Ultrastructural Observations in an Alcoholic Patient with Post-Surgical Pseudomonas Infection

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ABSTRACT

Gram negative bacteria were seen in the peripheral blood and within the neutrophils of a patient with bacteremic shock secondary to *Pseudomonas aeruginosa* infection. By electron microscopy, bacteria were present either in vacuoles or in the cytoplasm of neutrophils. When seen in the cytoplasm, they were surrounded by amorphous material which most probably represented fused lysosomal granules. In both cases, the microorganisms appeared morphologically normal. The presumption is that there was a pre-existing defect of neutrophilic lysosomal formation or function. These findings indicate the importance of studying neutrophil morphology and function in patients with persistent infections.

Introduction

In a study of 3500 routine blood smears, Zieve et al found the leukocytes of only 122 patients had vacuolization. Of these, 119 had bacterial infections. Neutrophilic vacuolization is therefore a cardinal feature of septicemia and one which is immediately available to the clinician, whereas results of blood culture may require 24 hours or more.

Neutrophils may phagocytize bacteria, which are then digested by cytoplasmic granules. The hydrolytic enzymes and peroxidase of neutrophils are known to destroy the bacteria. If digestion is impaired either by defective processes within the cell or by overwhelming infection, bactericidal activity may be defective. The bacteria can, then, be seen microscopically in the cytoplasm.

Patients with gram negative septicemia classically show leukocytosis, toxic granulation, and vacuolization of the neutrophils in the peripheral blood. In a three year period at Kings County Hospital, where approximately 400 complete blood counts (CBC) are done daily, intra-leukocytic gram negative organisms have
not previously been found in a peripheral blood smear.

This report describes a postoperative patient who developed overwhelming *Pseudomonas aeruginosa* infection and was treated with appropriate antibiotic therapy as judged by sensitivity test. Nevertheless, the patient developed shock, and at that time the neutrophils of the peripheral blood smear showed marked vacuolization and intracytoplasmic bacteria.

**Case Report**

A 34-year-old black man was admitted to Kings County Hospital with epigastric pain, nausea, and vomiting of one day's duration. He gave a history of chronic alcoholism. Laboratory data included hemoglobin, 14.6 gms; hematocrit 44 percent; white blood cell count 9.5 per cu mm with normal differential; blood urea nitrogen, 8 mg per dl; serum creatinine, 1 mg per dl; serum glucose, 178 mg per dl; and serum calcium, 7.8 mg per dl. Physical examination revealed marked epigastric tenderness, and a diagnosis of acute pancreatitis was made. Peritoneal and pleural taps showed bloody fluid.

On the sixth hospital day, the hematocrit fell to 26 percent and an exploratory laparotomy was performed. During this procedure, acute hemorrhagic pancreatitis was found and a distal pancreatectomy, splenectomy, cholecystectomy, and gastrectomy were performed. Following surgery, the patient's temperature rose to 105° F. The sputum culture revealed *Pseudomonas aeruginosa* sensitive to gentamycin and cephalothin. Although the patient was promptly treated with the antibiotics, the following days were "stormy." The fever persisted, and *Pseudomonas aeruginosa* grew in the drainage from the abdominal wound. The patient was again explored by laparotomy because of bleeding into the peritoneal cavity and upper gastrointestinal bleeding. *Pseudomonas aeruginosa* was cultured on several different occasions from endotracheal aspirate and from the purulent discharge of the abdominal surgical wound, but never from the blood.

On the 34th hospital day, a subphrenic abscess was discovered from which *Pseudomonas aeruginosa* was cultured. The blood revealed leukocytosis between 20,000 and 30,000 per cmm. Although antibiotic treatment of gentamycin and cephalothin continued, the fever persisted. *Pseudomonas aeruginosa* was again cultured from the purulent discharge and again it was sensitive to gentamycin and cephalothin.

On the 52nd hospital day, there was a leukocytosis of 16,000 per cmm, but a differential was not done. Despite all treatment the patient continued to deteriorate. On the 53rd hospital day, the blood pressure suddenly fell to 80/40, the patient developed oliguria, and the temperature fell to 95° F. Blood was drawn for a complete blood count. Soon after this, the patient went into deep coma and expired. Permission for autopsy was not obtained.

**Material and Methods**

Blood obtained from the patient on the 53rd hospital day was immediately brought to the hematology laboratory for a complete blood count. Hemoglobin was 3.8 g per dl, hematocrit was 11.9, and the white cell count was 16,700 per cmm. Smears were stained with Wright's and Gram stain. The differential count revealed 92 percent polymorphs, 5 percent lymphocytes, 2 percent monocytes, and 1 percent band. Approximately 400 μl of blood was left for ultrastructural studies. The buffy coat was collected and prepared for electron microscopy according to the methods previously described.3,4

**Results**

**Light Microscopy**

The examination of the peripheral blood revealed that 42 percent of the neutrophils contained Gram negative organisms (figure 1). In some neutrophils, the bacteria were seen in division. This phenomenon was also seen extracellularly. The neutrophils contained toxic granulations and/or vacuoles. In the majority of neutrophils the toxic granulations were few, large and unevenly distributed. The number of vacuoles varied from cell to cell; often there was only a single large vacuole, empty or containing bacteria (figure 2). Occasionally, many small vacuoles were seen within polymorphonuclear leukocytes which often lacked granules. The lymphocytes, monocytes, red cells, and the few platelets were normal.

**Electron Microscopy**

The nuclei of the neutrophils had a normal arrangement of the chromatin and heterochromatin. The cytoplasm had
scanty ribosomes and occasional vesicles of the rough endoplasmic reticulum. The amount of glycogen was also normal. The number of granules varied. In some neutrophils, they were abundant; in some, especially in those having many vacuoles, they were sparse or absent. The vacuoles were of various size and often contained bacteria. Many neutrophils presented one bacterium in the cytoplasm but occasion-
Figure 4. Neutrophil with phagocytized bacterium. Vacuole is not apparent, but a dense, amorphous material (arrow) is seen. N = nucleus; B = bacterium; Ly = lysosomes. Uranyl acetate; × 26,368.

Figure 5. Bacterium pinched in the center and surrounded by dense, amorphous material which is partially bounded by a discontinuous membrane (arrow). B = bacterium. Uranyl acetate; × 55,965.
ally two or three organisms were also seen. Rarely neutrophils were seen in phagocytic activity actively engulfing a bacterium (figure 3). The great majority of bacteria seen in the cytoplasmic vacuoles appeared unaltered.

The microorganisms were also seen in the cytoplasm where the vacuoles were not apparent (figure 4). In these cases, the bacteria were surrounded by amorphous material which most probably represents fused lysosomal granules as revealed by the presence of residual lysosomal membrane or residual membranes of the vacuole. Even in these instances, the bacterial body appeared intact. Of particular interest was the presence of dividing bacteria within the cytoplasm of few neutrophils (figure 5). Bacteria were also seen extracellularly, and no difference was noticed between phagocytized and non-phagocytized microorganisms.

Discussion

The post-surgical course in this patient was characterized by a severe intractable *Pseudomonas aeruginosa* infection, and the final clinical events were typical of septicemia. In general, infection of wounds by *Pseudomonas aeruginosa* is not unusual, but involvement of the respiratory tract is uncommon; in debilitated persons, fatal sepsis may occur. In our case, bacterial growth not only occurred at the site of the surgical wound but also in the respiratory and urinary tract. Terminally, *Pseudomonas aeruginosa* invaded the blood stream to such an extent that bacteria were seen both in plasma and within leukocytes.

The poor response of this patient to antibiotic treatment might have been the result of many factors. For example, in chronic alcoholics decreased marrow granulocyte reserve has been suggested. In our case, the sustained leukocytosis made it unlikely that there was any decrease in marrow granulocyte reserve. Although there is no good correlation between alcohol ingestion and infections, it is well known that alcohol ingestion may lead to abnormal granulocyte mobilization.

There are few microscopic descriptions of microorganisms within circulating leukocytes, and the intraleukocytic demonstration of bacteria in division is an unusual finding. The present authors are not aware of electron microscopy being performed in similar conditions.

The mechanism of cell division of gram negative bacteria including *Pseudomonas aeruginosa* has been a matter of debate. Steed and Murray mentioned that division occurs by constriction. In our case, the cells appear pinched in the middle, and septa are not seen. Later, however, Gilleland and Murray emphasized that although technically it is difficult to demonstrate, septation is the mode of cell division in gram negative bacteria, and constriction is a fixation artifact.

In the case just described, evidence is presented by microscopy that while the neutrophils retained their phagocytic activity, their function was altered because they had lost their bactericidal activity. In fact, by electron microscopy, all bacteria including those in division appeared unaltered within the cytoplasmic vacuoles and resembled those seen extracellularly. Similarly, in the cytoplasm itself, being surrounded by amorphous material of probable lysosomal origin, the bacteria were morphologically well preserved. This, perhaps, was due to the fact that the lysosomal enzymes had not yet initiated the process of bactericidal digestion or most probably were defective. The presumption is that there was a pre-existing defect of neutrophilic lysosomal formation or function.

In view of findings in cases like this one, it may be possible to elucidate the antibiotic unresponsiveness much earlier by electron microscopic studies of the peripheral blood as well as with studies to determine the function of the neutrophils.
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