Phagocytosis of Monocytes and Platelets in a Neutropenic Infant with Septicemia

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ABSTRACT

The case is described of a low birth weight infant who developed severe neutropenia, thrombocytopenia and monocytosis in the course of staphylococcal septicemia. Light microscopy examination of the blood revealed bacteria inside the monocytes. On electron microscopy, the monocytes were degranulated and distorted by the presence of large vacuoles containing bacteria. While most of the bacteria were intact, some showed capsular dissolution suggesting the effect of lysosomes. Rarely, the bacteria were also noted within the polymorphonuclear leukocytes and platelets.

Based on the finding of electron microscopy in this case, the phagocytic function of the monocytes, neutrophils and possibly the platelets was intact. In view of the fatal outcome, despite the previous findings, it is suggested that granulocyte transfusion be considered in low birth weight infants with persistent sepsis.

Introduction

Neutropenia is a reduction in the circulating neutrophils below 1500 per cmm. Infection and adverse drug reaction are commonest of the causes of neutropenia. A relative or absolute monocytosis is often observed during the recovery phase of neutropenia. In such cases, the monocytes may assume the phagocytic function which is carried out primarily by the neutrophils.

This case report describes a newborn with staphylococcal septicemia who developed severe neutropenia, monocytosis, and thrombocytopenia during the course of her infection. The examination of the peripheral blood by light microscopy revealed striking phagocytic activity by the monocytes. By electron microscopy, the coccii were not only noticed in the monocytes but also were seen in the few neutrophils that were found and in the platelets.
Case Report

A 850 g female infant was born to a 22 year old primigrávida by normal spontaneous vaginal delivery at 32 weeks gestation. The infant started on i.v. antibiotics with the clinical impression of sepsis. The blood culture grew Staphylococcus epidermidis and the antibiotic therapy was changed to i.v. methicillin. The infant developed hyperbilirubinemia and anemia at 48 hours of age, for which she received phototherapy and transfusion of packed red cells. On the third day of life, a post transfusion blood count revealed a hemoglobin of 13.6 g per dl, white blood cells (WBC) 8,700 per cmm and adequate platelets on the smears. The differential count revealed polymorphonuclear leukocytes of 69 percent; band cells, 2 percent; lymphocytes, 26 percent; and monocytes, 3 percent. The infant was given digitalis for congestive heart failure, owing to patent ductus arteriosus, and also received total parenteral nutrition. A repeat hemogram at the age of one week revealed a hemoglobin of 12.3 g per dl, hematocrit 37, WBC 5,900 with 56 percent polymorphonuclear cells; 7 percent band cells, 25 percent lymphocytes, 9 percent monocytes, and 3 percent eosinophils. The platelet count was 140,000 per cmm.

Further clinical course of the infant was complicated by pulmonary hemorrhage and anorxia leading to death on the 18th day of life. A blood count done a few hours prior to death showed a hemoglobin of 13.4 g per dl, WBC 4,400 per cmm with polys, 2 percent; lymphocytes, 76 percent; and monocytes, 22 percent. The platelet count was 40,000 per cmm. Premortem blood culture again grew Staphylococcus epidermidis.

Autopsy confirmed the diagnosis of patent ductus arteriosus and also showed bronchopneumonia with vasculitis, necrosis, and pulmonary abscesses. The bone marrow was hypercellular with a marked decrease in the number of late myeloid cells but with an increase in the number of early myeloid cells. There was also a decrease in the erythroid precursors and megakaryocytes.

Material and Methods

After complete blood count, films of the peripheral blood taken shortly before death were stained with Wright’s and gram stains. Two additional five ml aliquots of peripheral blood from the patient were used for electron microscoby. They were placed into two test tubes containing ethylenediaminetetraacetic acid (EDTA). One tube was kept in vertical position at 40° C for one hour. The plasma layer rich in white blood cells was aspirated and placed into a second test tube and centrifuged at 2000 rpm for 10 minutes in a Clay-Adams centrifuge. The plasma-supernatant was discarded, and sodium-cacodylate buffered glutaraldehyde, pH 7.4, was added for fixation.

The second test tube of blood was used to obtain a platelet pellet. The whole blood was then centrifuged for 12 minutes on an International refrigerated centrifuge. The platelet rich plasma was aspirated and placed into another test tube and immediately centrifuged for six minutes at 1000 rpm to eliminate the remaining erythrocytes and white blood cells. The supernatant containing only platelets was again aspirated and spun at 2000 rpm for an additional 10 minutes. The supernatant was then discarded and fixative added over the pellet. All the pellets were processed for electron microscopic examination.

Results

Light Microscopy

As described previously, monocytosis was observed on smears of the peripheral blood drawn a few hours prior to death. Majority of the monocytes showed cytoplasm vacuoles which contained one or more cocci that were gram positive. The presence of such vacuoles produced distortion of the cytoplasm and the nucleus. Thick sections from Epon blocks of the leukocyte pellet revealed that monocytes and few neutrophils contained vacuoles with one to six cocci. The lymphoid cell appeared morphologically normal. Examination of thick sections of the platelet fraction revealed minimal red cell contamination and no leukocytes.

Electron Microscopy

On electron microscopy, the monocytes revealed their characteristic morphology with convoluted nuclei with occasional inconspicuous nucleoli, the usual cytoplasmic organelles, and projections. However, some monocytes contained vacuoles...
with ribosomes and with one to five cocci inside the vacuoles (figure 1). The vacuolar membranes in some cells were not intact. It is possible that some cytoplasmic ribosomes might have entered through the interrupted vacuolar membranes or that the vacuolar ribosomes were of bacterial origin. The cocci were surrounded by
amorphous material and, for the most part, had an intact capsule. Only occasionally was the capsule of the bacteria not discernible, probably because of digestion from lysosomal enzymes (figure 2). Although neutrophils were rarely seen by electron microscopy, most of them had phagocytised cocci and showed obvious distortion of the cell owing to the presence of large vacuoles.

EXAMINATION OF THE PLATELET FRACTION

The majority of the platelets were morphologically normal. Approximately 20 percent of them had large vacuoles, and only nine platelets were seen containing cocci within the vacuoles (figure 3). Some vacuoles with cocci in them also contained amorphous material and various glycogen particles. There was a suggestion of phagocytic activity by the platelets, as shown by the presence of cytoplasmic projections close to the cocci (figure 4). The presence of cocci was variable: in some platelets there were only a few while in others the cocci were abundant. Similarly, the number of vacuoles was different from platelet to platelet. Some platelets, because of distortion produced by the vacuoles, were hardly recognized as platelets.

Discussion

Bacterial infections in the newborn are often associated with an increase in absolute band count and an increase in the ratio of bands to segmented neutrophils. However, neutropenia has also been de-
described in some infants with bacterial infections.  
In our case, while the neutrophil count was essentially normal for age at the outset, severe neutropenia, monocytoisis, and thrombocytopenia were noted after two weeks of antibiotic therapy. Thus methicillin or the underlying bacterial infection could have contributed to the hematologic abnormality. The present authors believe that the infection was the probable cause of neutropenia and thrombocytopenia.

In our patient, light and electron microscopy showed evidence of phagocytosis by the monocytes and the few neutrophils that were seen. Although there was a profound neutropenia, the phagocytic function of the neutrophils appeared to be intact, based on the findings of intracytoplasmic cocci seen with electron microscopy. In this case, monocytoisis was probably a compensatory mechanism in the phase of neutropenia. These monocytes exhibited active phagocytosis and lysis of the bacteria by their lysosomal enzymes.

Another interesting observation in this patient was the presence of cocci in the platelets. It has been shown that platelets can phagocytize latex particles and engulf bacteria. White considered this phenomenon as sequestration of the foreign material within the canalicular systems of the platelets. The platelet phagocytosis or sequestration in this case was probably an in vivo phenomenon as EDTA has been shown to prevent phagocytosis and clumping of both leukocytes and platelets. In the present case, it appears that some cocci within the platelets were lysed, probably by the platelet lysosomes.

In conclusion, in the present case there was observed a striking phagocytic activity by the rare polymorphonuclear leukocytes by the monocytes in the peripheral blood, and possibly by the platelets. This indicates that the defense mechanism of phagocytosis was intact. Therefore, it is suggested that granulocytes transfusion be considered in infants with sepsis who do not respond to appropriate antibiotic therapy. It is quite possible that in a case such
as this the transfused granulocytes having normal phagocytic function would act synergistically with antibiotic treatment.

References