

Serratia marcescens osteomyelitis in an infant

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ABSTRACT

Neutrophil dysfunction can result from oxidative burst defect or from glucose-6-phosphate dehydrogenase (G6PD) deficiency; we noted both in the same patient. A 4-month-old male infant with G6PD deficiency presented with swelling of the left middle finger, left leg, and right big toe. At 5 weeks of age he was hospitalized for fever for 2 days. A maternal uncle died at 5 years of age and a male maternal cousin died at the age of 21 months, both reportedly diagnosed with chronic granulomatous disease (CGD). On physical examination, he had a swollen erythematous left third finger, left distal leg swelling, and right big toe abscess. None of these areas was significantly tender. WBC was $18.7 \times 10^3/\text{mm}^3$ with 37% PMN and 5% bands. The x-ray films showed osteomyelitis in the left third proximal phalanx and the distal right first metatarsal. Culture from the toe abscess grew *Serratia marcescens*. His neutrophil oxidative burst was tested by the dihydrorhodamine-123 assay and was markedly suppressed, typical of CGD. The mother and maternal grandmother were found to be CGD carriers. He was treated with i.v. antibiotics for 4 weeks and was discharged on prophylactic trimethoprim, itraconazole and interferon γ , with substantial reduction in infections. Infection in this infant was unusual in its nature, in affecting multiple sites, and in its causative organism. Immune deficiency was suspected, particularly of the phagocytic component, but could not be attributed to his moderate degree of primary G6PD deficiency. Additional immunologic evaluation and the family history led to the diagnosis of X-linked CGD.

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CASE PRESENTATION

Chief Complaint

Swelling of the left middle finger, left leg, and right big toe.

History of Present Illness

A 4-month-old African American male infant presented to our hospital with swelling of the left middle finger, left leg, and right big toe and a fever of 102°F for a few days. His medical history was significant for Hgb C trait and glucose-6-phosphate dehydrogenase (G6PD) deficiency A[−] variant (202A/376G) both diagnosed *via* the State's newborn screen. At 5 weeks of age, he was hospitalized for 2 days for fever without

apparent cause. His mother had a negative test for HIV. A maternal uncle died at 5 years of age and a male maternal second cousin died at 21 months old, both reportedly were diagnosed with chronic granulomatous disease (CGD).

Physical Examination

His vital signs were within normal limits and afebrile. Developmentally, he was age appropriate and his length, weight, and head circumference were above the 50th percentile. He had a swollen erythematous, tender left third finger, left distal leg swelling, and right big toe abscess. The rest of the physical examination was within normal limits.

Laboratory Findings

Table 1 presents our laboratory findings. Of particular interest were his low G6PD level, leukocytosis, and elevated ESR and CRP. The x-ray films revealed osteomyelitis in the left third proximal phalanx and the distal right 1st metatarsal. Microbiological culture of the abscess of his big toe grew *Serratia marcescens* resistant to ampicillin and amoxicillin/clavulanate and sensitive to several second- and third-generation cephalosporins. Serum immunoglobulin

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Table 1 Laboratory findings in an infant with osteomyelitis

Previous laboratory data		
G6PD quantitative 77 U/10 ¹² RBC (nl: 146–376)		
G6PD deficiency A [−] variant (202A/376G)		
Hgb C trait		
Current laboratory evaluation		
WBC 18.7K/mm ³ ; 37% N, 5% B, 40% L, 12% E, 6% M		
Hgb 6.8 g/dL, Hct: 21.7%		
ESR 45 mm/hr		
CRP 4.59 mg/dL		
The x ray: osteomyelitis left third proximal phalanx and distal right first metatarsal		
Culture of big toe abscess: <i>S. marcescens</i> .		
IgG 1300 mg/dL (nl 217–704)		
IgA 43 mg/dL (nl 8–84)		
IgM 145 mg/dL (nl 35–102)		
<i>S. pneumonia</i> Abs protective to nine serotypes		
Tetanus antibodies 0.8 IU/mL (protective >0.15)		

Flow cytometry	Patient	Normal
CD4	29.4%, 3,416/mm ³	33–58%, 1500–5000/mm ³
CD8	8.5%, 988/mm ³	11–25%, 500–1600/mm ³
CD19	42.3%, 4,915/mm ³	14–39%, 600–3000/mm ³
CD4/CD8 ratio	3.5:1	1.7–3.9:1

levels showed elevated IgG and normal IgA and IgM. He also had protective levels of antibodies to pneumococcal and tetanus antigens. Neutrophil oxidative burst assay, using the dihydrorhodamine (DHR)-123 flow cytometry–based assay, was done at a mail-out laboratory. The results came back as uninterpretable because of a problem in differentiating granulocytes from other leukocytes. Repeating the test by the same laboratory on a new specimen was interpreted as normal, with an intermediate and normal population of granulocyte DHR fluorescence.

Clinical Course

He was treated with i.v. clindamycin and ceftriaxone for 28 days in the hospital. Early in the course of therapy, the swelling and erythema rapidly improved. Based on the initial laboratory report, we thought his infection was secondary to G6PD deficiency even though it was of a moderate degree. On discharge, prophylactic trimethoprim (without sulfa because of his G6PD deficiency) was prescribed. Six weeks later (6 months of age) he was readmitted with a fever (102°F) and a pustular rash over the majority of his body compatible with chicken pox. We discovered that he was not given any trimethoprim after hospital discharge. Blood and pustular cultures were negative for bacteria and viruses, and Tzanck smear was negative for varicella. He improved on acyclovir and clindamycin.

QUESTIONS

What Is the Differential Diagnosis?

This infant's infection was considered unusual regarding its nature (osteomyelitis), appearing in multiple sites, and because it was caused by *Serratia marcescens*. Therefore, primary immune deficiency should be strongly suspected. If so, what would be the likely immunologic disorder? Considering the patient's age and the nature of infection and its causative organism, the following should be considered: phagocytic defect, severe combined immunodeficiency, humoral defects, Wiskott-Aldrich syndrome, hyperimmunoglobulinemia E (Job) syndrome, complement deficiency, and G6PD deficiency.

What Additional Tests Would Be Needed?

Except for phagocytic defects, most of the foregoing disorders seemed to be unlikely in light of normal lymphocyte count, platelet count, immunoglobulin levels, and protective specific antibody titers. Because two male family members on the maternal side were diagnosed with and succumbed to CGD, this disease should remain highly suspect although the DHR assay was reported "normal." Neutrophil oxidative burst assay depends on maintaining the viability of the phagocytes at the time of the test. Outside the body, the cells are short lived and sub-

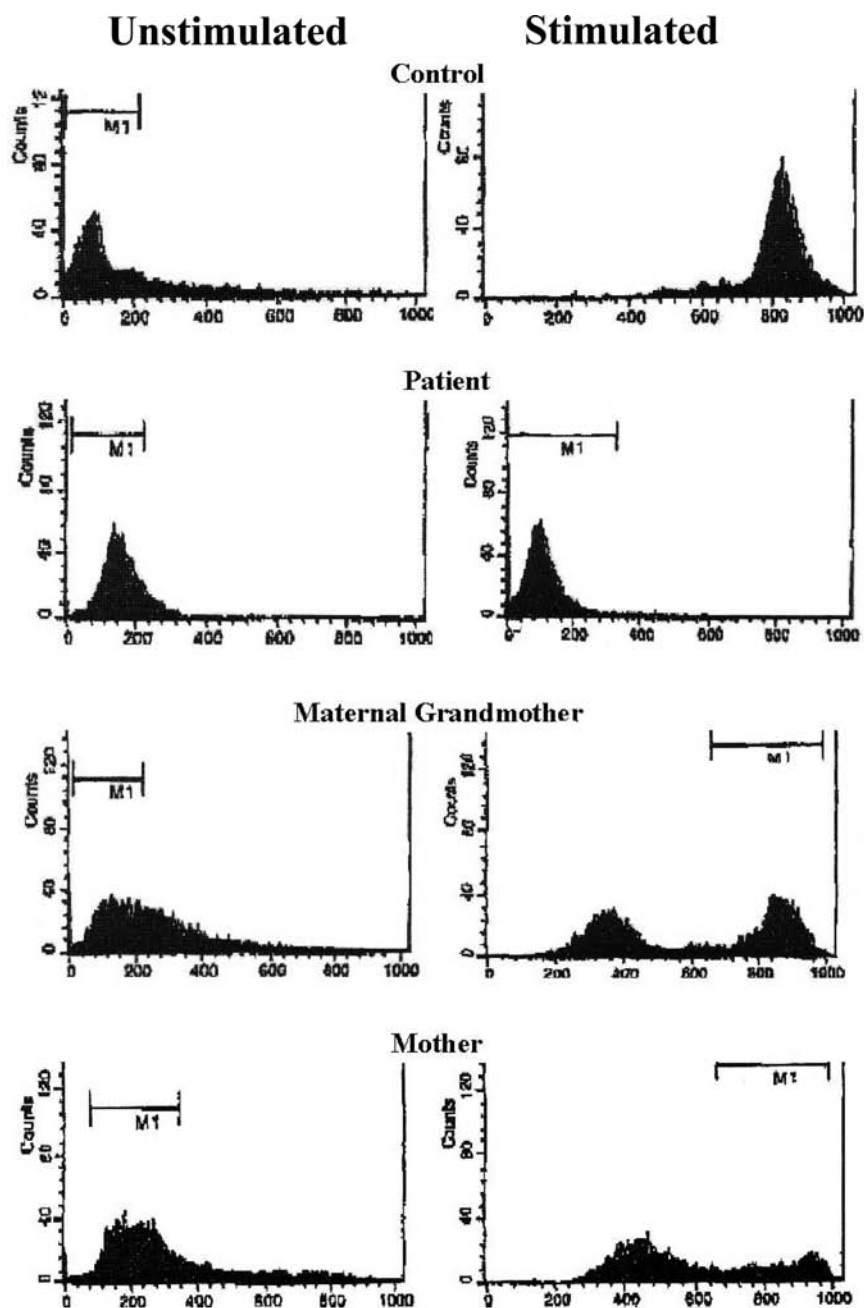


Figure 1. Neutrophil oxidative burst assay (DHR-123) on the infant, his mother, his grandmother, and normal control.

ject to environmental injury. Therefore, a repeat DHR on the infant's blood, together with the mother's and maternal grandmother's blood, was done at another laboratory. The patient's cells showed marked impaired oxidative burst capacity consistent with X-CGD (Fig. 1). The mother's and maternal grandmother's assays showed two distinct cell populations, one impaired and the other normal, consistent with CGD carrier state.

DEFINITIVE DIAGNOSIS

Based on the abnormal DHR assay findings in the infant and his mother and maternal grandmother, to-

gether with the strong family history, the diagnosis of X-CGD was made. He also had G6PD deficiency.

DISCUSSION

Osteomyelitis is uncommon in children. When it occurs in children <2 years of age, <10% are multifocal and only 4% of cases are caused by Gram-negative bacteria.¹ Most cases of childhood osteomyelitis occur in the long bones, with only 1–4% developing in the hands or feet. Because of the rarity of multifocal locations and the causative bacteria (*S. marcescens*) in our patient, primary immunodeficiency was strongly suspected.

Phagocytic cells in patients with CGD have a defect in the oxygen metabolic burst. The latter converts oxygen to superoxide, which then generates hydrogen peroxide, hypochlorous acid, and hydroxyl radical.² With a defect in the formation of superoxide, phagocytosed bacteria (primarily catalase positive) and fungi are not killed. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the enzyme that catalyzes oxygen to superoxide in the presence of NADPH, consists of six subunits.³ When the phagocyte is activated, all of the subunits associated with each other in a series of steps, forming NADPH oxidase. Approximately 70% of patients with CGD have the X-linked form and the remaining were autosomal recessive.^{4,5} The defect in the X-linked form is a mutation of the gp91^{phox} subunit of NADPH oxidase gene (CYBB on chromosome Xp21.1).² In the past, screening for CGD was by the nitroblue tetrazolium test, but the diagnosis can be missed because the test depends on visual inspection of a limited number of cells.⁶ Because DHR-123 is converted to rhodamine-123 in the presence of hydrogen peroxide, the recent development of the flow cytometric DHR assay provided a specific objective test. The DHR assay can identify the CGD genotype by the histogram pattern, typically showing no DHR shift in the X-linked form of the disease and a bimodal distribution in the carriers.⁶⁻⁸ Most patients with CGD present in the 1st year of life with recurrent severe infections, most frequently pneumonia, lymphadenitis, skin or liver abscesses, and osteomyelitis.² The predominant causative organisms are *Staphylococcus aureus*, *Aspergillus*, *Salmonella*, *Klebsiella*, *S. marcescens*, and *Burkholderia cepacia*.^{2,9} Chronic infection can cause granuloma formation that may lead to obstructive complications in the urinary and gastrointestinal tracts.

G6PD is an important cellular enzyme that catalyzes the initial step of the hexose monophosphate shunt, which is the only source of NADPH in the red blood cells. NADPH reduces glutathione which protects the red blood cells from oxidative stress.¹⁰ A reduction or absence of NADPH together with an increase in oxidative stress results in hemolysis. G6PD deficiency is an X-linked disorder that disrupts the first step of the hexose monophosphate shunt resulting in reduced production of NADPH. More than 300 G6PD deficiency mutations have been identified and are divided into three categories.¹¹ The most common category includes mutations associated with acute intermittent hemolytic anemia. The two other rare categories include those with no risk of hemolysis and those with chronic hemolytic anemia. When G6PD deficiency is severe (<5% of normal level), patients can present with a CGD-like clinical picture because of the reduced availability of NADPH in phagocytic cells, with subsequent impairment of NADPH oxidase activity.^{10,12} Under the influence of NADPH oxidase, NADPH con-

verts to NADP. Because the latter seems to be necessary for the stabilization of G6PD, CGD patients can have secondary G6PD dysfunction.¹³⁻¹⁶ Our patient, however, has primary deficiency of G6PD type A⁻, as confirmed by genotyping. It is unknown to what degree, if any, the G6PD deficiency in our patient is contributing to the phagocytic defect. It is unlikely for such a moderate degree of G6PD deficiency (about one-half of normal) can affect phagocytosis, because a G6PD activity as low as 23% does not affect neutrophil function.¹⁷

While awaiting the laboratory confirmation of CGD and obtaining recombinant interferon (rIFN) γ for therapy, the patient was admitted twice, once for fever without localization and once for pneumonia that was treated with antibiotics and antifungal agents. The addition of rIFN- γ (0.0015 mg, SQ three times a week), to trimethoprim (2.5 mg/kg bid), and itraconazole (2.5 mg/kg b.i.d.) prophylaxis resulted in marked reduction in infections. The only infection he had during the past 11 months was acute otitis media twice, the last was several weeks ago. We have been following this patient for 15 months so far.

The course of CGD varies widely; infants with X-linked CGD tend to present earlier and with more severe infections than patients with the autosomal recessive form.² Most patients do reasonably well on prophylactic antibacterial and antifungal agents together with rIFN- γ . The latter has been shown to reduce the rate of infection by 70%.² For patients who do not do well on the aforementioned regimen, definitive therapy with stem cell transplantation would be recommended.^{2,18}

Finally, it is worth noting that the diagnosis of CGD in this infant would not have been made if G6PD was considered the only underlying defect or if the DHR assay was not repeated. In fact, such an incident prompted us to develop the DHR assay in our institution's laboratory.

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