and foci of micronecroses without direct leukocyte infiltration (Fig. 1, b). The primary infection focus displayed hemorrhagic and suppurative reactions in both experimental sets, but were more pronounced in the infection induced by *Ps. aeruginosa* strain № 453.

The specific features of the migrational and bactericidal polymorphonuclear leukocyte activity induced by the biologically active agents of the *Ps. aeruginosa* strains were analyzed in parallel. *In vitro* the № 103A strain filtrate did not significantly affect the average rate of movement of the neutrophil population, while the 453 strain filtrate caused a pronounced reduction of cell mobility (Fig. 2).

The neutrophil cytotoxic activity varied depending on the *Ps. aeruginosa* strain used. A paraphase shift of the phagocytic activity levels was observed in the primary infection focus one day after infection (Fig. 3). An almost twofold decrease of phagocytic activity was revealed in the experiment with the 103A strain. In contrast, inoculation with the 453 strain enhanced neutrophil activity. The differences in the levels of neutrophil chemiluminescence markedly increased three days after inoculation. Strain № 453

infection was associated with a maximal increase of phagocyte activity, illustrating the state of hyperstimulation, while strain № 103A produced a low level of activity.

The findings indicate that the septic process, expressed in different morphological forms, is attended by dissimilar changes in polymorphonuclear leukocyte functional state. Strain № 453, which mainly causes the foci of suppuration, induces an increase of the cytotoxic activity of neutrophils and reduces their mobility. This mobility reduction, which can be observed for high chemoattractant concentrations, possibly governs the accretion of the cells in the infection foci. There is an evident correlation between a low neutrophil functional activity, probably arising from the action of strain 103A pathogenic factors, notably exotoxin A [6,7], and the virtual absence of pyometastases, which are peculiar to the septicemic form of the disease. The described features of the polynuclear cells determine in many respects the nature of formation of both the primary and the metastatic foci. Strain № 453 produced a suppurative focus (with a necrotic component when the concentration of the microorganisms was high), while the primary focus and micronecrotization of the parenchymatous organs induced by strain № 103A expressed a reduction, often to the point of disappearance, of the leukocyte reaction.

The so-called "areactive" necroses or angio-necroses, as well as the visualization of microorganisms without a corresponding reaction, usually con-

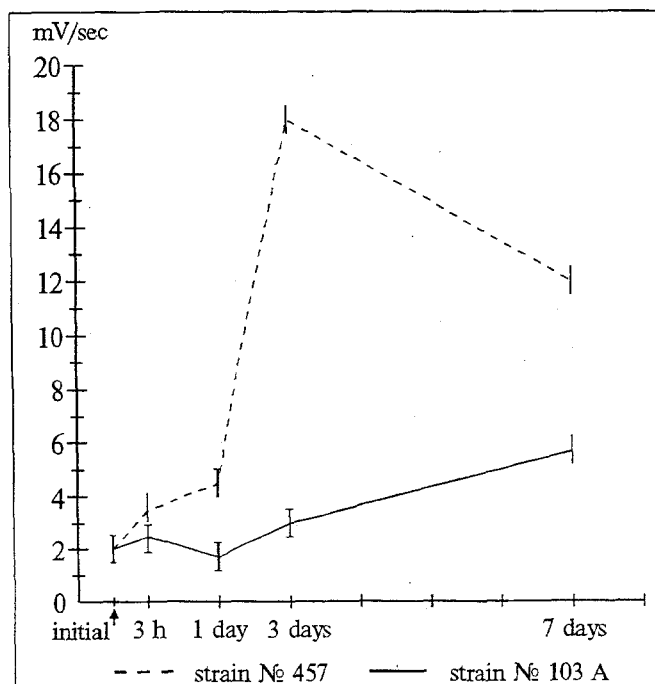


Fig. 3. Neutrophil chemiluminescence dynamics in primary focus during generalization of *Pseudomonas aeruginosa*-induced infection. Curve 1) strain № 453; 2) strain № 103 A.

sidered to be a manifestation of "post-mortem microbe dissemination in the tissues", cannot be verified with certainty without taking into account the species and the biological properties of the microorganism.

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Muscle Spindle Ultrastructural Features in a Replanted Limb

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Muscle spindles are an important structure of muscle tissue participating in motor function coordination. Reports on the role of various components of spindles in the preservation of receptor intactness and receptor activity recovery in disease are contradictory [2-6]. An entire array of problems concerning the structure of the spindle capsule and its role in receptor viability preservation, reinnervation possibility, intercellular including axon-Schwann cell interactions, and specific features of the microcirculatory bed require a thorough ultrastructural analysis. The aim of the present study was to examine the ultrastructure of muscle spindle components in a replanted limb.

MATERIALS AND METHODS

Replantation of the right hind limb was carried out in white male rats weighing 270-320 g. The surgical technique has been previously described [1]. Specific

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features of the model used were the absence of prolonged thermal ischemia (blood flow in the main vessels was intact during surgery) and preclusion of the effect of muscle injury on the course of regenerative processes: muscle below the tissue crossing site were examined. *M. ext. hallucis longus* was used to its small volume. Since the intrafusal fiber distribution is irregular, examinations of serial sections were carried out. The material for the investigation was collected under ether anesthesia one month after surgery (5 animals). The muscle fragments were fixed in cooled formol-sucrose solution and then in 1% OsO_4 buffered solution and embedded in araldite. Ultrathin sections were examined under a JEM-7A electron microscope.

RESULTS

Muscle spindles were found to survive one month after replantation. Some of them were rather well preserved. The majority showed signs of destructive degenerative changes of varying degree involving all the receptor components.