PATHWAYS OF LUNG INVASION BY *Pseudomonas aeruginosa* IN BURNS

B. V. Vtyurin and R. I. Kaem

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The problem of postoperative complications due to the development of pyogenic infection assumes particular importance in the treatment of burns with clinical manifestations of septicemia and of so-called bacterial shock. The study of these states is difficult because the role of the conditionally pathogenic microflora (***Escherichia coli***, *Pseudomonas aeruginosa*, etc.) is considerably enhanced in the development of surgical sepsis. While noting the important role of staphylococci as one of the principal agents of hospital infections, it must, however, be emphasized that during recent years the relative importance of the Gram-negative bacillary microflora among agents of hospital infections has considerably increased [6, 8]. The most important member of this group is now *P. aeruginosa*, which has begun to be isolated much more frequently as the agent of hospital infections in different clinical forms [12, 13, 16]. In severe burns inflammatory changes in the lungs and microbial dissemination in lung tissue are observed almost constantly [4, 5, 9, 10, 15].

The appearance of inflammatory processes of this kind in the lungs and microbial invasion are evidently due to a generalized lesion of the nonspecific barrier mechanisms, arising in patients with severe thermal burns; this is, moreover, an important pathogenetic factor in the development of early bacteriemia, bacterial toxemia, and sepsis [2]. The direct effect of *P. aeruginosa* and its metabolic products on cell structure of the alveolar epithelium and capillary endothelium is also of great importance for the development of these processes. However, the intimate mechanisms of bacterial penetration from the lung alveoli through the air–blood barrier into the bloodstream have been inadequately studied.

The aim of this investigation was to study the morphologic dynamics of invasion of the lungs by Gram-negative microflora and its interaction with the various components of the air–blood barrier and to identify the mechanism of penetration of bacteria into the blood on a model of thermal injury.

EXPERIMENTAL METHOD

Experiments were carried out on 22 noninbred male rats weighing 200-250 g, on which III-IV degree flame burns covering an area of 18-20% of the body surface were inflicted. Under superficial ether anesthesia, 4 h after burning, the animals were given an intratracheal injection of 1 ml of a 4-million suspension of a 24-h culture of *P. aeruginosa* (strain No. 87) in an aqueous solution of polyvinyl alcohol. Pieces of the lungs for electron microscopy were fixed in glutaraldehyde, postfixed with OsO_4, and embedded in a mixture of Araldite and Epon 812. Ultrathin sections were stained with lead citrate and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

On the second day after injection of the culture of *P. aeruginosa* the most conspicuous feature in the lungs of the burned rats was a marked hemorrhagic reaction. The alveolar septum was edematous, with separation of the various structures forming it and with marked capillary stasis. Type II cells of the alveolar epithelium were swollen, rounded, and contained an increased number of myelin inclusions, in some places with destruction of the basal cell membrane. In some parts of the alveolar wall, **destructive** changes were found, involving not only a group of alveolocytes, but also adjacent areas of the septal space and capillary wall. In zones of destruction many bacterial bodies were characteristically present, mainly

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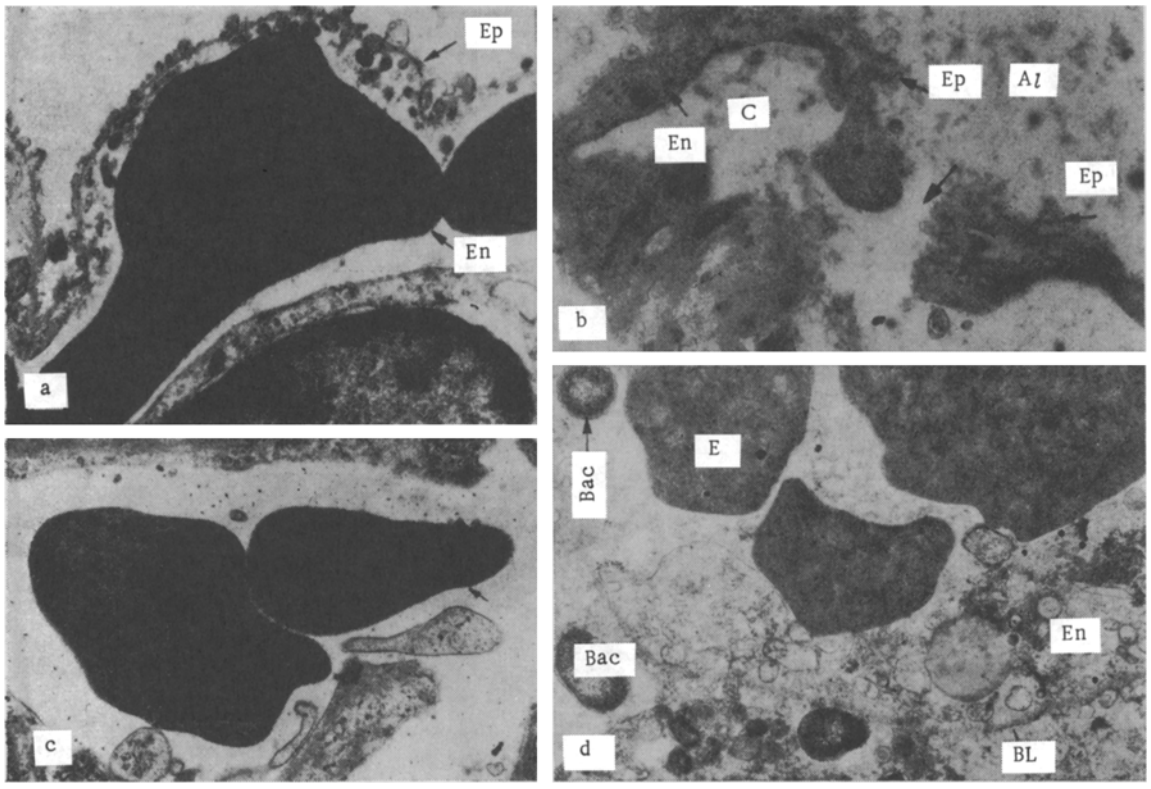

Fig. 1. Changes in alveolar wall of lungs after injection of *P. aeruginosa* suspension into animals with experimental burns. a) Lysis of cell membranes of alveolocytes (Al) and endotheliocytes (En), sludging of erythrocytes (E) in capillary; b) formation of hole (arrows) as a result of destruction of alveolar epithelium (Ep) and separation of processes of endothelial cells (En) in alveolar wall; c) escape of erythrocytes (E) into septal space; d) marked destruction of cells of alveolar wall, partial lysis of basal layer (BL) in zone of localization of bacteria (Bac); C) capillary lumen. 26,000 ×.

lying freely among macrophages, erythrocytes, and various homogeneous granules. Vacuoles of different sizes containing several bacteria, with no evidence of lysis or destruction of microbial bodies, were also observed. In those areas of lung tissue where the alveolar wall was formed by thin processes of type-I alveolocytes, and the capillary wall was covered with processes of endothelial cells, considerable areas of destruction were frequently observed: These were manifested by lysis and destruction of the plasma membranes of the cells and by the formation of vacuoles of different shapes and tiny granules at the site of processes of the alveolocytes and endothelial cells on the basal layer. The basal layer between them had the appearance of a thin granular strip, which in some places became extremely thin and could not be followed. Signs of sludging of erythrocytes were observed in the capillaries (Fig. 1a). Primary destructive changes were observed mainly in type-I alveolocytes. Changes in the plasma membranes led to destruction of the cell processes, denudation of the basal membrane, and separation of junctions between endothelial cells (Fig. 1b). As a result of separation of the cell junctions, the intercellular spaces became widened, and through them blood cells and plasma penetrated into the septal space or directly into the lumen of the alveoli (Fig. 1c). Disturbance of the integrity of cells of the alveolar wall was particularly marked in areas where bacteria were present. In these areas not only lysis of the processes of the alveolar and endothelial cells was present, but also destruction of the central part of these cells with evidence of karyolysis and karyopycnosis. Vacuolation of the cytoplasm, fragmentation of the nuclei, and destruction of the mitochondria often led to complete destruction and death of the cells (Fig. 1d).

Penetration of bacteria (*P. aeruginosa*) into the lumen of the alveoli can be fully explained: Due to the experimental conditions, after intratracheal injection of the suspension of *P. aeruginosa*, bacteria together with mucus penetrate into the alveoli and, becoming mixed with the polyvinyl alcohol, they are held up there for a certain time. The following pathways

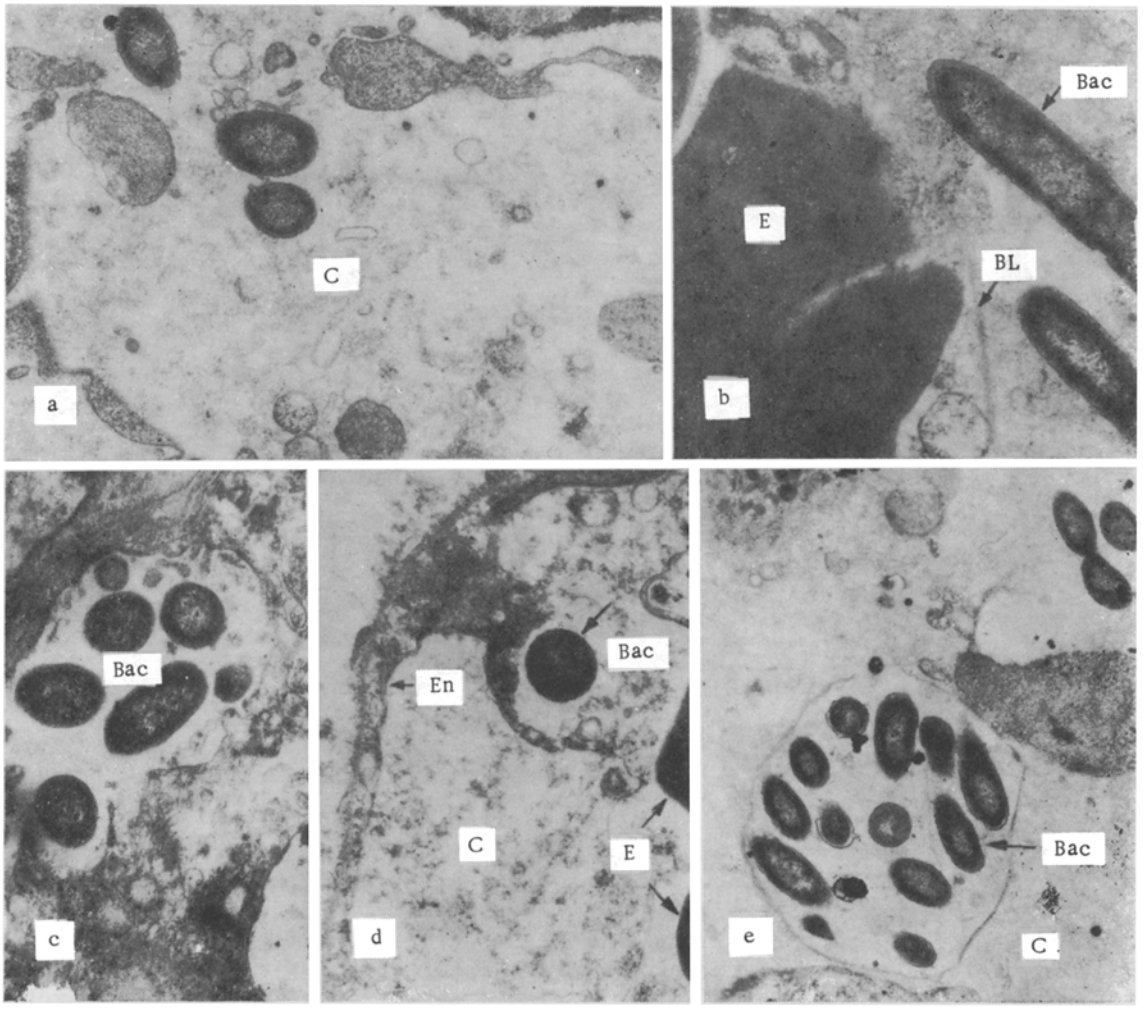

Fig. 2. Penetration of bacteria into bloodstream in areas of changes in air–blood barrier. a) Bacteria (Bac) present in region of separation of endothelial cell processes. C) Capillary lumen. b) Penetration of bacteria (Bac) into cytoplasm of lysed alveolocyte; c) localization of bacteria (Bac) in vacuole of endothelial cell (En); d) disturbance of integrity of endothelial plasma membranes (En) and escape of bacteria (Bac) into capillary lumen (C); e) solitary bacteria and bacteria included in vacuole (Bac) in capillary lumen (C) 28,000 ×.

of bacterial invasion from the alveoli into the capillary lumen and bloodstream can be postulated. First, as a result of disturbance of the integrity of the alveolar epithelium and basal membrane, and also of separation of the cell junctions and the appearance of wide intercellular spaces, bacteria can pass through the "holes" thus formed, penetrate into the bloodstream, and spread throughout the body with the blood flow. However, it must be noted that disturbance of cell junctions and rupture of the epithelial processes were observed comparatively infrequently together with destruction of the basal layer. The electron-microscopic picture much more frequently observed was one of invasion of microbial bodies from the septal space, in the presence of destructive changes in the endothelial cells with destruction of the capillary endothelial lining, through holes in the endothelium (Fig. 2a). Another possible pathway was that in which bacteria, in direct contact with cells of the alveolar wall, secrete toxic substances and gradually dissolve the plasma membrane of the cell, infiltrating into the cytoplasm of the alveolocytes. Often destruction of the basal layer was followed immediately by destruction of the endothelial cells of the capillary (Fig. 2b). In regions of the alveolar wall where changes of this kind were present, direct invasion of bacterial cells from the alveolar lumen into the capillary lumen also was possible.

Penetration of bacteria into the bloodstream through their ingestion by endotheliocytes, which are known to perform a phagocytic function, also was observed. As a result of incomplete phagocytosis, because of dystrophic changes in the endotheliocytes, bacteria contained

in the phagolysosomes as a rule preserved their structure and sometimes showed no evidence of destruction. Moreover, the number of ribosomes, lysosomes, and mitochondria was greatly reduced in endotheliocytes which contained vacuoles with bacteria in their cytoplasm, and the lamellar complex was almost invisible. In such areas translucency of the cytoplasm and destruction of cell membranes were observed. In such cases bacteria could be seen to escape from the endotheliocyte through its disturbed cell membrane. Escape of whole vacuoles with bacteria from a destroyed cell into the bloodstream of a capillary also was observed, after which they evidently circulated in the bloodstream and thus created a depot of infection in the blood (Fig. 2c, d, e).

When these data are analyzed it should be noted that dystrophic changes characteristic of severe burn trauma and disturbances of permeability of the alveolar wall, inhibition of phagocytosis, and depression of the leukocyte reaction favor the development of *P. aeruginosa* pneumonia after introduction of a subthreshold dose of the infecting agent into the animals. Under these circumstances irreversible changes take place in cells of the alveolar epithelium and capillary endothelium, and are most conspicuous in areas where bacteria are present and producing lysis of cell membranes, separation of cell junctions, and the formation of "holes" through which the bacteria penetrate into the bloodstream. There is evidence in the literature that the pre-existing capillary fenestrae, and also the "interendothelial spaces" and "transcellular canals" appearing under pathological conditions, constantly change their shape and size, and constitute a highly dynamic ultrastructural system. This evidently explains the phenomenon of continuous diapedesis and escape of microorganisms into the pericapillary space and bloodstream, observed in the present experiments, although open spaces and fenestrae were not always observed. This phenomenon is probably due to contraction and closure of most of the spaces and fenestrae during fixation.

Destructive changes in the alveolocytes are due mainly to the direct action of *P. aeruginosa*. Evidence of this is given by the presence of a halo of translucent, destroyed cytoplasm around the bacteria.

Disturbance of the permeability of the air—blood barrier and increased detachment of alveolar cells from the basal layer lead to "denudation" of the latter: It becomes discontinuous, loses its uniformity, and becomes less resistant, thus providing favorable conditions for invasion of the septal space of the lungs by bacteria and their accumulation in the intercellular lacunae, with the formation of distinctive "depots" of infection [1, 14]. Depression of the protective properties of the air—blood barrier, on the one hand, and the direct action of microbial toxins on cell membranes, on the other hand, evidently bring about invasion of the cell cytoplasm by bacteria. The presence of unchanged bacteria in phagolysosomes (the endocytobiosis phenomenon) leads to marked changes in the intracellular organelles, or even to fragmentation of the nucleus [2, 11]. Invasiveness of bacteria, as we know, is one criterion of their pathogenicity, reflecting their ability to multiply in cells and to overcome their protective adaptations [7, 8]. Pictures of incomplete phagocytosis which we observed (the presence of groups of unchanged bacteria in the phagosomes) indicate that after lysis of the cell membranes, cells of the tissue—blood barriers die, and the freely lying bacteria and unchanged bacteria included in vacuoles and phagosomes, on entering the bloodstream, give rise to bacteriemia and ultimately to the development of septicemia.

The process of bacterial invasion is complex and is linked with inadequate intralysosomal activity of the degeneratively changed cells, disturbance of phagocytosis, and the possible occurrence of endocytobiosis with multiplication of bacteria in degeneratively changed cells.

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