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—Review Article—
Children with Chronic Granulomatous Disease

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Patients with CGD are "experiments of nature." An abnormality of genetic coding of small part of a protein (cytochrome b) in the membrane of phagocytic cells results in abnormal oxidative metabolism of these cells. The metabolic defect is critical for production of reactive oxygen radicals, which are necessary for efficient intracellular killing of catalase-positive bacterial and fungal species within phagocytic vacuoles.

Patients with CGD suffer recurrent severe and often life-threatening infections with these same species of bacteria and fungi. Thus clinical evidence is provided for the importance of a normal oxidative response of phagocytic cells during the engulfment process for normal host defense against bacteria. Investigators, intrigued by this remarkable biochemical clinical correlation, have studied human granulocytes with the tools of modern molecular genetics. The abnormal gene has been located and defective-gene products have been identified in CGD patients. This knowledge has provided a basis for therapy of CGD patients with human recombinant interferon gamma, an immunomodulator which stimulates NADPH-oxidase activity in the abnormal granulocytes. Other treatment and replacement modalities are anticipated but most importantly these CGD patients have provided insights into the usually hidden mysteries of nature. We are very grateful to these patients as our teachers.

Key words : CGD, Chemiluminescence, NADPH-exidase, IFN-γ

Introduction and History

Host defense fails in patients with chronic granulomatous disease (CGD) because phagocytic leukocytes (neutrophils, eosinophils, monocytes, and macrophages) do not generate reactive oxygen radicals necessary for intracellular killing of phagocytized microorganisms. Patients suffer recurrent life-threatening bacterial and fungal infections and respond poorly to conventional antimicrobial therapy.

The first clinical description of CGD was by Janeway and his colleagues in 1954. A description of five patients with hypergammaglobulinemia and with severe recurrent infections was presented to the American Pediatric Society. In 1952 Bruton had described agammaglobulinemia and pediatricians began to recognize that defective host-defenses may be related to failure of response of a patient with serious infections to appropriate antimicrobial therapy.

In 1957 Good and his colleagues described a clinical syndrome which they call "a fatal granulomatosis of childhood". Children with this syndrome were severely ill and responded poorly to antibiotics in spite of hypergammaglobulinemia. Appropriate numbers of circulating leukocytes with normal morphology were present but abscesses and granulomas in the lymph nodes, subcutaneous tissues, lungs, liver, gastrointestinal tract, and bone produced severe morbidity and death at an early age. This clinical syndrome was considered a disorder of the reticuloendothelial system because of an exaggerated granulomatous response.

In 1967, Quie observed that peripheral blood granulocytes from patients with this syndrome failed to kill staphylococci even though phagocytosis by granulocytes was normal[1]. CGD granulocytes also failed to respond to phagocytosis with an oxidative, metabolic burst, and the association between this abnormal response of phagocytic cells and defective microbial killing was appreciated. Investigation of granulocytes from children with chronic granulomatous disease during the ensuing 20 years in Japan and throughout the world has remarkably increased our understanding of normal genetics, biochemistry, and physiology of the phagocytic system.

Clinical Manifestations of Patients with CGD

Patients with CGD suffer recurrent severe infection which respond poorly to antibiotics. The most common pathogens encountered in CGD patients are catalase-positive organisms, because catalase prevents the CGD phagocytes from using microbial-generated H_2O_2 for killing these pathogens. Etiologic agents associated with these infection are frequently *Staphylococcus aureus*, *Aspergillus fumigatus*, and a variety of Gram-negative entirc bacilli.

Chronic supperative lymphadenitis is a frequent clinical presentation, especially during the first years of life. Patients with CGD have skin lesions which characteristi-

cally heal slowly and granulomatosis of the skin may persist for weeks or months. Skin manifestations in older patients include healed scars of lesions or surgical procedures. Abscesses characteristically heal slowly and leave prominent scars. Patients with CGD may achieve a nearly normal life span and it is not surprising that infectious disease associated with an older age group is identified in CGD patient.

Pneumonia is the most frequent infectious disease diagnosis in patients with CGD. X-ray manifestations are highly variable but are usually associated with hilar-node enlargement. Lung abscesses are common. The liver is also a frequent target organ and abscesses usually require prolonged antimicrobial therapy, and surgical intervention. Invasive aspergillus infections of the lungs are a frequent cause of death in CGD patients.

Bone infections in CGD patients are usually caused by *Staphylococcus aureus*. However, osteomyelitis caused by *Serratia marcescens* or aspergillus occurs frequently and requires aggressive antibiotic therapy. Prolonged treatment is followed by return to normal function.

Obstructive lesions of the gastrointestinal tract or genitourinary tract are a consequence of the exaggerated granulomatous process stimulated by persistent infections in CGD patients. Corticosteroid therapy has been used in several CGD patients with obstructive lesions and may accelerate recovery.

The persistence of microorganisms or microbial products because of defective phagocyte function results in an exaggerated granulomatous response and prolonged morbidity.

Functional Abnormalities of Phagocytic Cells from CGD Patients

The observation of Quie and his colleagues in 1967 that granulocytes from CGD patients could not kill *Staphylococcus aureus* and other catalase-positive microbes and that the oxidative response during phagocytosis was defective suggested the importance of reactive oxygen radicals such as superoxide and hydrogen peroxide in intracellular microbial killing[1].

The diagnosis of CGD can be made by determining the capacity of neutrophils to generate reactive oxygen radicals when these cells are stimulated with either particulate or soluble stimuli. Measurement of the respiratory burst activity of granulocytes is a sensitive and direct way of diagnosing CGD and the nitroblue tetrazolium (NBT) test has become a standard method used for assaying respiratory burst activity in granulocytes. NBT is a yellow soluble dye which forms a purple precipitate when reduced by association with reactive radicals. This precipitate is visible by light microscopy in normal phagocytic cells responding to stimulation with a respiratory burst. NBT is not reduced by phagocytic cells from CGD patients since there is no respiratory burst. CGD carriers may be identified by the NBT test since CGD carriers have two populations of circulating granulocytes, i.e., granulocytes which reduce NBT and granulocytes which do not reduce NBT. These two populations of granulocytes are easily observed in peripheral blood smears from CGD carriers after incubation with opsonized bacteria or soluble stimulants.

The chemiluminescence response during phagocytosis or after stimulation with phorbol myristate acetate (PMA) is also useful for measuring the respiratory burst activity of granulocytes. Chemiluminescence measures light emission which is a consequence of the respiratory burst generated by cell-membrane NADPH-oxidase activity and therefore reliable for diagnosis of CGD.

Prenatal diagnosis of CGD is possible by incubating fetal blood with NBT or by using chemiluminescence assay to determine the respiratory burst of fetal granulocytes.

Genetic Classification of CGD Patients

Genetic transmission of CGD is X-linked in 60-70% of patients but CGD patients also demonstrate autosomal recessive inheritance[2)3)]. A majority of CGD patients have X-linked inheritance, therefore mothers and sisters of these boys are carriers and diagnosis of the carrier state is made by studying the microbicidal activity, NBT reduction, or chemiluminesence of their granulocytes. Microbicidal capacity, NBT reduction, and chemiluminescence may be near normal or almost absent in mothers and sisters of X-linked CGD patients. These observations are consistent with the Lyon hypothesis of random inactivation of the X-chromosome and certain carriers demonstrate extreme lyonization. Female CGD patients with few NBT-positive granulocytes and less than 10% of the expected chemiluminescence response to PMA. Stimulation, may have clinical manifestations of CGD as severe as X-linked male CGD patients.

Autosomal recessive inheritance of CGD has been documented in 20 to 40% of CGD patients. Both male and female siblinlg have clinical manifestations of CGD and consanguinity is not unusual in autosomal recessive CGD families.

The critical role of cytochrome b in the oxidative metabolic response has made measurement of this cellular component extremely valuable for determining the genetic basis of CGD. Patients with CGD can now be classified into five genetic forms. X-linked and cytochrome b negative patients are the majority (60-70%). X-linked and cytochrome b positive patients are rare. Twenty to forty percent of CGD patients are autosomal recessive and cytochrome b positive ; autosomal recessive, cytochrome b negative patients are rare (Table 1). The NAPDH-oxidase response to stimulation is abnormal. The oxidative

metabolic response in phagocytic cells is extremely complex and genetic defects involving one of many critical reactions necessary for a normal oxidative response result in the clinical phenotype known as CGD[4].

We recently reported 62-year-old X-CGD patient. The neutrophils, monocytes, and B-lymphocytes of this patient were found to be completely devoid of gp91-phox protein and mRNA expression as well as NADPH-oxidase activity, whereas his eosinophils were completely normal in protein expression and oxidative activity[6].

The gene regulating NADPH-oxidase has been precisely identified recently by Orkin et al[5]. An unusual patient with X-linked CGD contributed to this identification. In addition to CGD the patient also had Duchenne's muscular dystrophy, McLeod abnormality of erythrocytes, and retinitis pigmentosa. Deletion in the region of Xp21 of the short arm of the X-chromosome localized the chromosome site of these disorders. DNA probes which recognized restriction fragment length polymorphism provided evidence that the gene for X-CGD was Xp21.1. Results from hybridization techniques suggested that the protein coded by this gene was the gp91 protein of cytochrome b. The X-CGD protein therefore is a component of the neutrophil NADPH-oxidase system. The gp91 heavy chain must combine with a p22 light chain for a complete cytochrome b and for normal electron transport. The gene coding for the gp91 polypeptide is totally independent of the gene coding for the p22 polypeptide.

Biochemical Abnormalities of Phagocytic Cells from CGD Patients

The phagocyte NADPH-oxidase system is responsible for transfer of electrons from NADPH to molecular oxygen, which changes oxygen to an unstable radical known as "superoxide". The phagocytic cell NADPH-oxidase is dormant until activated by phagocytosis of bacteria or other opsonized particles. Activation may also result from stimulation with soluble factors such as chemotactic peptides or PMA. Electron transport between NADPH and molecular oxygen may require multiple factors by a critical component is cytochrome b. The role of cytochrome b in the respiratory burst of phagocytic cells was established when the spectral activity of cytochrome b was found to be absent in almost all patients with X-linked CGD and abnormal in rare patients with autosomal recessive CGD[7]. Patients with CGD have also been found to have low levels of flavin, a cofactor in the oxidase system and necessary for normal electron transport. The necessity for phosphorylation of phagocyte proteins for normal NAPDH-oxidase has also been established by studies on granulocytes from CGD patients.

At least four separate components of cytochrome b are necessary for activation of the NADPH-oxidase system for electron transport and for production of superoxide. There are two membrane components of cytochrome b : gp91 beta chain and p22 alpha chain. There are two cytosolic components of cytochrome b : p67 and p47. When granulocytes are activated, the cytosolic proteins become associated with membrane components of cytochrome b. Phosporylation of the p47 cytosolic protein appears to be one of the earliest events during activation. Phosphorylation in normal granulocytes may be triggered by separate pathways : one of these may be activation of specific surface receptors via formal peptides; another may be stimulation of a protein kinase without receptor requirements, i. e., stimulation with PMA ; and still another may be activation of the arachidonic acid pathway which occurs during phagocytosis of opsonized particles (Fig. 1).

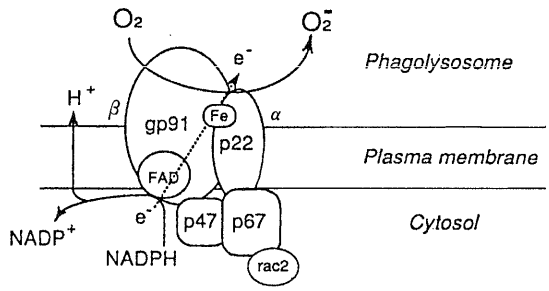

Fig. 1. The human neutrophil NADPH-oxidase complex consists of two cytochrome b membrane subunits, an α (p22) subunit and β (gp91) subunit, and cytosolic cofactors (p67) and (p47), required for the respiratory burst. A soluble ras family protein rac-2 is also required.

Table 1. Classification and Incidence of CGD on Molecular Defects of Neutrophil

Genetics	Incidence%		Cytochrome b	Defect
	Western country	Japan		
X-linked	50~55	76	Absent	Expression of cyt. b gp91 subunit
	5	2.3	Present	Function of cyt. b
Autosomal	30~35	8.0	Present	p47 cytosolic factor absent
	5	8.0	Present	p67 cytosolic factor absent
	5	5.7	Absent	Expression of cyt. b p22 subunit

Treatment of CGD Patients

The major effort in treatment of CGD patients is prevention and cure of bacterial and fungal infections. Once the etiology of an infectious process has been determined, vigorous aggressive treatment of infection is required.

Surgical help is frequently needed for obtaining a diagnosis and for drainage or excision of abscesses.

Infections are usually prolonged in CGD patients and therefore careful search for the offending microbial species is extremely important. Knowledge of the sensitivity of infecting microbes to antibiotics and ability of antibiotics to penetrate the infectious site are equally important considerations. Intravenous administration of antibiotics for several weeks is usually necessary to cure an infectious process.

The value of prophylactic antibiotic therapy with trimethaprim-sulfamethoxazole was first reported from Japan and is now standard practice throughout the world[8]. Patients who develop adverse reactions to sulfonamides may be treated with trimethaprim alone. Sulfasoxazole has been used in CGD patients because of improvement in the bactericidal capacity of CGD granulocytes.

An association between CGD and the Kell antigen system of erythrocytes exists and blood transfusions should be avoided if possible. Since certain CGD patients may not have these antigens on their erythrocytes, antibodies will develop after a transfusion and subsequent transfusions will be extremely hazardous.

Granulocyte transfusions are useful in patients with severe abscess lesions that do not respond to appropriate antibiotics or surgical drainage. Granulocyte treatments must include at least 10^9 cells in each transfusion and a minimum of 5-10 transfusions.

Bone marrow transplantation has been used to treat several patients with CGD[9]. Two populations of circulating granulocytes are observed in the circulation of successfully transplanted CGD patients. NBT-positive granulocytes are the progeny of the donor's cells and NBT-negtative granulocytes are the patient's cells. But little success because of Graft rejection and partial engrafment.

Treatment with recombinant human interferon gamma is an exciting new development in the care of patients with CGD. The X-CGD gene responds to interferon gamma, a lymphokine secreted by activated helper T-lymphocytes. Interferon gamma increases NADPH-oxidase activity of granulocytes and mononuclear phagocytes and therefore enhances the microbicidal activity of the cells[10]. The increased NAPDH-activity in interferon gamma-treated cells is a consequence of increased production of the gp91 submit of cytochrome b, with increased production of reactive oxygen radicals and enhanced intracellular killing of microbes. Recombinant human interferon gamma has been used for the treatment of leprosy or cancer in more than 1,000 patients and is relatively free of adverse reactions.

Treatment of CGD patients with interferon gamma has been reported[11]. A several years ago, a large international study with 128 CGD patients showed that prophylactic treatmeant with recombinant human γ-interferon (rhIFN-γ) administered reduced the number of new infections and improved the response to existing infections. There was no difference in neutrophil superoxide production and killing of *Staphylococcus aureus* between the rhIFN-γ and the placebo group[12]. The in vivo effect of rhIFN-γ is even greater than in vitro effects, which suggests that the cytokine acts on bone marrow progenitor cells. rh-IFN-γ treatment may also have an effect on non-oxidative bactericidal mechanisms of CGD granulocytes.

Attempts to provide gene therapy employing gene transfer of the X-CGD cDNA are in progress.

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