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HUMAN INFECTION FROM AN UNIDENTIFIED ERYTHROCYTE-ASSOCIATED BACTERIUM

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Abstract A 49-year-old splenectomized man had an infection from an unidentified, gram-positive, rod-shaped bacterium that adhered to the majority of his peripheral-blood erythrocytes. On transmission electron microscopy, the bacterium was seen to be extra-erythrocytic and was 0.2 μm wide by 1.0 to 1.7 μm long. It possessed a thick, granular cell wall, a trilamellar membrane external to the cell wall and prominent mesosomes. Attempts to cultivate the organ-

ism in vitro or to duplicate the patient's disease in splenectomized animals were unsuccessful. The patient's response suggested that the bacterium was susceptible to cell-wall-active antibiotics and to chloramphenicol but not to tetracycline. This bacterium may be the cause of other chronic, fever-producing, multisystem diseases of unknown origin. (N Engl J Med 301:897-900, 1979)

INFECTION agents that parasitize erythrocytes are rarely acquired by human beings in the continental United States. Only the protozoa causing babesiosis and malaria are accepted as human red-cell pathogens.^{1,2} In this report we describe an illness in which an organism involved the erythrocytes of a splenectomized, previously healthy, lifelong resident of the eastern shore of Virginia. Although the organism could not be isolated in vitro, its morphology and ultrastructure suggested that it was a unique gram-positive bacterium.

CASE REPORT

This 49-year-old man was in his usual state of health until approximately six months before he was hospitalized. His only previous hospitalization had been in 1947 when, after an automobile accident, his spleen ruptured and was removed. Since 1972 he had been seeing physicians intermittently for arthralgias without arthritis, and adequate relief had been achieved with a variety of analgesics. In November, 1975, he noted worsening of arthralgias and the onset of malaise, easy fatigability, anorexia, chills, drenching night sweats, evening temperatures to 39.4°C and purpuric lesions on his feet. By April, 1976, he was unable to work, had lost 9 kg in weight and was admitted to a local hospital. After six days of hospitalization failed to yield a diagnosis, the patient was transferred to the Medical College of Virginia hospitals for further evaluation.

The patient was a lifelong resident of the eastern shore of Virginia, had been a maintenance worker on the Chesapeake Bay bridge-tunnel for 10 years and had driven a milk truck for 17 years before that. He occasionally had part-time jobs as a carpenter and house painter. None of his co-workers, friends or family had had a

similar illness. He neither owned animals nor had any close contact with them; he had not traveled away from his immediate environment for 30 years, and he ate no unusual or poorly cooked foods. Although he frequently received insect bites, he could not remember specific tick, fly or mite bites.

On initial examination at the Medical College of Virginia hospitals, he appeared to be chronically ill and in no acute distress, with a temperature of 38.9°C and otherwise normal vital signs. The only abnormal findings on physical examination were generalized lymphadenopathy, purpuric, nodular, tender lesions on both feet that impaired ambulation, and petechiae on both lower extremities below the knee. Results on the admission chest x-ray film and electrocardiogram were normal, as were serum electrolytes, creatinine, bilirubin, glutamic oxalic transferase, alkaline phosphatase and results on urinalysis. Hemoglobin was 11.0 g per deciliter (6.8 mmol per liter), reticulocyte count 3.4 per cent (0.034), Westergren sedimentation rate 54 mm per hour (uncorrected; 54 arbitrary units), platelet count 388,000 (388×10^9 per liter) and white-cell count 12,000 cells per cubic millimeter (12.0×10^9 per liter) with 88 per cent neutrophils, 10 per cent lymphocytes and 2 per cent eosinophils. A peripheral-blood smear showed Howell-Jolly bodies and polychromasia consistent with splenectomy; red-cell morphology was otherwise normal. Small bacterium-like structures were seen on Wright's stain in association with 60 to 80 per cent of the patient's red cells (Fig. 1). The structures stained poorly with Gram's stain, but they appeared to be gram positive. Biopsy of the skin lesions revealed no vasculitis; collections of erythrocytes and mononuclear cells were present in the dermis. The organisms seen in the peripheral blood were also found in the skin-biopsy specimen in large numbers, predominantly outside the cells. The bone marrow was also heavily infiltrated with these organisms.

Because the organisms were gram positive and the patient reported a history of urticarial reaction to penicillin, therapy was begun with vancomycin hydrochloride, 750 mg given intravenously every 12 hours. The fever was gone within 36 hours, and the patient felt markedly improved. After four days of therapy the lesions on his feet were no longer painful, his arthralgias had disappeared, and only occasional organisms were identified in a peripheral-blood smear. After 16 days of vancomycin therapy severe phlebitis necessitated a change of antibiotics to cefazolin, 500 mg given intravenously every eight hours. Antibiotics were discontinued after 30 days of treatment. At the end of therapy the patient's hemoglobin was still 10.0 g per deciliter (6.4 mmol per liter) and his

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Figure 1. Smear of the Patient's Peripheral Blood Obtained on Admission, Showing Small Organisms (Arrows) Associated with Most Erythrocytes (Wright's stain, $\times 1500$).

sedimentation rate was 56 mm per hour, but he felt completely well and had gained 4.5 kg; no more organisms were demonstrable in his peripheral blood. The following studies were negative or within normal limits during his hospitalization: lupus erythematosus-cell preparation, antinuclear antibody, direct and indirect Coombs test, the Venereal Disease Research Laboratories test, latex agglutination for rheumatoid factor and complement components C3 and C4. Serologic tests for the following infections were negative or within normal limits on admission and showed no rise after four weeks: tularemia, leptospirosis, brucellosis, toxoplasmosis, Q fever, psittacosis, epidemic typhus, Rocky Mountain spotted fever, malaria (*Plasmodium vivax*, *P. falciparum* and *P. malariae*) and babesiasis (the latter two tests were performed by the Center for Disease Control). Skin tests for tuberculosis and streptokinase-streptodornase were negative, but tests for candida and trichophyton were positive, indicating intact delayed hypersensitivity to common antigens. Serum immunoglobulin levels were normal or elevated on two occasions. All routine cultures of blood, biopsy specimens and urine yielded no growth. An echocardiogram revealed no valvular dysfunction or evidence of vegetations.

The patient was discharged taking no medications and returned to work. He did well for three weeks, and his hemoglobin returned to normal, but he then began to experience a recurrence of his previous symptoms. In July, 1976, a peripheral-blood smear performed by his local physician again revealed organisms on erythrocytes, and he returned to our hospital. All signs, symptoms and laboratory findings were identical to those seen on his first admission. Treatment with oral tetracycline failed to cause any change in symptoms after four days, but intravenous cefazolin in combination with intramuscular streptomycin, 500 mg every 12 hours, again resulted in rapid disappearance of fever and of red-cell-associated organisms from peripheral-blood smears. The patient was discharged, feeling well, after six weeks of therapy. A sedimentation rate of 38 mm per hour was the only abnormal laboratory finding at the time of discharge.

A second relapse occurred two months later in early October, 1976. Because financial constraints prevented hospitalization, the patient was treated with oral cephalexin, 500 mg four times daily, as an outpatient for four months. Symptomatic improvement and the clearance of organisms were as dramatic as with parenteral

antibiotics. However, a third relapse was diagnosed by his local physician in early April, 1977, three months after therapy had been stopped. Evaluation and treatment were carried out at a local hospital. A bone-marrow aspirate disclosed abundant organisms. Because the bone marrow was a potential reservoir for the organism, therapy was begun with chloramphenicol, which is known to reach high concentrations in bone marrow. The antibiotic was administered intravenously for 10 days in doses of 1 g every four hours and was then given orally for one month in doses of 250 mg every six hours. Symptomatic improvement was slow, with the temperature gradually decreasing to normal on the sixth hospital day. After three weeks of chloramphenicol therapy repeat bone-marrow aspirate and peripheral-blood smear revealed no organisms. After a month of treatment, the patient's hemoglobin had decreased from 13.9 g per deciliter (8.6 mmol per liter) to 12.4 g per deciliter (7.7 mmol per liter), and chloramphenicol was replaced with oral cephalexin. Cephalexin alone was continued for 10 months until March, 1978. Since then, the patient has remained well, and a sedimentation rate of 10 mm per hour was recorded in March, 1979.

All attempts to culture the organism from fresh heparinized blood or hemolyzed blood were unsuccessful. The following culture mediums were used: broth, semisolid and solid mediums supplemented with amino acids and 20 per cent fresh whole human blood (from both normal donors and the patient); specialized mediums for isolation of leptospira, *Legionella pneumophila*, mycobacteria and mycoplasma; embryonated eggs; and monolayers of vero or hep-2 tissue-culture cells. The cultures were incubated aerobically, anaerobically and with 10 per cent carbon dioxide at both 28°C and 37°C. Inoculation of the patient's blood into normal mice and into five species of splenectomized animals (rats, guinea pigs, dogs, rabbits and rhesus monkeys) failed to produce disease or an association of organisms with circulating erythrocytes. Intradermal injection of blood into rhesus monkeys failed to produce skin verrucae.³ Serums taken both before and after therapy were not specific for *Bartonella bacilliformis* as tested by the indirect fluorescent-antibody technic (performed by Dr. J. Feeley of the Center for Disease Control).

APPEARANCE OF THE MICROORGANISM

Optical Microscopy

In smears of peripheral blood, short rods, staining bluish-purple with Wright's or Wright-Giemsa stains, blue to purple with Gram's stain and black with Gomori's methenamine-silver nitrate stain,⁴ were easily seen, especially with Gomori's stain. These organisms, although sometimes lying free, were usually in close association with erythrocytes and seemed to adhere to them. They were not apparently within any cells, nor adherent to any white cells. In tissue sections and bone marrow, they were present in the interstitium and were not associated with macrophages, platelets or endothelial cells. No vasculitis was present in either skin or bone marrow, despite an abundance of organisms.

Electron Microscopy

Direct negative staining⁵ of thawed whole blood revealed short rods 0.2 μm wide by 1.0 to 1.7 μm long, most of which appeared to adhere to red cells. Within the rods, areas of tubules or vesicles located centrally or subterminally resembled the mesosomes⁶ that are prominent in gram-positive bacteria. Examples of division (binary fission) were sometimes seen at these sites. There was no evidence of pili, flagella or other surface structures.

In sections stained and embedded in Spurr's medium⁷ the rods were in crypts or involutions of the red cells. These effects were probably artifacts of handling or processing; there was no evidence of erythrocyte damage by the bacteria. The rods were in close proximity to the red-cell membrane but separated from it by a constant gap of 10 to 20 nm. The red-cell membrane was intact, and no organisms were intraerythrocytic. Cross-sections of the rods (Fig. 2, left) revealed an outermost triple layer that resembled the red-cell membrane and had the same measured width (6.6 to 7.5 nm). In order inward, the components were then a granular cell wall 14.0 to 16.2 nm wide; a densely staining 3.0 to 3.2-nm layer that may have represented an inner lamella of the cell wall; an electron-lucent region approximately 4.0 nm wide that probably corresponded to periplasm; a triple-layered cytoplasmic membrane 7.5 nm wide; and a densely stained protoplasmic cylinder that in some sections revealed ribosomes and electron-lucent regions characteristic of the bacterial nucleoid. No other details were defined, but another method for treatment of fresh heparinized blood (Karnovsky fixation⁸ and Epon-Araldite embedding, performed by Dr. F. Murphy of the Center for Disease Control) revealed internal membranes resembling mesosomes (Fig. 2,

right). No membrane external to the cell wall was detected, however, with this technic for fixation and embedding. All in all, the ultrastructural features (size, nature of cell wall, nucleoids and prominent mesosomes) were characteristic of a prokaryotic microorganism that was a gram-positive bacterium.^{9,10}

DISCUSSION

Although protozoa that infect red cells, such as plasmodia and babesia, are well known human pathogens, the association of bacteria with human erythrocytes is rare. The only bacteria known to attack human red cells are *Bartonella bacilliformis*, an organism limited by its insect vector to a narrow geographical area in South America,¹¹ and an unidentified "bacterium-like organism" seen in several patients with the hemolytic-uremic syndrome.¹² The bacterium found in our patient was different from these organisms in several respects. First of all, both these organisms could be cultivated on artificial mediums, whereas ours could not; secondly, both were pathogenic for some animals, whereas no disease developed in any of six animal species inoculated with our patient's blood; thirdly, morpho-

Figure 2. Electron Micrographs from Fixed Sections of Peripheral Blood.

The photograph on the left shows a cross-section of the organism in an infolding of the erythrocyte. The om denotes outer membrane, cw cell wall, il inner layer of cell wall, cm triple-layered cytoplasmic membrane, n nucleoid, and rm red-cell membrane (glutaraldehyde-osmium fixation in Spurr's embedment, $\times 123,000$).

The photograph on the right shows a longitudinal section of the organism adherent to a red cell, with a mesosome (m) and the division site (arrow) (Karnovsky-osmium fixation and Epon-Araldite embedment, $\times 66,000$).

logic and staining characteristics were dissimilar; and, finally, our patient's serum did not react with *B. bacilliformis* in an indirect fluorescent-antibody test.

Although they are rare in human beings, bacterium-like erythrocyte parasites are found in many animal species and may cause hemolytic anemia and systemic disease.¹³ The most common among animals are haemobartonella and eperythrozoon, members of the family bartonellaceae, and anaplasma, a member of the family anaplasmatidae. Although these organisms have not been cultured on artificial mediums, Kallick et al.¹⁴ have identified structures that are morphologically and antigenically similar to haemobartonella on the red cells of some patients with systemic lupus erythematosus. Our patient's organism could be distinguished from these animal parasites by electron-microscopical evidence of a thick cell wall and of cytoplasmic nucleoid material — structures absent from haemobartonella-like organisms.¹³

A unique feature of our patient's organism, not found in other prokaryotic cells, was the outer membrane external to the cell wall. It was not possible to determine whether this membrane was produced by the bacterium or acquired from a mammalian cell. The close association of the bacteria with erythrocytes implies that metabolically active red cells were necessary for bacterial replication. This characteristic may explain the inability of the organism to grow on artificial mediums and suggests that it requires a vertebrate host to be maintained in nature.

There were several interesting aspects of the patient's illness. In the first place, the absence of a spleen may have increased his susceptibility to infection and prolonged the course of his disease, as in splenectomized animals infected with babesia, haemobartonella, eperythrozoon or anaplasma.^{13,15} Secondly, the cure produced by chloramphenicol after repeated failures with cell-wall-active antibiotics suggests that chloramphenicol was reaching a reservoir for the organisms that the other agents failed to penetrate. In view of the known ability of chloramphenicol to penetrate the bone marrow in high concentrations,¹⁶ the overwhelming number of organisms seen in this location and the absence of any other obvious site, it seems likely that the bone marrow was the site of the reservoir.

Additional infections with this unusual bacterium may have occurred before and gone undiagnosed since the clinical syndrome in this patient resembled that of a connective-tissue disease. Furthermore, patients with intact spleens may have had a less severe illness and fewer organisms in the peripheral blood. Examination of bone-marrow aspirates and peripheral-blood smears stained with both Giemsa and Gomori's methenamine-silver stains in cases of multisystem disease of unknown cause may uncover more infections with this bacterium.

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