Adults with Chronic Granulomatous Disease of "Childhood"

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Patients with chronic granulomatous disease of childhood have an inherited defect in polymorphonuclear neutrophil (PMN) bactericidal activity and suffer from recurrent severe infections. The onset is usually in infancy with a fatal outcome nearly always by adolescence. Four adult male siblings, aged 28, 30, 32 and 40 years, had the onset at age six of serious bacterial infections involving the lungs and lymph nodes followed by a marked decrease in the frequency of infections by their mid-twenties. Sequelae include pulmonary fibrosis, ill-defined polyarthritis and glomerulonephritis.

The diagnosis of chronic granulomatous disease (CGD) of childhood was made by showing the characteristic defects in PMN function. Despite normal morphology and the ability to ingest microbes, postphagocytic PMN failed to (1) reduce nitroblue tetrazolium (NBT) dye, (2) consume oxygen and produce hydrogen peroxide and (3) stimulate the hexose monophosphate shunt. Family studies demonstrated X-linked recessive inheritance. PMN glucose-6-phosphate dehydrogenase (G-6-PD) levels were normal. Although these oxidative abnormalities of the patients' PMNs were marked and typical of "classic" CGD, patients' PMNs killed and iodinated bacteria slightly better than cells from previously studied patients with CGD.

Additional bactericidal mechanisms which might explain the longevity of the patients were searched for in phagocytic cells. To see if nonoxidative bactericidal factors were important, the patients' PMNs were studied anaerobically. However, like normal PMNs, the patients' cells showed impaired bacterial killing anaerobically. In contrast to normal monocytes and cultured macrophages which reduced NBT, patients' cells failed to reduce the dye. CGD should be considered in adults with a history of severe infections and unexplained pulmonary fibrosis.

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Typical chronic granulomatous disease (CGD) of childhood is an inherited disorder that becomes manifest in infancy [1]. It is characterized by severe and recurrent infections of lung, bone, liver, skin and lymph nodes, with granulomas of involved organs. The basis for this susceptibility to infection resides in the inability of polymorphonuclear neutrophils (PMNs) to kill certain bacteria normally [2]. This defect in killing has been linked to defects in the oxidative metabolism of PMNs, such as lack of postphagocytic oxygen consumption, hydrogen peroxide and superoxide production, hexose monophosphate shunt

activity and iodination of bacteria. In addition to studies of bactericidal capacity, the ability of PMNs to reduce nitroblue tetrazolium (NBT) to the purple formazan is often used to screen for these defects in PMNs in patients suspected of having CGD. In children with CGD, there is virtually no NBT reduction by PMNs. Most children with CGD die by adolescence [3–4].

Several adults (older than 15 years) have been described with defects in PMN bactericidal activity and absent NBT reduction similar to that found in children with CGD [5–13]. Questions suggested by these adult patients include: (1) Do they have the same defect in PMN function as children with CGD? (2) Are there facets of phagocyte function that may explain longevity? (3) Is the disease transmitted to the children of adult patients with CGD?

We have extensively studied four adult brothers with the CGD syndrome in an attempt to answer these questions and to better understand the pathophysiology of CGD.

METHODS

Subjects. These consisted of the four brothers, their immediate family and healthy volunteer subjects. All subjects gave informed consent and were withdrawn from medications two weeks prior to study, except for one (Case 3) who continued to take his antihypertensive drugs.

Bacteria. Staphylococcus aureus (Strains 502A and Wood 46), Escherichia coli (0111-B4), Streptococcus faecalis and Serratia marcescens were used after incubation for 18 hours in trypticase soy broth at 37°C.

Preparation of PMNs and Macrophages. PMNs were obtained by Dextran® sedimentation of peripheral venous blood. The supernatant was centrifuged at 200 g for 12 minutes, the cells were washed in Hank's Balanced Salt Solution without bicarbonate (HBSS, Microbiological Associates) and then resuspended in HBSS with 10 per cent autologous serum [7]. For metabolic studies, red blood cells were lysed with water [14]. Macrophages were derived from cultures of peripheral blood monocytes, obtained by Ficoll-Hypaque separation [15], washed three times in HBSS and resuspended in 3 ml of Medium 199 (Microbiological Associates) with 10 per cent autologous serum, to which 100 U/ml of penicillin and 10 μg/ml of gentamicin were added. The monocyte-rich cell suspension was then cultured in Lab-Tek flaskettes (Miles Laboratories, Inc.) for four days at 37°C in a moist 5 per cent carbon dioxide incubator.

Aerobic and Anaerobic Leukocyte Bactericidal Activity. For aerobic studies, 4 ml of a PMN suspension containing 5 $\times$ 10^6 bacteria and 5 $\times$ 10^6 PMNs were tumbled end over end at 37°C in plastic tubes. Samples were removed at 0, 30, 60 and 120 minutes. For anaerobic studies, PMN-bacterial suspensions were placed in air-tight flasks and washed with nitrogen for 2 hours at 0°C, by which time oxygen saturation was less than 1 per cent. No bacterial killing occurred at 0°C. The flasks were then transferred to a 37°C shaker bath in which incubation allowed phagocytosis and killing to occur. Samples were aspirated through air tight rubber stoppers at 0, 60 and 120 minutes. In both aerobic and anaerobic experiments all removed samples had total, sediment and supernatant bacterial counts performed after differential centrifugation. PMNs from healthy adults were obtained as controls and were always run in parallel with the PMNs of the patients [16].

Nitroblue Tetrazolium Dye Reduction. The ability of PMNs and macrophages to reduce NBT was semiquantitatively assessed on glass slides by the method of Gifford and Malawista [17]. Quantitative NBT determinations were performed by the method of Baehner and Nathan [18], except that the PMNs were collected by dextran sedimentation, and boiled staphylococci were used instead of latex particles. Results are expressed as the postphagocytic change in optical density/15 min/2.4 $\times$ 10^6 PMNs measured at 515 mμ.

Phagocytosis of Labeled Bacteria. Modifications of the methods of Downey and Diedrich [19] were employed. Staphylococci were incubated 18 hours in trypticase soy broth with 10 μCi/ml of C^{14}-labeled amino acid mixture, then washed in saline solution. Five $\times$ 10^8 bacteria/ml were added to 5 $\times$ 10^6 PMNs/ml, which were tumbled at 37°C and 0.2 ml samples removed at 0, 5, 10 and 20 minutes. The samples were placed in 4 ml of iced HBSS with 10 per cent fetal bovine serum and centrifuged at 100 g for 5 minutes at 4°C. The cell buttons were washed, digested with Protosol® (New England Nuclear, Boston) and counted in a Beckman LS250 scintillation counter.

Oxygen Consumption. Suspensions of 3 $\times$ 10^7 PMNs/ml in HBSS with 10 per cent autologous serum were placed in the chambers of a polarographic oxygen monitor. After an initial base line was obtained, 10^9 boiled staphylococci were added, and the rate of oxygen consumption was measured [20].

Hydrogen Peroxide Production. ^{14}C-1-formate oxidation was measured to quantitate hydrogen peroxide production [21]. A suspension of 10^7 PMNs and 0.5 μCi of ^{14}C-1-formate in HBSS with 25 per cent autologous serum was placed in 10 ml siliconized flasks. Two-tenths milliliter of 10 per cent potassium hydroxide was placed in a plastic well and suspended through an air-tight rubber stopper over the flask. One-tenth milliliter (10^9) of heat-killed staphylococci was added for a final volume of 1.0 ml. After 30 minutes shaking at 37°C, the reaction was stopped by adding 0.5 ml of 1N hydrochloride to the flask to liberate carbon dioxide. The flask was incubated for 15 minutes more, after which the plastic well containing potassium hydroxide and absorbed $^{14}CO_2$ was counted in a liquid scintillation counter.

Hexose Monophosphate Shunt Activity. This was determined by measuring the oxidation of ^{14}C-1-glucose by methods essentially as described for determination of ^{14}C-1-formate oxidation. One microcurie of ^{14}C-1-glucose was used in a final volume of 2 ml, consisting of 0.5 ml autologous serum, 10^9 1 μ polystyrene balls and 10^7 PMNs in HBSS. In addition, the ability of 2 mM methylene blue to stimulate shunt activity was ascertained as an indirect measure of glucose-6-phosphate dehydrogenase (G-6-PD) activity.

G-6-PD Activity. Determinations of G-6-PD activity were performed after the method of Kornberg and Horecker [22] as modified by Chan et al. [23]. The final assay mixture

contained 1 ml of 0.1 M tris buffer at pH 8.0, 0.2 ml of 0.002 M NADP, 0.1 ml of 0.2 M magnesium chloride, 0.1 ml of 0.02 M G-6-P and 1.5 ml distilled water. The reaction was started in a quartz curvette with the addition of 0.1 ml of the leukocyte extract. Results are recorded as the change of optical density/min/10^9 PMNs from readings made at 340 mμ at 25°C in a Beckman Spectrophotometer.

Iodination of Protein by Leukocytes. The test was performed by the method of Root and Stossel [24] with minor modifications. Five × 10^6 PMNs/ml were inoculated with 5 × 10^8 preopsonized heat-killed Staph. aureus and 0.2 μCi of $Na^{125}I$ (1.9 mol). The reaction was stopped after 20 minutes, and the washed trichloroacetic acid precipitates were counted in a Beckman 300 gamma counter. In some experiments, 1 mM sodium azide was added to the initial mixture to inhibit myeloperoxidase activity.

CASE REPORTS

Case 1. This patient is a 28 year old white male unemployed barber who had recurrent otitis media in early childhood but was otherwise healthy until age 6 when bilateral pneumonia developed following measles. From then until age 18, he had 16 episodes of pneumonia for which he required hospitalization and antibiotic therapy. During adolescence intermittently draining inguinal lymph nodes developed which have since resolved. In 1965, at age 18, a chest roentgenogram revealed bilateral pulmonary fibrosis. A scalene node biopsy specimen showed hyperplasia. Serum agglutinins against thermophilic actinomycetes were absent. Rheumatoid factor was 1+. Discharge diagnosis then was idiopathic pulmonary fibrosis. Beginning in early 1974, at age 27, the patient experienced recurrent episodes of fever and migratory polyarthritis involving the knees, elbows, shoulders, hands and back, for which he required multiple hospitalizations. In addition, the patient has been chronically disabled by dyspnea with the slightest exertion, associated with a daily cough productive of a scant amount of white sputum.

Physical examination revealed a very thin, chronically-ill appearing white man, using accessory muscles of respiration. Small 2 to 4 mm lymph nodes were palpable in the neck, axillae and groin. The chest was resonant to percussion with fine bilateral rales, greater in the upper lung fields. Cardiac examination was within normal limits. The liver and spleen were not palpable. Joint stiffness was noted without signs of arthritis.

The hematocrit value was 40 per cent and the white blood cell count was 5,600/mm^3 with a normal differential. Urinalysis disclosed no abnormalities. The erythrocyte sedimentation rate was 47 mm/hour. Antinuclear antibody (ANA) was 4+ positive. The patient had negative rheumatoid factor, hepatitis-associated antigen, anti-DNA and cryoglobulins. Serum hemolytic complement was 48 U/ml (normal 34 to 48 U/ml), and circulating immune complexes were absent. Quantitative immunoglobulins showed an immunoglobulin A (IgA) level of 400 mg/100 ml (normal 60 to 300 mg/100 ml), an immunoglobulin G (IgG) level of 1,810 mg/100 ml (normal 635 to 1,400 mg/100 ml) and an immunoglobulin M (IgM) level of 115 mg/100 ml (normal 41 to 248 mg/100 ml). $Alpha_1$ antitrypsin levels, sweat chlorides and antistreptolysin O (ASO) titers were normal. The vital capacity was 35 per cent of predicted, the 1 second forced expiratory volume (FEV_1) was 25 per cent and the carbon monoxide diffusion was 19 to 20.3 ml/min/mm Hg. Arterial blood gases showed an oxygen tension (pO_2) of 66 mm Hg, a carbon dioxide tension (pCO_2) of 30 mm Hg and a pH of 7.41. Roentgenograms of affected joints disclosed no abnormalities. Skin tests to Candida (dermatophyton-O), trichophyton and intermediate, purified protein derivative were negative; streptokinase-streptodornase (SK-SD) tests were reactive. A chest roentgenogram revealed bilateral pulmonary fibrosis (Figure 1-1) with little change from films taken in 1965. During this hospitalization, the patient's fingers and knees transiently became tender and red. After discharge 40 mg of prednisone daily was given with only minimal diminution in his arthritic complaints and no improvement in his respiratory status.

Case 2. This patient is a 30 year old white male part-time farmer who was healthy until age six, when he sustained a traumatic pneumothorax complicated by bilateral pneumonia. From then until age 25, the patient had yearly hospitalization for pneumonia. From age 12 to age 25, he also had chronically draining submandibular lymph nodes. Since age 25, the patient has had yearly attacks of acute bronchitis without changes on the roentgenogram. Chest films obtained in 1965, at age 20, revealed bilateral pulmonary fibrosis with an enlarged heart. Lung biopsy at that time demonstrated granulomas and chronic interstitial fibrosis; the diagnosis of farmer's lung was entertained. However, his serum contained no antibodies to thermophilic actinomycetes. From age 20 to 28, he experienced increasing dyspnea on exertion, with a 2 to 3 pound weight loss per year. Skin tests were nonreactive to SK-SD, Candida, trichophyton, intermediate and second-strength purified protein derivative (PPD). Serum levels of $alpha_1$ antitrypsin were normal. The patient was maintained with prednisone, 40 mg daily, and isoniazid (INH) with no improvement in his respiratory symptoms.

After the propositus was identified, the patient was reevaluated in 1975 at age 30. On physical examination, he was a thin, chronically-ill appearing white man. Respiration was 14/min; temperature was 37°C. He had a diffuse follicular rash over the trunk and was diffusely hyperpigmented. Shotty lymph nodes were present in the neck and groin. Diffuse bilateral moist rales were heard in the chest, greater in the upper lung fields. A pectus excavatum deformity was present. Cardiac examination revealed a grade 3/6 systolic murmur at the upper left sternal border; the first and second heart sounds were normal. Hepatosplenomegaly was absent. The hematocrit value was 40 per cent and the white blood cell count 6,000/mm^3 with a normal differential. Erythrocyte sedimentation rate was 25 mm/hour. Chest roentgenogram revealed bilateral pulmonary fibrosis (Figure 1-2). The vital capacity was 31 per cent, FEV_1 normal and carbon monoxide diffusion 13.5 to 23.7 ml/min/mm Hg. Arterial blood gases revealed a pO_2 of 75 mm Hg, a pCO_2 of 42 mm Hg and a pH of 7.36. The IgA level was 500 mg/100 ml, IgG 1,800 mg/100 ml and IgM 160 mg/100 ml. Dermatology consultants believed the patient's rash was characteristic of Darier's disease (an uncommon benign disorder of keratinization) which was also noted in his mother at the same time. A cardiology consultant stated that the patient's murmur was consistent with a pectus excavatum deformity.

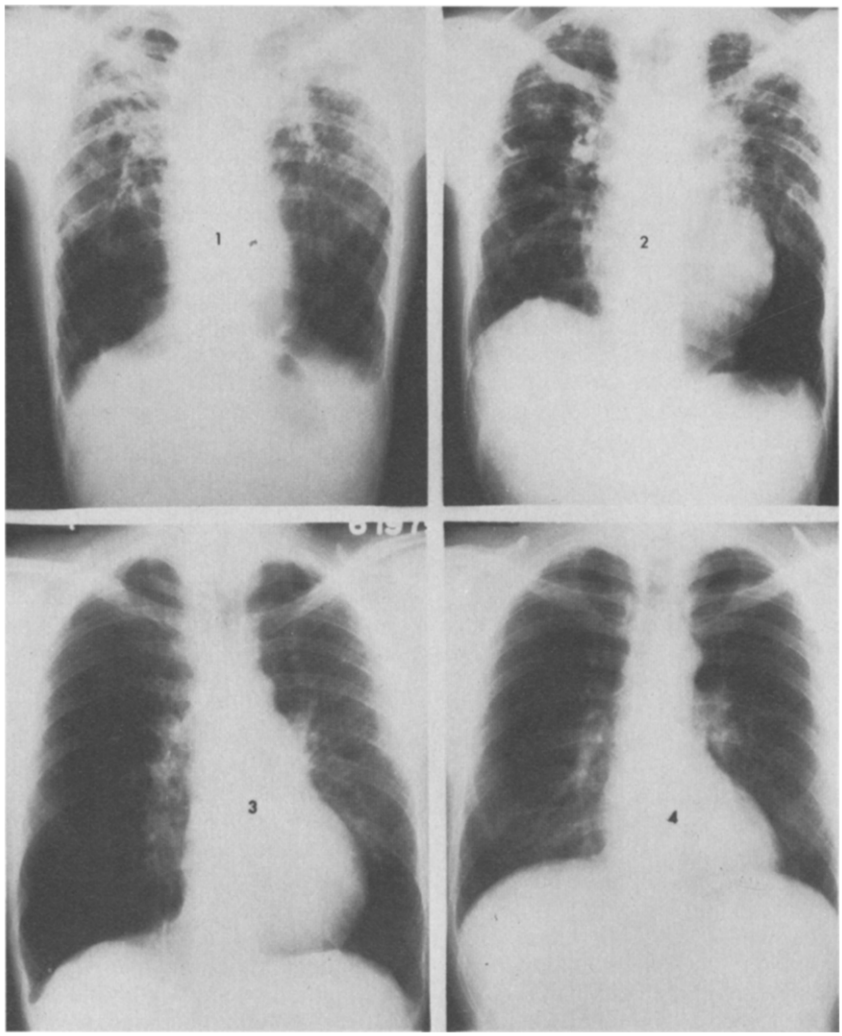

Figure 1. *Chest roentgenograms of patients. Pulmonary fibrosis is demonstrated in Cases 1, 2 and 3. In Case 4 the film shows no abnormalities.*

Case 3. This patient is a 32 year old white male farmer who was well until age five when he acquired "Bright's disease" with swelling of the hands and feet, which spontaneously resolved. At age six, he contracted measles, complicated by bilateral pneumonia. From then until age 27, he had yearly episodes of pneumonia. He had chronic shortness of breath by age 16. At age 22 the patient experienced three to four days of headache and lassitude, and was found to have painless hematuria. At age 30 orthopnea, paroxysmal nocturnal dyspnea and ankle edema developed; on examination, hypertension, proteinuria and hematuria were noted. Evaluation of his renal disease at that time revealed normal-sized kidneys, a creatinine clearance of 17 ml/min, normal ASO titers, negative ANA and latex fixation, normal renal scan, serum complement of 48 and a 24-hour urinary protein of 1.7 g. The renal disease was attributed to chronic glomerulonephritis of unknown etiology; a renal biopsy was not performed. Antihypertensive therapy was initiated with good control of his blood pressure. The patient's pulmonary status has slowly deteriorated, such that he now has dyspnea at rest. A short course of steroid therapy in 1974 did not diminish his respiratory symptoms.

On physical examination, the patient was a slightly obese man in no distress. Blood pressure was 220/110 mm Hg supine. The skin was ruddy, dry and scaly. There was no lymphadenopathy. Fundi were benign. The chest had bilateral diffuse moist rales, with decreased breath sounds at the base and occasional expiratory wheezes. The cardiac border was at the anterior axillary line; there were no murmurs, clicks or gallops. There was no hepatosplenomegaly or peripheral edema. The hematocrit value was 50 per cent and the white blood cell count 7,600/mm^3 with a normal differential. Urinalysis disclosed 1+ protein and occasional red blood cells but no casts. The blood urea nitrogen level was 31 mg/100 ml, the creatinine level 2.0 mg/100 ml and the creatinine clearance 32 ml/min. The alkaline phosphatase was 115 IU (normal 35 to 85 IU), total bilirubin 0.6 mg/100 ml and 5′ nucleotidase 18 IU (normal <19 IU). The uric acid level was 8.6 mg/100 ml. Further tests yielded a negative ANA and latex fixation, and a serum hemolytic complement of 58 U/ml.

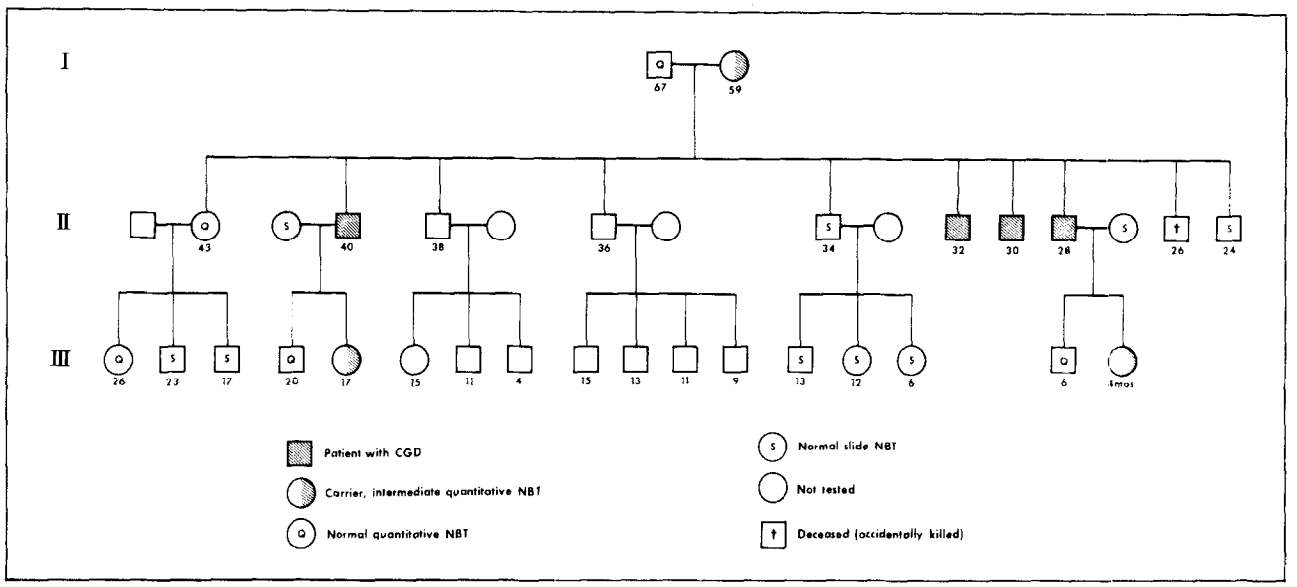

Figure 2. *Family pedigree of the four brothers with NBT test data.*

A chest film revealed bilateral pulmonary fibrosis and an enlarged heart (Figure 1–3). An electrocardiogram showed left ventricular hypertrophy by voltage and a right conduction delay. The forced vital capacity was 45 per cent and FEV_1 34 per cent of predicted. Arterial blood gases revealed a pO_2 of 68 mm Hg, a pCO_2 of 42 mm Hg and a pH of 7.44. The patient was unreactive to the following skin tests: SK-SD, Candida, trichophyton and intermediate PPD. Quantitative immunoglobulins revealed an IgA level of 960 mg/100 ml, an IgG level of 1,360 mg/100 ml and an IgM level of 115 mg/100 ml.

Case 4. This 40 year old white male construction worker was healthy until age eight when bilateral pneumonia developed. From then until age 14, he experienced yearly episodes of severe pneumonia, for which he required hospitalization and, when available, antibiotic therapy. At age 12, because of his pulmonary disease, he was hospitalized for six weeks at a tuberculosis sanitorium but was discharged after all cultures were negative. Since age 14, the patient has only had one upper respiratory tract infection per year, without evidence of pneumonia. At age 29, he had acute rheumatic fever without carditis, and he has been receiving penicillin prophylaxis since. From age 38 to the present, the patient has had intermittent swelling of a right submandibular lymph node, requiring incision and drainage. He currently operates heavy machinery and feels completely well.

He was admitted to our Clinical Research Center for study in 1975. Physical examination revealed a healthy-looking white man. Ther was a recent incision scar over the right submandibular area, but no adenopathy was noted here or elsewhere. The remainder of the examination was within normal limits. Laboratory evaluation revealed a normal hematocrit value, white blood cell count, urinalysis, electrolytes, liver function tests, creatinine and electrocardiogram. Pulmonary function tests were within normal limits, as was the chest roentgenogram (Figure 1–4). Quantitative immunoglobulins revealed an IgA level of 500 mg/100 ml, an IgG level of 1,720 mg/100 ml and an IgM level of 108 mg/100 ml. Skin tests were reactive to Candida, trichophyton and SK-SD; the intermediate PPD was negative.

RESULTS

Cell Morphology. Stained smears of patients' PMNs appeared normal. Observation of patients' PMNs by phase contrast microscopy demonstrated normal locomotion and engulfment of particles. Cultured macrophages from the patients were similar in appearance to control macrophages. Granules of patients' PMNs stained positively for peroxidase [25].

NBT Tests. The four brothers had no PMNs which reduced NBT; normal subjects and carriers had at least 10 per cent NBT-positive cells. Similarly, none of the brothers' macrophages reduced NBT, whereas greater than 15 per cent of normal macrophages were NBT-positive. The patients' phagocytizing PMNs failed to show any increase in quantitative NBT reduction above resting, compared to an increase of 0.15 ± 0.015 SEM OD_{515} by normal PMNs ($n = 17$). Carriers were identified by quantitative NBT reduction intermediate between that found in normal subjects and the brothers' PMNs. The mother showed an increase of 0.042 OD_{515}, the 17 year old daughter an OD_{515} change of 0.037, and the four month old daughter an increase of 0.003 OD_{515}. Results of NBT testing in the family are shown in Figure 2. Demonstration of the carrier state in the mother of the brothers and in their daughters establishes an X-linked recessive mode of inheritance.

PMN Bactericidal Activity. Normal PMNs killed Staph. aureus more effectively than the brothers' PMNs as shown in Figure 3. Similar results were obtained with two other catalase-positive bacteria, Esch. coli and

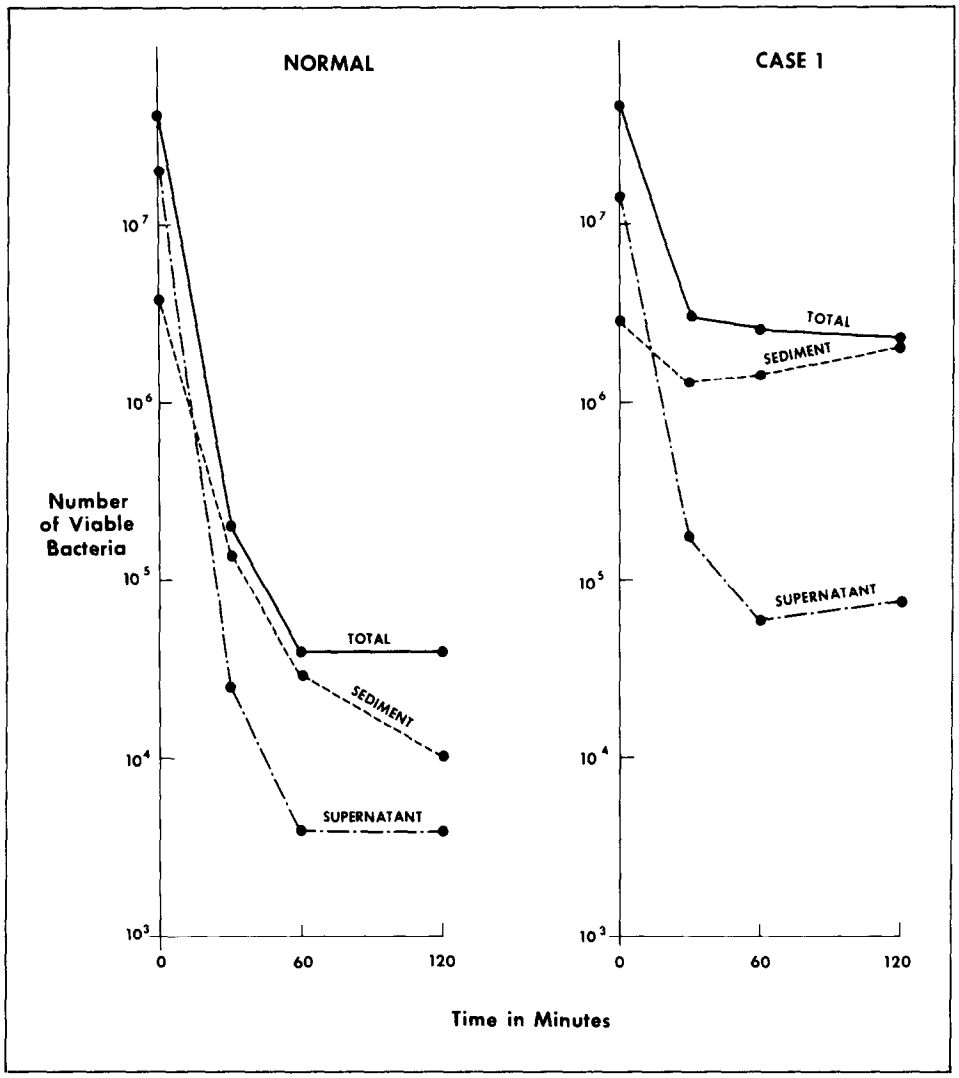

Figure 3. *Representative total, sediment (PMN-associated) and supernatant (PMN-free) bacterial counts in simultaneously run study of function of PMNs from patient and normal subject. This study demonstrates abnormal aerobic bactericidal capacity of PMNs from the patient (Case 1) for Staph. aureus.*

Serratia marcescens; however the peroxide-producing, catalase-negative Strep. faecalis (Enterococcus) was killed normally (Figure 4). Under anaerobic conditions, patients and normal PMNs killed bacteria similarly, with poor bactericidal activity against Staph. aureus (Figure 5) and good activity against Strep. faecalis.

Phagocytosis of Bacteria. Although patients' PMNs had significantly decreased numbers of cell-associated C^{14}-labeled bacteria at isolated time periods, no significant differences were consistently found (Figure 6). Therefore, uptake of bacteria by the brothers' PMNs was essentially normal.

PMN Metabolism. Results are summarized in Table I. In contrast to normal PMNs, patients' PMNs failed to increase oxygen consumption after phagocytosis. Hydrogen peroxide production, as measured by the oxidation of ^{14}C-1-formate, was also reduced. The activity of the hexose monophosphate shunt, measured by the oxidation of ^{14}C-1-glucose in patients' PMNs, was also markedly impaired. However, the shunt could be stimulated by the addition of methylene blue, indicating the presence of leukocyte G-6-PD. Actual G-6-PD levels were normal in Case 1: 2.32 ± 0.32 OD_{340} versus 2.46 ± 0.45 OD_{340} in eight normal subjects.

Protein Iodination by PMN. The ability of patients' PMNs to iodinate protein after phagocytosis was only one-third that of normal PMNs (Figure 7). Iodination could be completely blocked by sodium azide, indicating that the halogenation process was mediated by myeloperoxidase and hydrogen peroxide.

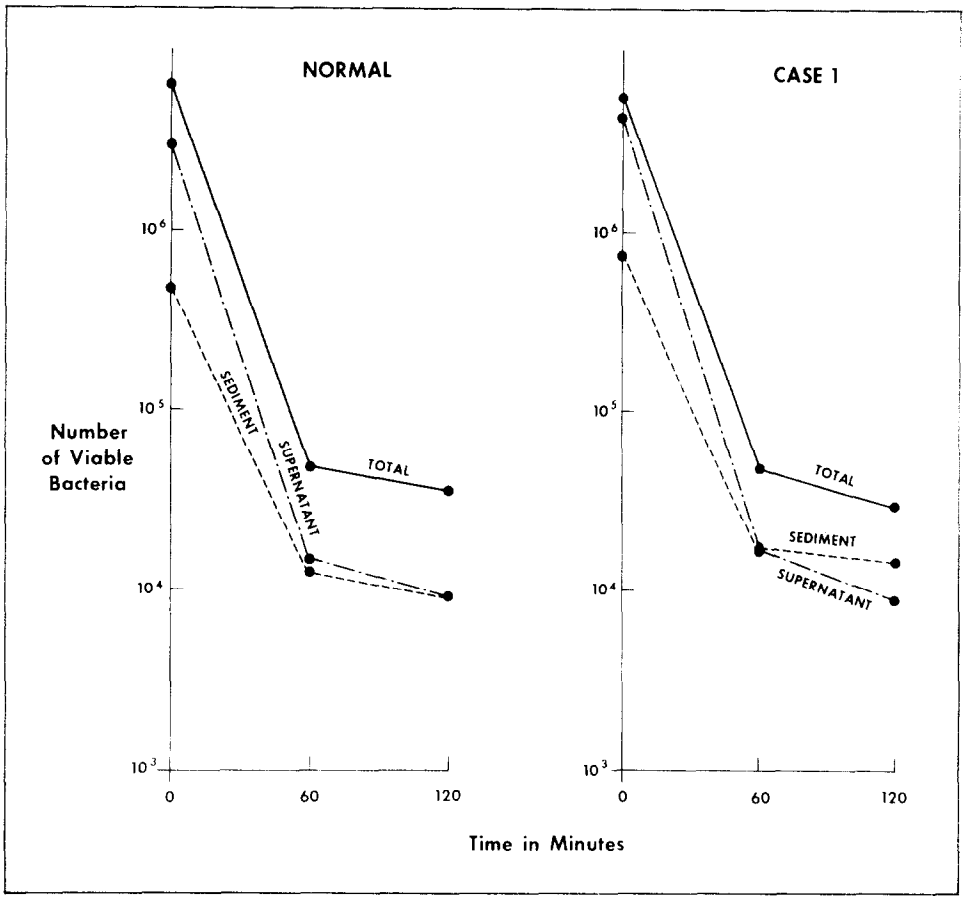

Figure 4. *Differential centrifugation tests demonstrating normal aerobic bactericidal capacity of PMNs from patient (Case 1) for Strep. faecalis (Enterococcus).*

COMMENTS

Many of the brothers' clinical and laboratory features closely parallel those of children with CGD. In both groups, there are frequent infections of lungs and lymph nodes, pulmonary fibrosis on chest film, and hypergammaglobulinemia. Granulomas are seen in children with CGD and were found in a lung biopsy specimen of one of the brothers. Both groups inherit the disease as an X-linked recessive. However, there are important differences. Unlike the four brothers, most patients with CGD have the onset of their disease in infancy, have infections of other organs in addition to those in the lung and lymph nodes, have hepatosplenomegaly, and experience anemia and leukocytosis [1]. Two of the brothers were found to be anergic to common skin test antigens, although most patients with CGD have normal cellular immunity [26]. One of the brothers had arthritis, a finding not seen in children with CGD, although it has been described in some of the CGD carrier mothers [13,27]. Of course, the most striking dissimilarity is that most children with CGD die by adolescence.

Similarities and differences were also found in the phagocytic cell function of the brothers and of children with CGD. PMN morphology, peroxidase content, G-6-PD levels and phagocytosis were normal. As in children with CGD, postphagocytic metabolic studies revealed almost no NBT reduction, oxygen consumption, hydrogen peroxide production and hexose monophosphate shunt activity in PMNs from the brothers. However, unlike the nearly zero iodination and aerobic killing of catalase-positive bacteria by typical CGD neutrophils, PMNs from the brothers showed some killing and iodination of bacteria, albeit diminished from normal. Such intermediate levels of killing and iodination are also seen in PMNs from carriers of CGD [28], since roughly one half of their PMNs have the CGD defect as predicted by the Lyon hypothesis. However, unlike the four brothers and patients with CGD, carriers are not susceptible to frequent infection and their "good" PMNs reduce NBT. We believe, therefore, that there are enough similarities among the brothers and children with CGD to classify these brothers as having a variant of CGD.

A history of frequent infections plus deficient PMN bactericidal activity against Staph. aureus is not sufficient to make a diagnosis of CGD, since there are adults

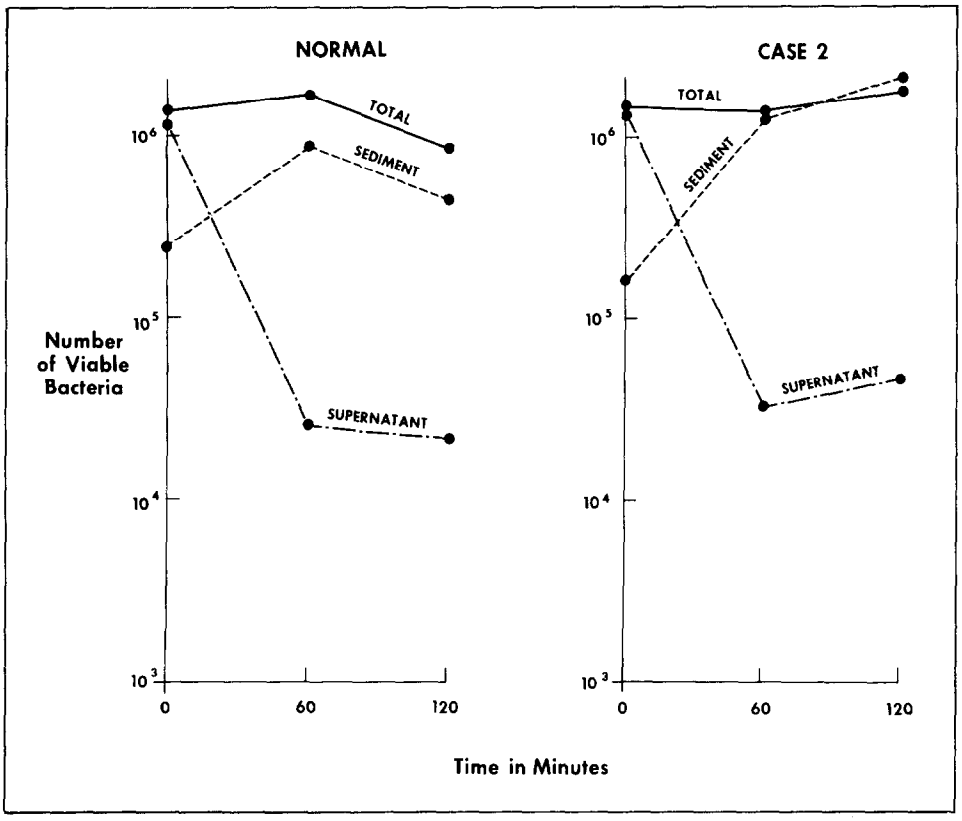

Figure 5. *Differential centrifugation tests showing equivalent impaired bactericidal capacity against Staph. aureus by normal PMNs and PMNs from patient (Case 2) in an anaerobic environment.*

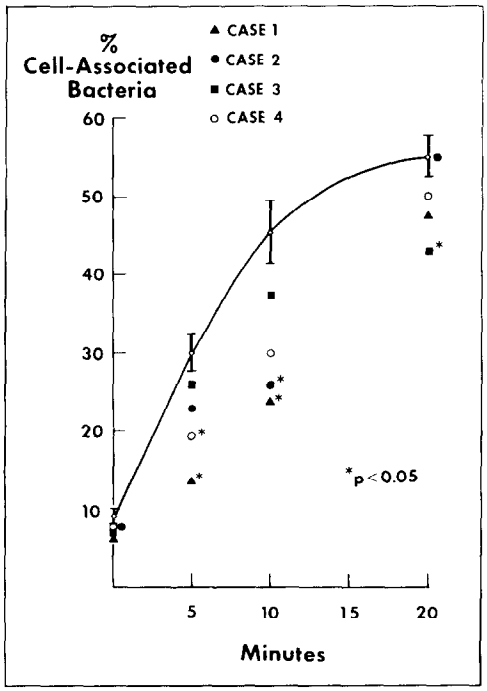

Figure 6. *Phagocytic uptake of C^{14}-labeled bacteria. The solid line was obtained using normal PMNs with the means and SEM shown (n = 10). The isolated points (see key) represent PMNs from the individual patients. For each time period, the per cent of bacteria associated with PMNs from a patient was compared to that associated with normal PMNs using the Student's t test.*

TABLE I PMN Metabolic Studies

Study	Normal (mean ± SEM)	Per Cent Increase After Phagocytosis			
		Case 1	Case 2	Case 3	Case 4
Oxygen consumption	1,335 ± 169 (n = 4)	25	0	100	0
^{14}C-1-formate oxidation	112 ± 5 (n = 4)	0	16	14	19
^{14}C-1-glucose oxidation	513 ± 96 (n = 4)	100	35	50	16
^{14}C-1-glucose oxidation with methylene blue	343	645	. . .	. . .	. . .

with isolated staphylocidal defects but with normal killing of other catalase-positive bacteria, normal NBT reduction and normal PMN oxidative metabolism, which clearly separates them from patients with CGD [29,30]. Granulomas are probably not necessary features of the disease, since there are a few children with typical CGD in whom no granuloma was found at autopsy [1]. Certain minimum criteria are necessary to establish a diagnosis of CGD. These include a history of frequent infections, demonstration of absent NBT reduction by PMNs, the finding of impaired killing of catalase-positive bacteria and the confirmation of normal leukocyte G-6-PD activity.

Three adults with leukocyte G-6-PD deficiency have been described with increased susceptibility to infection and with defects in PMN killing and oxidative metabolism similar to those observed in children with CGD [31,32]. These adults have also had erythrocyte G-6-PD deficiency with hemolytic anemia. However, a normal reticulocyte count alone may not adequately exclude G-6-PD deficiency. The 52 year old patient of Cooper et al. [31] had no detectable G-6-PD in her neutrophils, compared to low levels in her red blood cells. It is theoretically possible to have absent leukocyte G-6-PD activity predisposing to infection but adequate erythrocyte levels with little or no hemolysis. Thus, to exclude

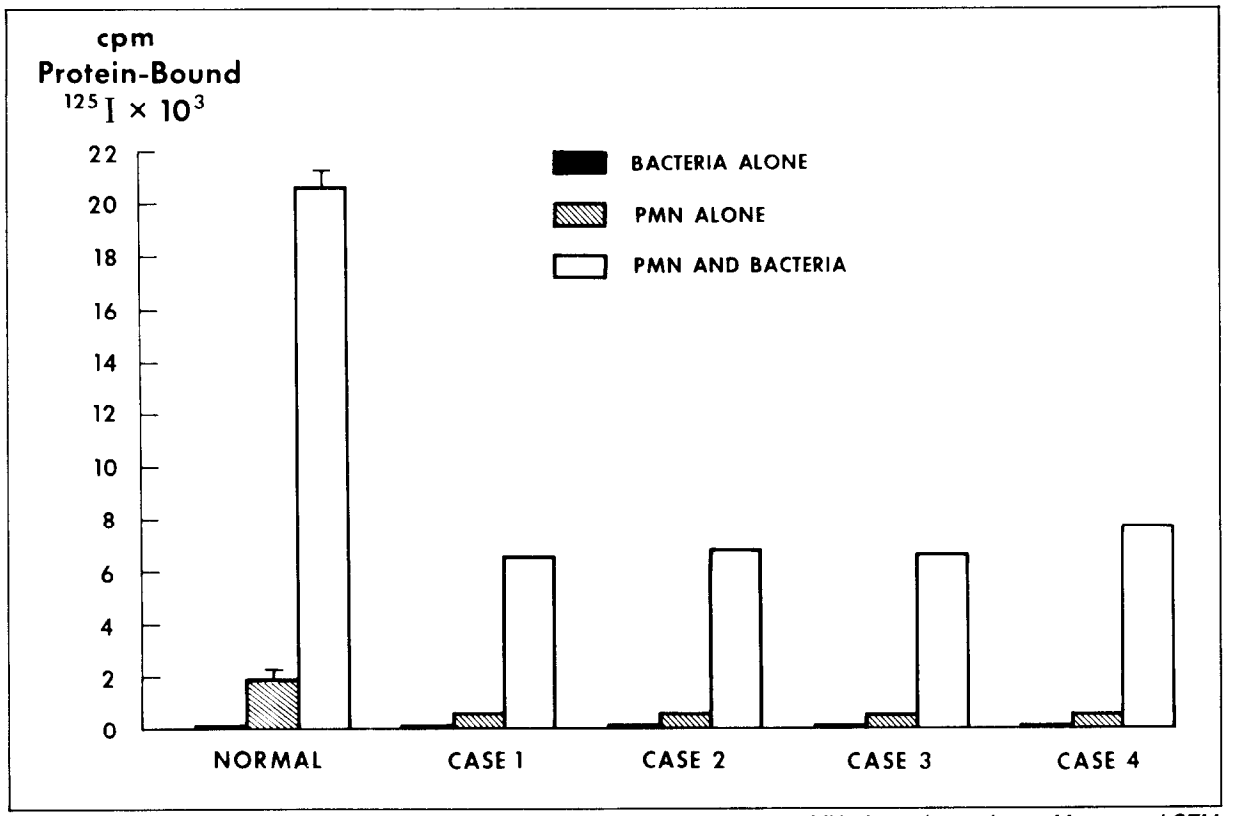

Figure 7. *Protein iodination by leukocytes, showing diminished iodination by PMNs from the patients. Means and SEM are indicated for five normal donors.*

leukocyte G-6-PD deficiency, either actual measurement of G-6-PD levels or stimulation of the hexose monophosphate shunt with methylene blue should be performed.

Three of our patients have pulmonary fibrosis, with respiratory insufficiency, whereas most adults with CGD have been spared this development. The pulmonary disease in this family was previously diagnosed as farmer's lung, sarcoidosis and idiopathic familial pulmonary fibrosis. It is most probable that their lung disease is a result of repeated destruction of lung tissue by infection. Thus, CGD should be added to the long list of causes of pulmonary fibrosis and be screened for by the NBT test in patients with a history of frequent pulmonary infections.

The mode of inheritance of CGD in adults has been difficult to establish, since studies of the PMNs of the parents have usually disclosed no abnormalities. X-linked recessive inheritance of adult CGD has only been established in three families by demonstrating the carrier state in the mother of the affected males [6–8] and in one family by showing that both sisters of a 25 year old man were carriers [5]. The data from NBT reduction by PMNs in the brothers' family allows confirmation of an X-linked recessive mode of inheritance through three generations. As predicted, the sons of the brothers with CGD are normal and the daughters are obligate carriers.

It seemed logical to look for possible compensatory mechanisms in host defense that would offset the defect in PMNs and allow the patient to survive to adult age. Increased immunoglobulins were noted, yet these levels were no higher than those found in children who died with CGD [1] and probably reflect the humoral response to repeated challenge by infectious agents. Enhanced cellular immunity also does not appear to be the compensatory mechanism since two of the brothers were anergic to commonly used skin test antigens, suggesting impairment of cellular immune response which has rarely been observed in CGD [1,33]. The macrophages of the four brothers showed no NBT reduction, suggesting defects similar to those found in their PMNs. However, the mechanisms of bacterial killing by macrophages have not been fully elucidated and may not be completely linked to oxidative metabolism and NBT reduction [34].

Enhanced phagocytosis by PMNs from patients with CGD has recently been reported [35]. We were unable to confirm this and thus agree with most workers that the ingestion phase is normal [2,4]. Under anaerobic conditions, normal PMNs show impaired killing of certain bacteria, but they can kill other bacteria efficiently [16]. Since the brothers' PMNs do not consume oxygen during phagocytosis, we thought that they might have enhanced capability to kill microbes in the absence of oxygen. However, no such enhancement was observed. Although subnormal, the bactericidal capability of their PMNs probably best explains their prolonged survival. PMNs from most other adults with CGD also show some bacterial killing, whereas PMNs from children who died with CGD exhibit almost no killing of catalase-positive bacteria.

PMN oxidative metabolism in adult CGD has shown equivalent impairment of oxygen consumption, hydrogen peroxide production, hexose monophosphate shunt activity and NBT reduction to that found in fatal CGD [5,7–9,12]. In the brothers, these almost total defects in oxidative metabolism did not correlate with the level of bacterial killing observed. In fact, only the partial defect in iodination of bacteria by the brothers' PMNs paralleled their partial bactericidal defect. Therefore, the iodination assay appears to be the most predictive of bactericidal activity of the tests of oxidative function. Particularly intriguing is the oldest brother with CGD. The bactericidal and metabolic defects in his PMNs are equivalent to those of his younger brothers, but he seems to have "outgrown" his susceptibility to infection, has no lung disease and is essentially healthy. Clearly, then, CGD is a heterogeneous syndrome of variable expression, with the possibility of survival to adult age. Once adulthood is attained, amelioration of the severity and frequency of infections may take place, despite persistence of the metabolic and bactericidal defects in PMNs.

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