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Chronic granulomatous disease: a case report

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Chronic granulomatous disease (CGD) is a rare inherited disorder caused by defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex of phagocytic leukocytes. The leukocytes of the CGD patients cannot produce adequate amount of superoxide and other oxygen metabolites which are toxic to microorganisms. As a result, the phagocytes fail to kill the ingested microorganisms, especially those with catalase activity. Typically, CGD patients suffer from recurrent pyogenic infections starting from the first year of life. We report a young boy who had experienced recurrent perianal abscess, osteomyelitis and bacterial enterocolitis. Flow cytometric analysis revealed defects in the neutrophil respiratory burst pathway and defined the carrier state of his mother and younger sister. He received antimicrobial prophylaxis at our out-patient clinics and remained well at present. We try to make clinical physician keep in mind the diagnosis of CGD by presenting this typical case. In the meantime, we review the recent literature regarding the advances in diagnosis and management of CGD.

Key words: Chronic granulomatous disease, flow cytometry, phagocyte respiratory burst

In 1957, Berendes *et al* described the first case of chronic granulomatous disease (CGD) with the initial presentation of hypergammaglobulinemia, lymphadenopathy, granulomatous infiltration of organs, and recurrent infections [1]. CGD was later recognized as resulting from impairment in phagocyte respiratory burst due to defects in the leukocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. CGD patients usually develop recurrent and life-threatening pyogenic infections early in life. The common pathogens involved are mainly catalase-positive microbes, including *Staphylococcus aureus, Klebsiella* spp., *Escherichia coli, Aerobacter* spp., *Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Serratia marcescens*, *Candida albicans*, *Aspergillus* spp., and some other fungi [2]. Granulomatous inflammation is characteristic in these patients and usually involves lung, skin, lymph node, liver, spleen, bone, and intestine. The incidence of CGD ranges from 1 to 4 in a million according to different literature [3-5]. The pattern of inheritance of this disease can be X-linked recessive or autosomal recessive.

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Case Report

A 27-month-old boy suffered from left forearm pain due to falling down and admitted to our hospital two weeks later. Closed fracture of the left distal ulna had been noted by radiography and short arm splint was applied in a local medical clinic. Five days prior to admission, fever, severe left forearm swelling and a subcutaneous nodule over left upper arm developed. Physical examination disclosed multiple small subcutaneous nodules with redness and tenderness over the cheeks and all four extremities. He weighed 11 kg and had a body length of 82 cm, both of which fell below the tenth percentile curve of the normal population. The white blood cell count was 22,100/mm^3 with 59% segment, 33% lymphocyte, 6% monocyte, 1% eosinophil, and 1% basophil in differentiation. The hemoglobin concentration was 8.2 g/dL and the platelet count was 459,000/mm^3. The C-reactive protein (CRP) level was 5.26 mg/dL (normal range < 0.8 mg/dL). Radiographic findings showed an osteolytic lesion with periosteal reaction, which were compatible with osteomyelitis of the left ulna. Debridement was performed and the tissue culture revealed *Salmonella* group D. Culture of pus from the subcutaneous nodules yielded the same microorganism. Intravenous ceftriaxone (100 mg/kg/day in two divided doses) therapy was continued for 6 weeks and the clinical

response was good. Tracing back his history, there was no family history of any genetic disorder. He had suffered from recurrent perianal fistula with abscess since birth. At the age of 14 months, right tibial osteomyelitis caused by *Serratia marcescens* had attacked him and complete recovery had been noted after debridement and thorough antimicrobial therapy. Besides, he had experienced repeated episodes of subcutaneous abscess, oral thrush, lymphadenitis and enterocolitis. The documented offending micro-organisms included *Candida parapsilosis, Klebsiella pneumoniae, E. coli*, and *Salmonella* group D.

Under the suspicion of immunodeficiency disorder, he received a series of immunological screening tests, including cellular and humoral immunity and complement system, which revealed negative results except a reverse CD4 and CD8 ratio (CD4/CD8 = 0.7). Human immunodeficiency virus antibody test was negative by enzyme-linked immunosorbent assay (ELISA). He was discharged and followed in our out-patient clinics without a definite conclusion about immunodeficiency. Five months later, bilateral submandibular masses (sized 1.5 cm x 1.5 cm x 1.5 cm) with local redness and tenderness developed and he was readmitted to our hospital. Aspiration of the submandibular masses was performed and the pus culture yielded *S. marcescens*. With the suspicion of CGD, a nitroblue tetrazolium dye reduction (NBT) test was performed by slide method (Fig. 1). Neutrophils from the patient showed no NBT reduction after phorbol myristate acetate (PMA) stimulation. Some neutrophils from the mother showed normal NBT reduction response but other cells showed very weak or no dye reduction. Flow cytometric assays were thus performed to detect granulocyte respiratory burst on the patients and his parents (Fig. 2). There was no respiratory burst-induced fluorescence in granulocytes from the patient in response to stimulation. There were two distinct populations of granulocytes from the mother. One population showed positive fluorescence response to respiratory burst stimulation while the other population showed no fluorescence response. His younger sister had the same flow cytometric pattern as his mother (not shown in figure 2). The test for his father was normal. Phagocytosis test of the polymorphonuclears (PMNs) of the patient was normal. The neonatal screening test for glucose-6-phosphate dehydrogenase (G6PD) deficiency was negative. According to these tests, the diagnosis of CGD was established and its possible inheritance pattern may be X-linked recessive.

Intravenous ceftriaxone (100 mg/kg/day in two divided doses) was prescribed for 10 days and then it was shifted to oral cefixime (8 mg/kg/day in two divided doses) therapy for 1 week after discharge. His recovery was excellent and antimicrobial prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX) was initiated. During the course of hospitalization, anemia due to iron deficiency and heterozygous ß-thalassemia with nucleotide 654 C to T mutation were also documented.

Discussion

Immunodeficiency disorders should be considered when patients experience frequent or severe bacterial

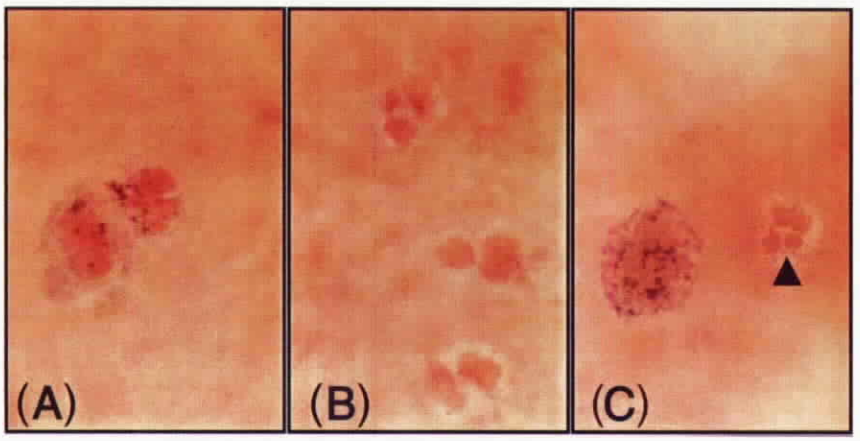

Fig. 1. NBT test (slide method) was performed for the patient and his mother under PMA stimulation. While the granulocytes from the healthy controls (**A**) shows positive response (shown as blue granules), the granulocytes from the patient (**B**) shows no NBT reduction. Some maternal granulocytes show normal NBT response, but other granulocytes (arrow head) show no response under stimulation (**C**).

infections beginning early in life, especially when uncommon pathogens are encountered. Failure to thrive is another characteristic of these patients. CGD is the most common inherited disorder of phagocyte dysfunction [6]. Phagocytes from CGD patients display normal chemotaxis, ingestion, and degranulation, but the microbial killing is deficient. Mutations in the genes encoding the components of the NADPH oxidase complex account for the pathogenesis of CGD. X-linked recessive CGD, accounting for about half of all cases, results from mutations in the gp91-*phox* gene on chromosome Xp21.1. The other forms are inherited as autosomal recessive traits. The genetic defects resulting from mutations in the p47-*phox* gene on chromosome 7q11.23 represent approximately one-third of all cases. The remaining cases result from mutations in the genes encoding p22-*phox* on chromosome 16q24 or p67-*phox* on chromosome 1q25 [2-5].

Recurrent pyogenic infections usually develop in the first year of life in CGD patients, but they may not become evident until adulthood, particularly in milder cases (variant CGD). The catalase-positive organisms are the most common offending pathogens in these patients. Although *S. aureus* is consistently the most frequent isolate, a review in 1975 (Lazarus and Neu) noted that it accounted for only 9% of fatal infections, while gram-negative bacilli caused 80% of infection associated deaths in the same series [3]. The diagnosis of CGD is often suggested by its characteristic clinical presentation or a typical family history. Infections that are unusual with respect to the locations (e.g., hepatic abscess), microorganisms (*S. marcescens, Aspergillus fumigatus, Burkholderia cepacia*), or severity are particularly suggestive [4]. Infections of the lung, skin, and lymph nodes usually predominate in CGD patients. In this index case, skin, bone, alimentary tract, and lymph nodes were involved and the identified pathogenic microorganisms include *S. marcescens, C. parpasilosis, K. pneumoniae, E. coli,* and *Salmonella* spp.

Early clinical and laboratory diagnosis is essential for the survival of CGD patients. In a multicenter survey

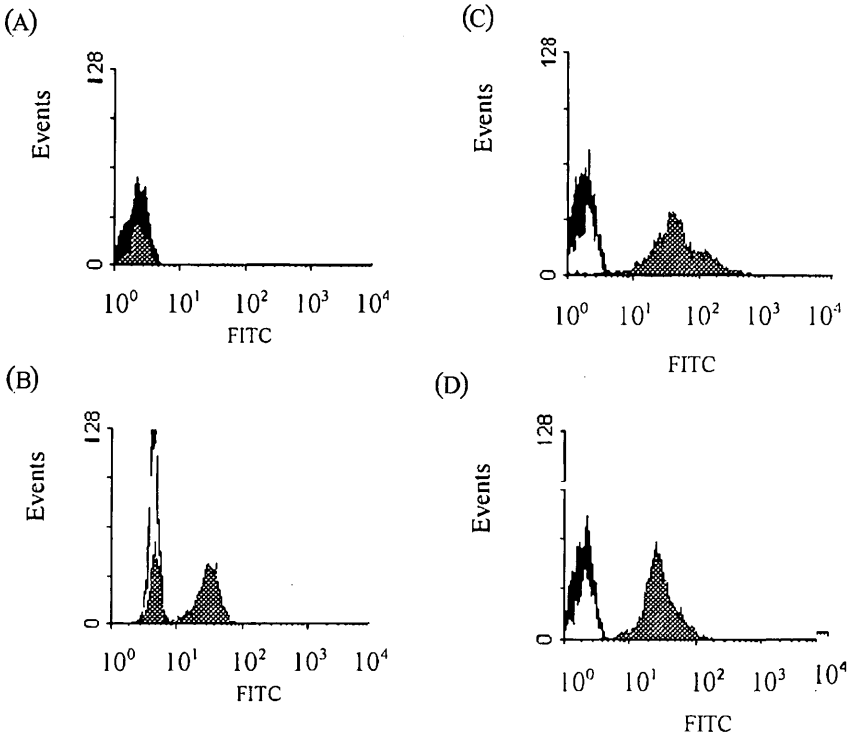

Fig. 2. Composite fluorescence histograms obtained from unstimulated (unshaded) and PMA-stimulated (shaded) PMNs from members of his family. The patient's granulocytes (**A**) show no respiratory burst in response to stimulation. The maternal granulocytes (**B**) contain a population of cells that are without respiratory burst activity. This indicates mosaicism in the cell population. His father's granulocytes (**C**), as well as the cells from a healthy control (**D**), show normal respiratory burst activity (FITC = fluorescein isothiocyanate).

of Forrest *et al* in 1988, the mean age at diagnosis was 3.6 years [3]. With the recent advance in laboratory diagnostic measures, the diagnosis can be made much earlier. A laboratory diagnosis of the disease is made by demonstrating complete absence or marked reduction in phagocyte respiratory burst. NBT test, chemiluminescent assay, spectrophotometric assay and flow cytometry are useful to detect this abnormality. In Taiwan, these tests are available only in some reference laboratories of medical centers. NBT test and flow cytometry were applied to the diagnosis in this index case. In 1983, Bass *et al* published the first method that used the dye, 2', 7'-dichlorofluorescein (DCF) and flow cytometry to measure the respiratory burst. DCF would diffuse into the cells and be oxidized into a fluorescent product by hydrogen peroxide produced by the stimulated phagocytes. The fluorescent intensity can be measured by flow cytometry quantitatively [7]. We also use this method to detect the superoxide activity, which reflects NADPH oxidase activity indirectly. Recent data showed that dihydrorhodamine (DHR) 123 is an even sensitive indicator for the diagnosis of CGD and the detection of its carriers [8]. The advantages of flow cytometric assay over NBT test are its objectivity, minimal specimen requirement, superiority in detecting an X-linked carrier, and thus the ability to indicate the likely underlying molecular defect [7-11]. Besides, flow cytometric assay may be particularly useful in cases of unusual presentation or in the identification of variant forms of CGD such as the X-linked variant, described by Woodman *et al* [10]. The current assays are sensitive to identify the carrier state of X-linked CGD in females, however, they may fail to detect the X-linked origin of the disease in some families because new mutations in germline cells and extreme lyonization exist [12]. We cannot define the exact inheritance pattern as X-linked recessive in our case unless we utilize further gene analysis.

G6PD deficiency should be included in the differential diagnosis of CGD due to similar clinical picture of both diseases. NADPH, a substrate for the respiratory burst, is generated by two enzymes of the hexose monophosphate shunt, G6PD and 6-phosphogluconate dehydrogenase. As expected, severe G6PD deficiency in leukocytes results in a diminished respiratory burst. Although the leukocyte and the erythrocyte G6PD are encoded by the same gene, the former enzyme deficiency is extremely rarer than the latter. One of the reasons is that leukocytes should have a severe G6PD deficiency (less than 5% of the normal) before the respiratory burst function is adversely affected. Furthermore, the levels of G6PD in the short-lived leukocytes usually do not decay low enough to cause clinical symptoms. Low erythrocyte G6PD level and recurrent infections are characteristics in the patients of severe leukocyte G6PD deficiency [4]. The erythrocyte G6PD level is normal in our index case, so the diagnosis of G6PD deficiency can be excluded.

Antimicrobial therapy, both prophylactic and therapeutic, remains the mainstay of management for CGD. TMP-SMX is particularly useful in prophylaxis because of its broad spectrum of activity, low toxicity, and bactericidal activity in CGD granulocytes. For patients who are unable to tolerate TMP-SMX, most clinicians substitute with a beta-lactamase-resistant semisynthetic penicillin such as dicloxacillin. Prolonged use of antimicrobial agents and adequate surgical intervention are necessary for infection control. Interferon gamma has been proven to be an effective adjunctive immunotherapy in CGD patients [13-15]. In the case of poorly controlled infections, granulocyte transfusions may be benefit when combined with other therapies [4,12]. Bone marrow transplantation and gene therapy provide a definite cure for CGD patients. Gene therapy reconstituting the NADPH oxidase activity in CGD patients is now available and has an exciting success in recent literature [16,17].

The vast majority of CGD patients died from severe infection in the 1950s, when the disorder was first described. Early diagnosis and aggressive management have resulted in an obvious decline in the mortality rate of CGD. The overall mortality rate has been estimated as two patient deaths/year/100 cases in recent literature [2]. Despite the improving prognosis, genetic counseling is of the greatest value in CGD families. We are currently developing techniques for carrier detection and mutation analysis for CGD patients in this country. Molecular genetic techniques will allow early, even prenatal, diagnosis of this potentially fatal disease. These new diagnostic techniques will further improve the outcome of the patients with CGD.

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