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Chronic Granulomatous Disease: Fatal Septicemia Caused by an Unnamed Gram-Negative Bacterium

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A 2-year-old boy with proven X-linked chronic granulomatous disease was placed under continuous co-trimoxazole prophylaxis. He remained free of infection for 4 years. At age 6.25 years, he suddenly developed a fever with no localizing signs and died 16 days later in septic shock. A gram-negative, catalase-positive, halophilic, aerobic bacterium was cultured from blood, bone marrow, and ascitic fluid. This organism could not be identified in microbiological laboratories in Europe and the United States. Its biochemical features indicate that it may belong to a species which has not previously been described.

Chronic granulomatous disease (CGD) is an inherited disorder of phagocyte function. Neutrophilic and eosinophilic granulocytes, monocytes, and macrophages are able to phagocytize normally, but are not able to kill catalase-positive bacteria and fungi (2, 4, 13). The basis of this immune defect is the inability to transform molecular oxygen into microbicidal oxygen radicals such as O_2^-, OH^-, H_2O_2, and OCl^- (3). The phagocytized organisms are protected from the killing action of most antibiotics and of the serum (5), and by these vehicles, they are transported into every organ, thus giving rise to recurrent and disseminated infections. The secondary defense reaction of the body is local granuloma formation. Organisms isolated from infected lesions include *Staphylococcus* spp., gram-negative bacteria such as the *Enterobacteriaceae* and *Pseudomonas* spp., and fungi (6, 10). Catalase-negative bacteria, however, pose no problem, as they produce the hydrogen peroxide required for their own destruction within the phagocytic vacuole (8, 11).

Clinical experience has shown that continuous co-trimoxazole therapy is able to effectively prevent bacterial infections (9). We report here on a boy with CGD who developed a fulminant and fatal septicemia due to an unidentified, catalase-positive, gram-negative, halophilic, aerobic bacterium while under prophylactic co-trimoxazole therapy.

CASE REPORT

The patient, born April 20, 1973, was a white male of Italian descent. During the first 2 years of his life he had been plagued with many recurring infections manifested as bloody-mucoid diarrhea, otitis media, bronchitis, furunculosis, and pyuria and had been hospitalized on several occasions. Upon admission to the Children's Hospital in Zürich at the age of 2 years, the diagnosis of X-linked CGD was suspected and confirmed by the Nitro Blue Tetrazolium (NBT) test. The rate of reduction of NBT by phagocytic cells was 0% in the patient, 65% in his mother, and 97% (normal) in his father (Fig. 1). From the time of diagnosis the boy received prophylactic continuous co-trimoxazole, 240 mg twice daily. He remained free of infections for 4 years. At age 6.25 years, he was readmitted because of high fever (40.7°C), anorexia, malaise, and dry cough which had been present for 10 days.

Upon admission, he appeared to be well developed (height, 113.5 cm = 25th to 50th percentile; weight, 17.85 kg = 10th percentile). There were numerous pinhead-sized nodules on the skin of his face, neck, and shoulders. In the neck region there were tiny pustules, and on both sides of the palate there were mucosal ulcers, approximately 5 mm in diameter, with suppurating margins. Lymph nodes in the neck and inguinal regions were pea sized. The liver was palpable 1 cm below the costal margin, but the spleen was not palpable.

Laboratory findings were as follows. Upon admission the hemoglobin was 111 g/liter, reticulocytes were 1.6%, leukocytes were 6.5×10^9/liter with 70% neutrophils (stabs, 54%; segs, 16%), and the sedimentation rate was 61/114 (1 h/2 h). Blood (aerobic and anaerobic, done eight times) and urine cultures were negative. A stool culture showed normal intestinal flora. The pustule swab grew *Staphylococcus epidermidis*, and the throat swab grew alpha-hemolytic *Streptococcus* spp. Serological tests were negative for *Brucella* spp., *Francisella tularensis*, *Leptospira* spp., *Mycoplasma pneumoniae*, and *Toxoplasma gondii*, for influenza, adeno-, herpes simplex, cytomegalo-, coxsackie-, and echoviruses, and for *Candida albicans*, *Aspergillus fumigatus*, *A. nidulans*, and *A. niger*. Alkaline phosphatase, serum glutamic oxalacetic transaminase, and serum glutamic pyruvic transami-

nase were normal. The Mantoux test with 10 IU was negative. Streptozyme, rheumatoid, and lupus erythematosus tests were negative. The serum immunoglobulin G and immunoglobulin A were elevated (151 and 148 IU/ml, respectively). Immunoglobulin M was normal (101 IU/ml). The blood group was A Rh pos. Kell:K-k+ Kp (a-b+) (exclude phenotype Ko and McLeod). The chest X ray showed clear lung fields with prominent hilar and mediastinal lymph nodes. A skeletal scintigram with Technetium methylene diphosphonate gave no hint of osteomyelitis, but the lymphatic system in a Gallium scintigram showed increased density in the hilar, mediastinal, and abdominal regions.

During hospitalization, high fever (up to 40.7°C) persisted (with a remittent course due to antipyretics), but no localizing signs of the infection could be detected. Since it had started during co-trimoxazole therapy, this drug was discontinued on the third hospital day. Two days later, sudden shock developed, with signs of sepsis, disseminated intravascular coagulation, and renal failure. The leukocyte count fell to 1.3×10^9/liter (metamyelocytes, 7%; stabs, 88%; segs, 2%; and lymphocytes, 3%), the platelet count was 75×10^9/liter, and the humoral coagulation factors were diminished (prothrombin time, Quick method, 20%; partial thromboplastin time, 100 s; factor I = 0.6 g/liter, II = 48%, V = 22%, VII = 19%, X = 54%, with positive alcohol test). Creatinine was 14 mg/liter, and blood urea nitrogen was 310 mg/liter.

Blood and bone marrow cultures were taken, and then rifampin, cefotaxime, amikacin, co-trimoxazole, amphotericin B, and flucytosine were given. In addition, granulocytes from a relative, fresh plasma, and platelets were transfused. In spite of this intensive therapy, the boy's condition rapidly deteriorated in the following 24 h, and necrotic purpura of the hands, feet, and ears developed. An exploratory laparotomy for a possible abdominal abscess revealed 0.5 liter of ascitic fluid, but no evidence of an abscess. Soon afterwards, cardiac arrest led to death. Postmortem examination was refused.

The day after death, a gram-negative bacterium was isolated from cultures of blood, bone marrow, and ascitic fluid. It could not be identified in microbiological laboratories in Zürich, Copenhagen, London, Paris, and Los Angeles. Its characteristic features are described.

MATERIALS AND METHODS

Most of the medium preparation and the procedures used in determining the biochemical characteristics have been previously described (7, 15). Whenever possible, dehydrated media from commercial sources were used. NaCl tolerance was determined by using nutrient broth (Difco Laboratories, Detroit, Mich.) and various concentrations of NaCl (0% and 6 to 12%) in 4-ml amounts. The media were contained in screw-capped tubes (16 by 125 mm). Each tube was inoculated with 1 drop of an 18- to 24-h heart infusion broth culture and incubated at 35°C for up to 48 h and then examined for growth or growth inhibition. The flagella broth consisted of tryptose (Difco), 10 g; dipotassium phosphate (anhydrous), 1 g; sodium chloride, 2.5 g; and glass-distilled water, 1,000 ml (pH 7.0).

Colonial morphology and hemolysis of blood were determined on heart infusion agar (HIA) (Difco) containing 5% defibrinated rabbit blood. The plates were incubated for 36 to 48 h at 35°C in a candle jar atmosphere. A Gram stain was done on an 18- to 24-h HIA culture by Hucker's modified procedure (16). For the flagella stain, the strain was growth both on tryptone-glucose-yeast extract agar and in flagella broth overnight at 25°C; the simplified silver-plating method of West et al. (16) was used.

Biochemical tests are listed in Table 1. Tests for acid production from carbohydrates were done in oxidation-fermentation base (Difco) and fermentation broth base. The carbohydrates were filter sterilized and added to the sterile basal medium at a final concentration of 1%.

Growth at 25, 35, and 42°C was determined on both tryptone-glucose-yeast extract agar and HIA. Growth on Thayer Martin medium was determined by streaking a loopful of an 18- to 24-h heart infusion broth culture onto a commercially prepared Thayer-Martin medium plate (BBL Microbiology Systems, Cockeysville, Md.).

For the L-lysine and L-ornithine decarboxylase and the L-arginine dihydrolase tests, the media were supplemented with NaCl (0.2 ml of a 20% NaCl solution was added to 1.5 ml of medium), and these media were inoculated heavily with a loopful of growth from an 18- to 24-h blood agar plate containing HIA and 5% defibrinated rabbit blood.

Antimicrobial agent susceptibility testing was done by the microdilution broth method with Mueller-Hinton broth supplemented with calcium and magnesium as recommended by the National Committee for Clinical Laboratory Standards (12).

RESULTS

At 18 to 24 h on HIA with 5% rabbit blood, there was fairly heavy confluent growth, but no isolated colonies were visible (to the unaided eye); there was no change in the color of the blood agar. However, by 36 to 48 h, the colonies were 1.5 mm in diameter, convex with entire edges, semitranslucent, smooth, glossy, and slightly stringy in consistency. There was a lavender-green coloration and a clearing of the blood agar under the confluent growth. On tryptone-glucose-yeast extract agar, the strain had heavy growth at 25°C, light growth at 35°C, and no growth at 42°C; however, on HIA, it grew heavily at 25 and 35°C, but did not grow at 42°C. On HIA, this isolate was a pale-staining, gram-negative, small coccoid organism with a few slightly crescent-shaped forms. It was not acid fast. No motility was demonstrated in the motility medium or in a wet preparation, and no flagella were detected. The isolate did not grow on a blood agar plate which was incubated anaerobically for 3 days. It was DNase positive.

Because the cellular morphology of this organism was similar to that of *Francisella tularensis*, a fluorescent-antibody test was done. This organism stained 3 to 4 plus with *F. tularensis* conjugate.

TABLE 1. Biochemical characteristics of unidentified organism

Test or substrate	Reaction[a]
Triple sugar iron agar	
Acid slant	−
Acid butt	−
H_2S	+
Growth on MacConkey agar	−
Oxidase, Kovacs	+
Catalase	+
Nitrate reduction	−
Urease (Christensen's agar)	−
Gelatin hydrolysis	+
Indole	−
Growth in	
Nutrient broth, 0% NaCl	−
Nutrient broth, 6% NaCl	+
Nutrient broth, 8% NaCl	−
Motility	−
L-Lysine decarboxylase	−
L-Arginine dihydrolase	−
L-Ornithine decarboxylase	−
Acid from (oxidation-fermentation medium)	
Glucose	$+_w$
Xylose	−
Mannitol	−
Lactose	−
Sucrose	$+_w$
Maltose	$+_w$
Fermentation of glucose	−

[a] +, Positive in 1 or 2 days; −, no reaction; $+_w$, weakly positive in 1 or 2 days.

Results of the biochemical tests are given in Table 1. The acid reactions in oxidation-fermentation glucose, sucrose, and maltose were weak at 1 to 2 days, as well as at 3 to 7 days. No acid reaction was observed in the fermentation broth base. This organism did not grow in nutrient broth to which NaCl had not been added, but it did grow in nutrient broth with 6% NaCl. The upper limit of the NaCl tolerance was 7%. Also, this isolate was able to grow on Thayer-Martin medium.

The organism was resistant to ampicillin (minimal inhibitory concentration [MIC] = >32 μg/ml), cefamandole (MIC = >64 μg/ml), cephalothin (MIC = >64 μg/ml), clindamycin (MIC = 16 μg/ml), colistin (MIC = 32 μg/ml), co-trimoxazole (MIC = 8 μg/ml for trimethoprim and 152 μg/ml for sulfamethoxazole), and penicillin (MIC = >16 μg/ml). It was moderately susceptible to carbenicillin (MIC = 64 μg/ml), chloramphenicol (MIC = 2 μg/ml), erythromycin (MIC = 2 μg/ml), and ticarcillin (MIC = 32 μg/ml) and susceptible to amikacin (MIC = ≤1 μg/ml), cefotaxime (MIC = ≤1 μg/ml), cefoxitin (MIC = ≤1 μg/ml), gentamicin (MIC = ≤0.25 μg/ml), kanamycin (MIC = ≤1 μg/ml), and tetracycline (MIC = ≤1 μg/ml).

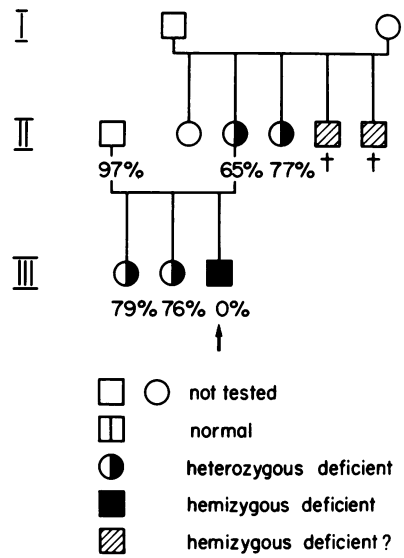

FIG. 1. Pedigree of patient with CGD. The percentages are the percentage of NBT-positive granulocytes, the crosses indicate death, and the arrow indicates the patient.

The diagnosis of X-linked CGD was established in our patient by the negative NBT test (Fig. 1) and deficient killing of catalase-positive, co-trimoxazole-resistant *Staphylococcus epidermidis* by his granulocytes (Fig. 2). The NBT test on his mother showed a mosaic of 65% NBT-positive and 35% NBT-negative granulocytes, resulting in intermediate values (Fig. 1). The bacterial killing test on the mother's granulocytes also showed intermediate values (Fig. 2). There was deficient depolarization of granulocyte transmembrane potential, presumably due to deficient activation of granulocyte NADPH oxidase (17).

DISCUSSION

Prophylactic administration of co-trimoxazole has improved the prognosis of patients with CGD (9) since it suppresses most usual acute bacterial infections. Co-trimoxazole is effective against the bacterial spectrum prevalent in CGD except for *Pseudomonas* spp. It is accumulated in granulocytes (sulfamethoxazole, 1.7 times; trimethoprim, 4.1 times [1]) and does not impair the functions of chemotaxis, phagocytosis, and degranulaton, which are intact in CGD. In addition, intestinal anaerobes are not eliminated, and therefore, resistant organisms rarely overgrow the gut flora. This protection is, however, not absolute, as illustrated by the case described. Our patient died of sepsis due to a co-trimoxazole-resistant bacterium.

This unidentified organism caused fulminant septic shock which was irreversible even after

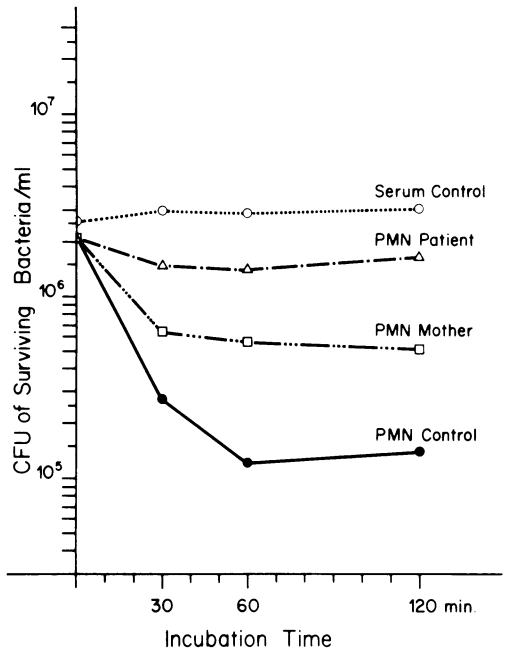

FIG. 2. In vitro bactericidal assay with *Staphylococcus epidermidis* and granulocytes (PMN) of the patient, of his mother, and of a normal control. CFU, Colony-forming units.

the administration of amikacin and cefotaxime, to which it was sensitive in vitro, and the transfusion of leukocytes.

This aerobic, catalase-positive, NaCl-requiring, gram-negative small coccoid organism has apparently not been described. Although it closely resembles *Francisella tularensis* morphologically and stains with the corresponding specific fluorescent antibody, it differs in other characteristics, i.e., H_2S production in TSI Agar (BBL Microbiology Systems), growth in 6 to 7% NaCl, gelatin hydrolysis, and reaction on HIA with 5% rabbit blood agar. It also grows more readily than *F. tularensis* does on most laboratory media. The biochemical characteristics of this organism are somewhat similar to those of *Pseudomonas putrefaciens* group 2 (14); however, it can be differentiated by its inability to grow on MacConkey agar, oxidation of sucrose and maltose, lack of nitrate reduction, a negative ornithine decarboxylase test, and no detectable motility.

The General Bacteriology Branch, Bacterial Diseases Division, Center for Infectious Diseases, Centers for Disease Control, has received for identification several similar cultures that were isolated from human clinical specimens in the United States: six from blood, two from lymph nodes, one from pleural fluid, and two from a lung biopsy. Only a limited amount of clinical information accompanied the cultures from these 11 patients. For at least three patients, however, it was indicated that the clinical diagnosis or an associated illness was CGD. A comparative study of this isolate and these similar cultures needs to be made. The pathogenicity and the relationship of this group of organisms to *F. tularensis* should be investigated further. Also, genetic studies are needed to determine whether this bacterium should be classified in one of the presently recognized genera.

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