THE ROLE OF INDIVIDUAL FEATURES OF MICROORGANISMS IN THE PATHOGENESIS OF SEPSIS DUE TO *Pseudomonas aeruginosa* (EXPERIMENTAL STUDY)

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Relations between the microorganism and host, and the role and importance of each of them in its onset and development still remains the central problem in the pathogenesis of sepsis even today [2]. As many observations have shown, in most cases the agent of sepsis is one of a limited group of microorganisms, consisting mainly of representatives of a conditionally pathogenic flora: *Staphylococcus, Pseudomonas aeruginosa, Escherichia coli, Proteus,* and so on. This fact is evidence that besides altered reactivity of the host [1], an important role in the development of the septic course of a disease is played also by the biological properties of the microorganisms. The investigation described below was devoted to a study of this role, using an experimental model of sepsis developed by the present writers.

EXPERIMENTAL METHOD

Experiments were carried out on 132 mature noninbred male albino rats weighing 160-200 g. There were four series of experiments in which three strains of *Ps. aeruginosa* (17a, 453, and 103) from the Cultures Museum of the L. A. Tarasevich Research Institute of Standardization and Control of Medical and Biological Preparations were used. The infectious process was induced by giving a single intramuscular injection of 0.3 ml of a suspension of a 24-h culture of the microorganisms in 10% $CaCl_2$ solution containing $8 \cdot 10^9$ microbial cells in 1 ml. Strain 17a was used in two modifications: "recent" and "old," having undergone passage in artificial media for more than 30 days. Enzyme-biochemical properties of each strain were studied: hemolysin, lecithinase, urease, gelatinase, the presence of pigments, and the character of growth on media were studied. For visual, histologic, and electron-microscopic investigation animals were killed between 2 and 14 days after infection; animals which died also were autopsied. The primary focus and internal organs (liver, kidneys, lungs, spleen, lymph nodes, adrenals) were fixed in 10% neutral formalin solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, azure and eosin, and toluidine blue. Material for electron-microscopic investigation was fixed in 1% glutaraldehyde and 2% OsO_4 solution, and embedded in a mixture of Epon 812 and Araldtite resins. Ultrathin sections were studied in the JEM-100B microscopes, under magnifications of between 20,000 and 45,000.

EXPERIMENTAL RESULTS

The study of the enzyme-biochemical parameters revealed a clear difference between all the strains of *Pseudomonas aeruginosa* studied (Table 1).

Table 2 gives data on the course and outcome of *Ps. aeruginosa* sepicopyemia, which show that different strains of bacteria cause septicemia and sepicopyemia with the same frequency, and that animals with septicopyemia survived longer.

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TABLE 1. Enzymic and Biochemical Properties of Different Strains of *Ps. aeruginosa*

Strain	Hemolysin	Lecithinase	Gelatinase	Urease	Pigment	Character of growth on media
17a "recent"	—	—	—	—	+—	Growth with surface film on liquid media
17a "old"	+	+—	—	—	—	Growth with surface film on liquid media
453	—	+	+	+	+—	Grows less well on artificial media
103	+	+—	—	—	—	Colonies on solid medium with indistinct borders, on liquid media growth without a surface film

Legend. —) Negative reaction, +—) weak positive reaction, +) positive reaction.

TABLE 2. Outcome of Sepsis Induced by Different Strains of *Ps. aeruginosa*

Series of experiment	Strain of Ps. aeruginosa	Total number of animals in series	Number of animals which died				Number of animals surviving or killed			
			with signs of septicemia		with signs of pyemia		with signs of septicemia		with signs of pyemia	
			abs.	%	abs.	%	abs.	%		%
I	17a "recent"	40	20	83	4	17	4	25	12	75
II	17a "old"	30	11	74	4	26	3	20	12	80
III	453	48	18	49	19	51	0	0	11	100
IV	103	25	17	100	0	0	5	63	3	37

In the experiments of series I (injection of strain 17a "recent") 60% of the animals died during the experiment. In 17% of dying and 75% of surviving and killed rats septicopyemia was observed with abscesses of different sizes and with multiple hemorrhages into the lung parenchyma. The time course of development of the abscesses showed the appearance initially of small foci of necrosis, from 0.1 to 0.15 cm in diameter, in the lung tissue with a small number of predominantly disintegrating neutrophilic leukocytes. Small septic, necrotic areas of vasculitis were observed, containing an abundant gram-negative bacillary microflora. On the 8th-10th day the metastatic foci were increased in size to 1.2-1.5 cm, the necrotic tissue was abundantly infiltrated by neutrophilic leukocytes, and active proliferation of fibroblasts was observed at the periphery of the abscesses.

The mortality in the experiments of series II (injection of strain 17a "old") was 50%. In 55% of these animals (26% of those which died and 80% of those which survived and were killed) pyemic foci were observed in the kidneys, and were discovered microscopically as early as 2-3 days after infection, and larger foci from 0.1 to 0.8 cm in diameter were recorded visually on the 8th-14th days. In the late stages of observation, multiple micronecroses were found in the liver of individual animals, and microorganisms were detected in them electron-microscopically. In this period marked proliferation of fibroblasts, forming a loose, stratified membrane, with microorganisms in its substance, was observed around the pyemic foci in the kidneys.

In the experiments of series III (receiving an injection of strain 453) the mortality was 77%. Pyemic foci were found exclusively in the kidneys in 60% of animals (in 50% of those which died and 100% of those which survived and were killed). The diameter of the abscesses was 0.3-1.5 cm. A characteristic feature of this series of experiments was the formation of a granulation barrier around the pyemic foci on the 10th-14th day with the formation of a pyogenic capsule.

In the experiments of series IV (injection of strain 103) the mortality was 65% and in 92% of cases the morphological course of the sepsis was of the septicemic type. In individual animals small foci up to 0.1 cm in diameter appeared in the spleen and kidneys of individual animals.

Marked disturbances of the microcirculation with cloudy-swelling degeneration of the cells were observed in the parenchymatous organs. Infection of the animals with different strains of *Ps. aeruginosa* was accompanied by a varied response of the immunocompetent organs. The septic process induced by strains 17a and 453 was manifested as a sharp increase (two-threefold) in size of the lymph nodes (inguinal) and spleen. Hyperplasia of the reactive centers of the follicles was observed on account of both T- and B-dependent zones. In animals infected with strain 103, no enlargement of the lymph nodes or spleen was observed. By contrast with the other strains tested, microorganisms of this type gave rise to an infectious process accompanied by hypoplasia of the reactive centers of the lymph nodes and lymphatic follicles of the spleen.

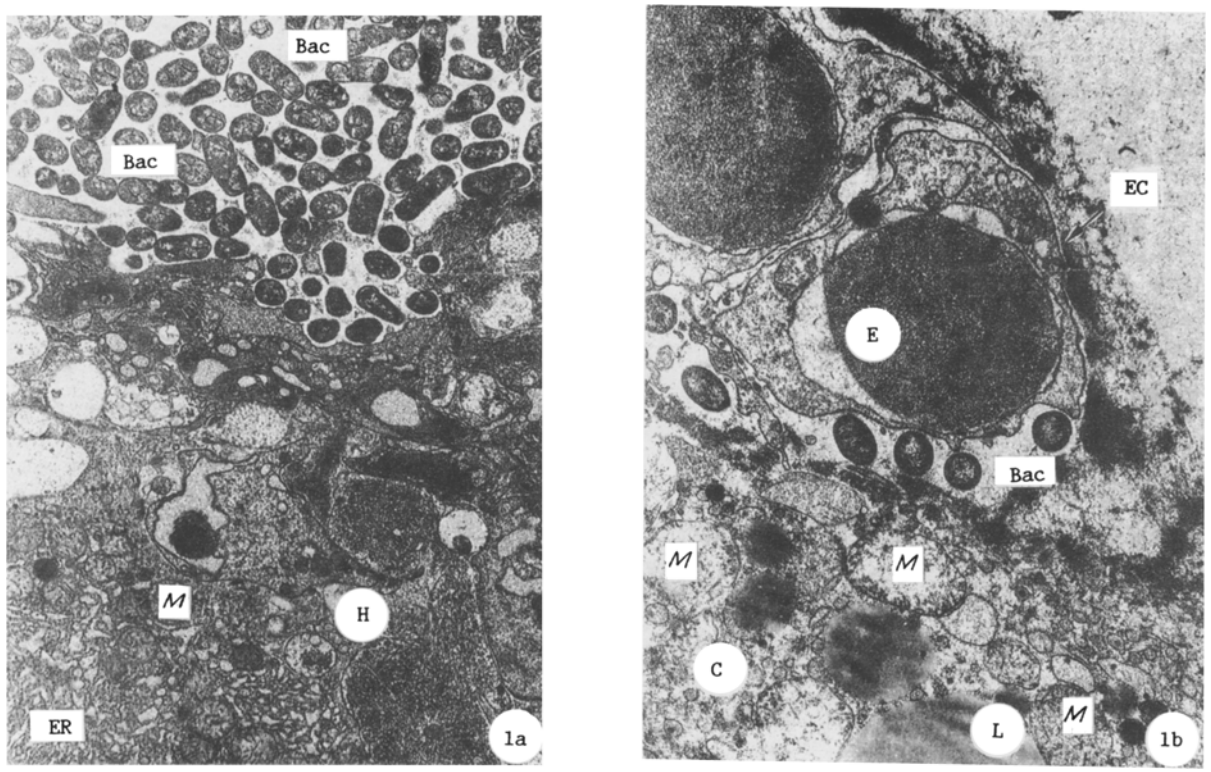

Fig. 1. Sepsis caused by injection of *Ps. aeruginosa,* strain 17a "old" on 2nd day of experiment. a) Accumulation of many bacterial cells (BAC) in intercellular space in liver (h — hepatocyte), where numerous mitochondria (M) and endoplasmic reticulum (ER) can be seen. 18,000×; b) single bacterial cells (BAC) in widened pericapillary space. E) Erythrocyte in capillary lumen. EC) Endothelial cell. C) Cell containing swollen mitochondria (M) and lysosomes (L). 20,000×.

Electron-microscopic investigation of bacterial invasion showed that in all cases penetration of the microorganisms was observed in areas of marked microcirculatory disturbances, manifested as capillary stasis and sludging of erythrocytes. Destruction of the endothelial lining and of the basal layer was observed in these sites, with the escape of microorganisms into the pericapillary space, where accumulation and multiplication of bacteria were observed, forming a "depot" of infection. At the same time marked destruction and lysis of the surrounding cells could be seen (Fig. 1a).

Investigation of the primary focus in all experiments revealed a hemorrhagic and suppurative response, more marked after injection of strain 453 of *Ps. aeruginosa.* The absence of signs of demarcation of the focus in the early stages led to an increase in its size to 1.5-2 cm and to the development of a superficial ulcer.

Histological and electron-microscopic investigations revealed multiple foci of necrotic vasculitis, septic emboli in the blood vessels consisting of Gram-negative bacteria, and the development of perifocal areas of necrosis with signs of destruction of the leukocytes infiltrating them, in primary and metastatic foci. After 12-14 days a tendency was noted for the inflammatory focus to be bounded by the formation of granulation barrier (Fig. 1b).

The results of this investigation show that the septic process induced by strains of *Ps. aeruginosa* with different biological properties differ in their course. Differences are observed in the development of the suppurative-necrotic process in the primary focus, the level of mortality, the form of sepsis (septicemia or septicopyemia), differences in the dynamics of growth of the secondary pyemic foci, and manifestations of different organotropism of strains of the same species of bacteria, and differences in the response of the immunocompetent organs. Correlation also was found between the appearance of individual features of the septic process and "aging" of the strain, accompanied by changes in its biological properties. Correlation between activity of the process in the primary focus and generalization of the infection also was evident. Clearly it is not just the properties of the host, but also the biological properties and pathogenicity factors of the microorganisms [3-7] that determine the particular type of course of sepsis.

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PREVENTION OF OPERATION WOUND SEPSIS BY CO_2 LASER RADIATION: EXPERIMENTAL STUDY IN VIVO

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Operation wound sepsis in emergency and cold abdominal surgery is due mainly to contamination of the subcutaneous cellular tissue by an infectious agent brought in during the operation from the infected peritoneal cavity or from the lumen of hollow organs. For instance, appendectomy for acute destructive appendicitis is complicated by operation wound sepsis in 26-94% of cases [4, 6, 7, 9]. In complicated cholecystitis removal of the gall bladder is accompanied by operation wound sepsis in every fourth patient [8, 10].

The degree of infection of the wound determines the frequency of sepsis: in clean operations sepsis arises in 2.2-5.5% of cases, but in operations with considerable bacterial contamination, in between 31.0 and 55.5% [1, 12].

There is as yet no method of preventing operation wound sepsis that is absolutely effective. By the use of known methods it is possible to secure more or less complete elimination of microorganisms from the wound surface. However, some microorganisms penetrate into the subcutaneous cellular tissue and become inaccessible for general methods of treatment [5]. A jet of physiological saline, pulsing under high pressure, has a more deeply penetrating action [11].

Considering the ability of radiation of a CO_2 laser to exert a thermal action not only on superficial, but also on deeper layers of tissues, we undertook experimental and clinical investigations of laser prevention of operation wound sepsis. The optimal conditions for laser scanning were 15-100 W/cm^2/sec. The efficacy of prevention of operation wound sepsis in acute destructive appendicitis was 100% [2, 3].

The aim of this investigation was to establish the theoretical basis for laser prevention of sepsis of contaminated wounds and to determine the parameters of effective action by lasers on microorganisms without any damaging action on the host's tissues.

EXPERIMENTAL METHOD

A production line model of the "Skal'pel'-1" laser surgical apparatus with continuous emission and with an output power from the light guide of 20 ± 2 W, was used for the investigation. Operations on the animals were performed under general ether

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