ULTRASTRUCTURAL ANALYSIS OF CHANGES IN THE LUNG PARENCHYMA IN THE INITIAL STAGE OF EXPERIMENTAL SEPSIS

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Pseudomonas pyocyanea is a conditional pathogen. Nevertheless, it is nowadays a frequent agent causing sepsis [1-3].

There have been many studies of the pathological anatomy of sepsis due to *Ps. pyocyanea* [2, 4, 5]. However, the fine structural changes in the early stages of sepsis have not yet been studied. This is a problem of great importance to the clarification of our ideas on the pathogenesis of sepsis and the dynamics of its development.

The object of this investigation was to study the initial ultrastructural changes in the lung parenchyma as the first barrier to the hematogenous spread of bacteria in experimental *Ps. pyocyanea* sepsis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g. Under ether anesthesia a gauze swab, soaked in a suspension of a culture of *Ps. pyocyanea,* isolated from a burned patient and adapted to rats by repeated passage, was implanted subcutaneously in the middle third of the dorsal region. After implantation of 3×10^{10} microbial cells, suspended in 1 ml of isotonic sodium chloride solution, 75% of the animals developed bacterial sepsis. The criteria of sepsis were mortality, and the results of qualitative and quantitative bacteriological, histological, and immunoluminescence investigations of organs. The early stages in the development of sepsis were fixed conventionally at 10 and 24 h after infection. In a separate bacteriological investigation of different zones of the lungs 24 h after infection, bacteria were detected in two-thirds of cases. Positive seedings were obtained most frequently from the posterior third of the left lung. This zone of lung tissue was subjected to light-optical and electron-microscopic investigation. Altogether 27 animals were used: 20 rats 10 h and seven rats 24 h after infection. The lung tissue of five rats, into which the corresponding dose of heat-killed culture of *Ps. pyocyanea* was implanted, also was studied.

EXPERIMENTAL RESULTS

The lungs 10 h after infection were aerated, pink in color, and in some animals showed indistinctly outlined foci of congestion. Histological investigation showed that the venules and capillaries of the lung tissue were dilated and congested with blood, and stasis of erythrocytes was present in many of them. Pavementing of the leukocytes was observed in the lumen of these blood vessels. Around the venules and, to a lesser degree, around the arterioles cuffs of histiocytes and macrophages appeared. The walls of the arterioles and venules were swollen and edematous, and signs of separation into layers and loosening of their fibrous structure were observed. The alveolar septa, especially those around the circumference of the arterioles and venules showing the greatest changes were thickened on account of swelling of all the cells composing them, of edema, and also of an increase in the number of histiocytes and macrophages in them. In histological sections stained by Goldman's method many polymorphonuclear leukocytes (PNL) were visible in the alveolar septa. The latter cells were extremely unevenly distributed: In some alveolar septa concentrations

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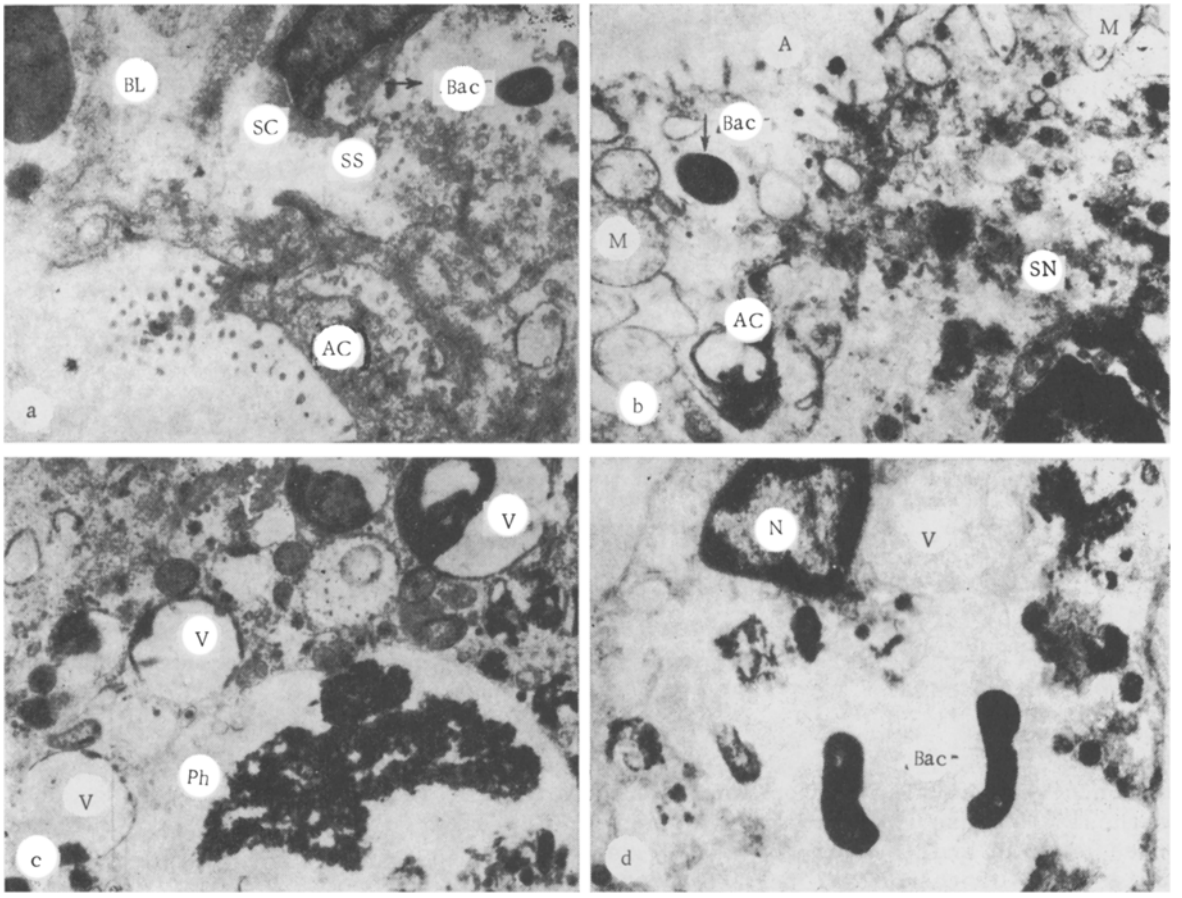

Fig. 1. Changes in alveolar wall cells after subcutaneous injection of *Ps. pyocyanea*. a) Single bacteria (Bac) lie in the widened septal space (SS), uneven loosening of basal layer (BL) in alveolar (AC) and endothelial cells 10 h after infection. SC) Septal cell; b) swelling of mitochondria (M), vacuolation of cytoplasm of alveolar cells (AC) 24 h after infection. A) Alveolus, SN) septal necrosis; c) increase in number of vacuoles (V) and phagolysosomes (Ph) in an alveolar macrophage; d) bacteria present in translucent zone of cytoplasm of alveolar macrophage. N) Nucleus. 14,000 ×.

of PNL were observed, in others they were uniformly distributed. PNL were most constantly found in the subpleural regions of lung tissue. Considering a characteristic feature of PNL (microphages), namely their ability to exhibit chemotaxis relative to bacteria, there is reason to suppose that their distribution is a unique marker of the presence of bacteria in the alveolar septa. Thickening of the alveolar septa was combined with their closer approximation, so that the lumen of the alveoli was reduced or even disappeared. In the lungs only 10 h after infection, multiple foci of so-called dyscirculatory atelectasis thus appeared. In the interstices of the lungs and in the alveolar lumen diapedesis of erythrocytes could be seen on a small scale. Single bacteria were found in histological preparations only during the study of semithin sections. They were located in the perivascular space or in the alveolar lumen. No bacteria were found in the cytoplasm of macrophages. Consequently, 10 h after infection the changes in the lung tissue could be described as a picture of interstitial pneumonia in response to the harmful action of bacteria penetrating by the hematogenous route from the primary focus of infection.

The lungs 24 h after infection showed little change as before. Their congestion was a little increased. Very small hemorrhagic foci could be distinguished. Histological investigation showed that the changes in the lungs were similar to those at the previous time but more pronounced. The increase in diapedesis of the erythrocytes was particularly marked: Areas of hemorrhagic infiltration of the alveolar septa and alveoli filled with blood could be seen.

On electron-microscopic investigation of the alveolocytes and endothelial cells of the capillaries 10 h after infection the following changes were observed: the capillary lumen was widened and packed with erythrocytes, the number of pinocytotic vesicles in the endotheliocytes was increased, and swelling of the mitochondria with translucency of their matrix were present. The basal layer of the endothelial cells in some areas was loose in structure and in places it did not cover the plasma membrane. Considerable changes in the intracellular organelles and vacuolation of the cytoplasm were observed in the septal cells. Vacuolation of the cytoplasm and an increase in the number of vacuoles containing myelin inclusions also were observed in the alveolocytes. Single bacteria could be seen in the intercellular space among concentrations of small granules and vacuoles (Fig. 1a).

Investigation of the ultrastructure of the rats' lungs 24 h after infection revealed marked congestion and stasis in the blood capillaries. In some areas of the endothelial lining disturbances and rupture of the cytoplasmic membranes with separation of the intercellular junctions were present. Through the openings thus formed erythrocytes and bacteria could penetrate into the intercellular space and accumulate in the altered macrophages and septal cells. The basal layer of the endothelial cells was uneven, and in some places it was loose in structure and separated from the plasma membrane. Heaps of erythrocytes and residues of destroyed cells were present in the capillary lumen. In the adjacent septal cells changes in the nuclear membrane and destruction of organelles were observed (Fig. 1b).

An increase in the number of phagolysosomes with various inclusions, including with bacteria, and also large vacuoles with finely granular masses could be seen in the alveolar macrophages (Fig. 1c). In some alveolar macrophages nearly the whole space of the cytoplasm was filled with large vacuoles containing bacteria; often the walls of these vacuoles were destroyed and translucent spaces were formed in their place. Bacteria were often seen in these translucent zones in the form of rectangular bodies, whose cytoplasm was bounded by a

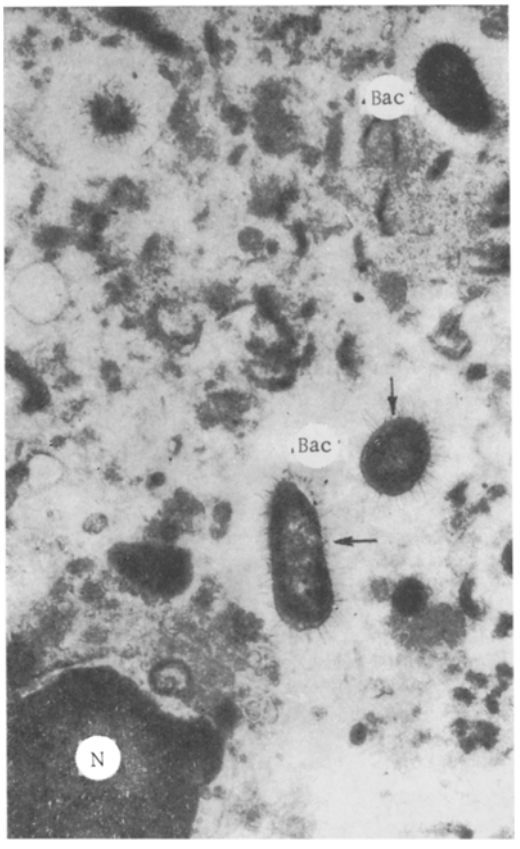

Fig. 2. Destruction of organelles, homogenization of chromatin of the nucleus (N), and presence of bacteria (Bac) in translucent areas of cytoplasm of a septal cell 24 h after infection (16,000 ×).

single membrane. The granular cytoplasm of the bacteria contained translucent areas with thin strands of DNA nucleotides. On their outer membrane micropili could be clearly seen (Fig. 1d). These formations are often regarded as evidence of marked pathogenicity of bacteria.

Destruction of membranes and translucency of the matrix were observed in the septal cells together with fragmentation of the tubules of the endoplasmic reticulum, and disappearance of ribosomes; numerous small granules and vesicles packed the cytoplasm of the cells unevenly. In the middle of the translucent areas of cytoplasm there were bacteria with conspicuous micropili on their outer membrane. Homogenization of chromatin granules could be seen in the nuclei. The outer layer of the nuclear membrane was unevenly separated, and tiny granules and vacuoles were present in the widened perinuclear space (Fig. 2).

After injection of killed bacteria the changes described above in the ultrastructure of the alveolar and septal cells were not observed.

After subcutaneous implantation of a culture of living *Ps. pyocyanea* cells into rats considerable changes in the blood capillaries, septal cells, and alveolocytes were thus observed 10 h after infection, and single bacteria could be seen in the intercellular space of the alveolar wall. The degree of the destructive changes after 24 h showed an increase almost to irreversibility. This was manifested primarily in the septal cells and alveolar macrophages: lysis of the cell membranes, homogenization of the chromatin, and destruction and lysis of the intracellular organelles took place in these cells, and ultimately led to their necrosis. The cause of the changes in these cells was most probably the direct action of the bacteria and their toxins on the intracellular organelles. This is shown by their absence after injection of a killed culture of *Ps. pyocyanea* and the constant presence of living bacteria with micropili in the region of these changes. Entry of bacteria into the lung from the primary septic focus induces disturbance of the microcirculation in the lung with the development of stasis in the capillaries and with destructive changes in their endothelial cells. From that time favorable conditions are created for bacteria to penetrate into the septal spaces of the alveolar wall, and then into the septal alveolar cells. After lysis of the cell membranes and death of the cells, bacteria liberated from the destroyed phagolysosomes, together with cell debris, enter the alveolar cavity or the intercellular space, from which they can penetrate through gaps in the endothelium into the capillary blood stream, leading to progression of the pathological changes in the lung tissue.

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