Understanding Chronic Granulomatous Disease

CGD is caused by the inability of neutrophils to fight bacterial or fungal infections; however, many treatment options are available, and a gene therapy curative option is being explored.

By Bob Geng, MD, MA

THE FIRST DESCRIPTION of chronic granulomatous disease (CGD) was in 1954 by Charles Janeway, MD. The common paradigm at the time was that immunodeficiency characterized by recurrent infections was a function of low immunoglobulin (IgG) levels. However, Dr. Janeway described several male patients who had recurrent infections and an enlarged liver and spleen, but had elevated IgG levels. Over the next several years, more cases of similar presentation along with granuloma formation and severe inflammation were reported. In the late 1960s, Staphylococcus was discovered to be the predominant form of infection in CGD patients, and while neutrophils in CGD could ingest these organisms without difficulty, they could not digest them. In the 1970s, the insufficient production of superoxide due to defect of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was discovered for CGD. The genetic discoveries of specific defects leading to CGD were made in the 1980s and 1990s, which greatly improved our understanding of the disease.

Cause of CGD

CGD is a disease that results from the inability of the cells in the innate immune system (neutrophils and monocytes) to make superoxide compounds that can effectively kill bacteria or fungus that the immune cells ingest. Neutrophils are the main cells of the innate immune system, and they respond quickly to bacterial or fungal infections. Normally, neutrophils are able to produce an "oxidative burst" from the production of hydrogen peroxide from superoxide that kills the bacteria. An enzyme called NADPH oxidase, which is made up of six different proteins, accomplishes this process. Mutations in five of the six components can lead to CGD. These six components are gp91phox, p22phox, p47phox, p67phox, p49phox and Rac2 (mutation in the sixth component causes a different disease). When the neutrophil is in a resting state before encountering any bacteria or fungus, these components are separate. Once a normal neutrophil is activated through encountering bacteria or fungus, these components combine together to initiate the "oxidative burst" process that kills the ingested organism. Mutations in any one of the components will lead to a defective NADPH oxidase that is incapable of producing the necessary compounds to kill the ingested invading organism.

Since neutrophils cannot effectively clear out the bacterial or fungal infections in CGD, the immune system forms granulomas to wall off the infections in an effort to contain them. The formation of the granulomas is the phenomenon that gives rise to the name CGD.

Epidemiology of CGD

The prevalence of CGD is estimated to be approximately one out of 200,000 live births. But, this statistic may be an underestimate because it is not systematically screened like severe combined immunodeficiency. Diagnosis can occur at any age because of different times of symptom manifestation, but generally the majority of diagnoses are made in children. However, autosomal recessive forms of the disease may present with a milder phenotype with delayed diagnosis into adulthood.

The most common form of CGD is the X-linked type, meaning the defect is located on the X chromosome, so the condition is only seen in males (Figure 1). The X-linked form (gp91phox) accounts for 65 percent of CGD cases. There are three major autosomal recessive forms (which need two copies of the same defect to manifest clinical presentation of disease), with the defect on chromosome 7 (p47phox) accounting for 25 percent of all known CGD cases. The remaining two forms of autosomal recessive CGD (p22phox and p67phox) each account for less than 5 percent of known cases. There are no known autosomal dominant forms of the disease.

Clinical Presentation of CGD

Most CGD patients present symptoms in early childhood, but presentations later in adulthood also occur. Later onset of disease can be the result of the autosomal recessive mutation. Delayed diagnosis may also occur because symptoms are better controlled in developed countries with lower levels of bacterial/fungal exposure, as well as use of strong antibiotics for infections. The predominant presentations of CGD are infections that can occur in the liver, skin, lungs and lymph nodes (Figure 2). In the U.S., CGD-related infections are generally from Staphylococcus aureus, Nocardia species, Serratia marcescens, Burkholderia cepacia and aspergillus. In many other places in the world, tuberculosis, Bacillus Calmette-Guerin (BCG from vaccination) and Salmonella are common causes of infections in CGD patients. With the exception of Staphylococcus aureus, all the other pathogens rarely cause disease in non-CGD patients. Fungal agents such as aspergillus cause common infections in CGD patients, and initial presentation can be difficult to appreciate, leading to delay in diagnosis.

Staphylococcal liver abscesses occur in a significant number of CGD patients, and they can be difficult to manage. The

Figure 1. X-Linked CGD

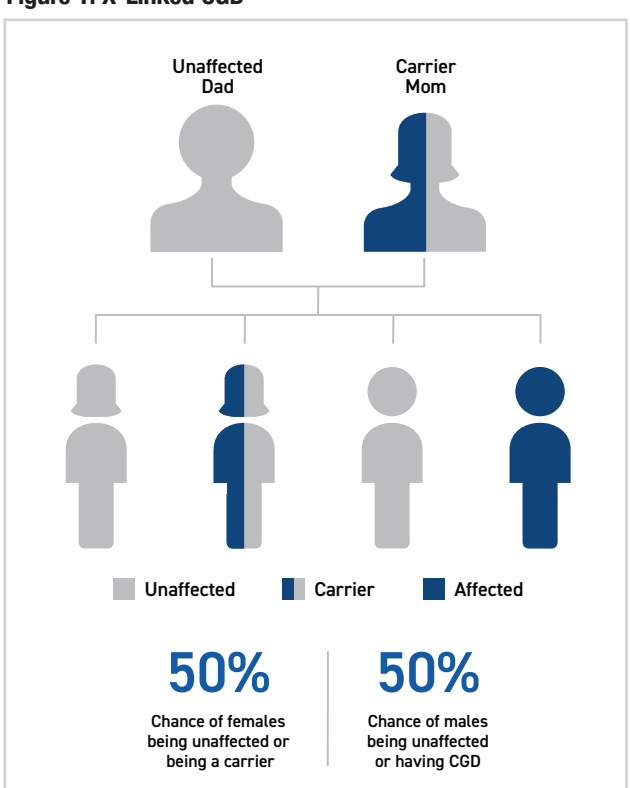
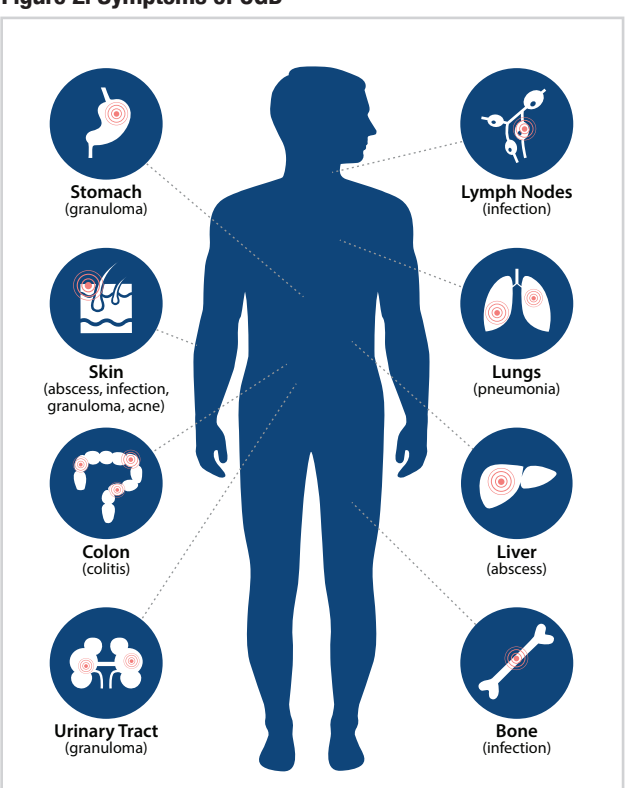


Figure 2. Symptoms of CGD



abscesses cause increases in liver enzymes and, over time, lead to structural complications such as elevation of pressures in the portal veins and abnormal enlargement of the liver and spleen. These complications are often associated with lower platelet counts, which coupled with worsening liver function often lead to significant morbidity and mortality.

Symptoms of both urinary and gastrointestinal obstruction can also be prominent in CGD patients due to the presence of granulomas. Formation of granulomas in the stomach can lead to gastric outlet obstruction, resulting in early satiety, nausea, decreased gastric emptying and severe vomiting. Blockage from granulomas can occur anywhere along the gastrointestinal tract such as the esophagus, stomach, small intestines, large intestines or rectum, leading to signs and symptoms of obstruction. Granulomas in the bladder can lead to obstruction in the urinary tract, leading to complications of chronic kidney disease and difficulty voiding urine.

Aside from severe infectious complications and structural blockage from granulomas, another significant clinical manifestation seen in CGD is from severe inflammation. The infectious agents that are exposed to the compromised innate immune system of CGD patients lead to excessive inflammation. This poorly controlled inflammatory response can directly impact the function of the lungs, bladder and gastrointestinal tract, leading to severe illness and disability. Excessive inflammation can also lead to signs and symptoms of skin disease and poor wound healing, as well as complications in oral health such as gum disease and ulcer formation.

Carrier Patients

Female carriers of the X-linked form of CGD generally do not present with infections. However, if the level of normal functioning neutrophils falls below 5 percent to 10 percent, they can develop CGD-type infections. As these individuals age, they can develop autoimmune complications of disease due to excessive inflammation such as discoid lupus, oral ulcers and photosensitive rashes. Therefore, a more nuanced way to understand these individuals is to transition from the perception of them purely as carriers toward monitoring potential evolution to heterozygote (having two different alleles of a particular gene or genes) symptomatic patients. These patients should be followed longitudinally for development of both autoimmune and infectious complications.

Diagnosing CGD

There are three main ways to test for CGD. The oldest and most well-known test is the nitroblue tetrazolium test (NBT). With this test, a special dye is used to stain neutrophils on a microscope slide. The cells that have normal reducing capacity to form superoxides form an insoluble bluish black compound, whereas the abnormal cells that have abnormal capacity to produce superoxides will not develop a color change. Therefore, a CGD patient's neutrophils will not stain normally, leading to diagnosis. However, this test is rarely performed today due to interoperator variability, its inability to detect more subtle forms of disease and its difficulty in quantifying degree of disease severity.

The NBT has been largely replaced by the dihydrorhodamine test (DHR), which functions by assaying the amount of hydrogen peroxide produced by neutrophils. DHR is a flow cytometry test, meaning that it is a quantitative test based on the counting of response of individual cells. This assay relies on the measurement of fluorescence following the oxidation of dihydrorhodamine by activated neutrophils. Inability to generate this fluorescence leads to the diagnosis of inadequate oxidative capacity of the neutrophil. In addition to diagnosing CGD, the DHR test can also distinguish between the X-linked form versus autosomal recessive forms. The DHR test can also detect female carriers of the X-linked form.

On the DHR test, neutrophils from normal individuals will have a low level of fluorescence in the resting state and significantly elevated level of fluorescence following stimulation. Neutrophils from classic X-linked CGD will demonstrate no change in degree of fluorescence following stimulation. Neutrophils from autosomal recessive CGD will result in a wider variation and lower degree of fluorescence compared to neutrophils from normal individuals following stimulation. Neutrophils from asymptomatic carriers of X-linked CGD can classically demonstrate two peaks following stimulation, showing that these individuals possess both normal cells and abnormal cells. Neutrophils from potentially symptomatic X-linked carriers can appear differently with a smaller peak in fluorescence following stimulation, followed by a much larger wider peak that shows most neutrophils are not responding adequately to stimulation.

The third method of detection of CGD is through direct genetic testing. For the X-linked form, the defect is in the CYBB gene on chromosome Xp21. The most common autosomal recessive form is from a defect in the NCF1 gene on chromosome 7. The other remaining autosomal recessive types are the result of defects of the CYBA gene

on chromosome 16 and the NCF2 gene on chromosome 1q42. The challenge with genetic testing is that not all testing companies sequence the NCF1 gene, which is responsible for the most common autosomal recessive form of disease. It is important to evaluate genetic testing and the DHR test along with the clinical presentation to help formulate diagnosis.

Managing CGD

There are several aspects to treating CGD. Acute infectious and inflammatory complications need to be managed with the appropriate antimicrobials, as well as immunosuppressive medications. Antimicrobials need to be selected based on their activity against the particular offending bacteria or fungus. Immunosuppressive medications need to be used to manage autoimmune complications. Liver abscesses in CGD need to be managed by a combination of both antibiotics and steroids for inflammation reduction. In general, steroids can help reduce the complications associated with the formation of granulomas and the hyper-inflammation seen in CGD following exposure to infectious agents. Biologic immunosuppressive medications should be used with caution in CGD patients due to concern for increased risk of serious infections.

The cornerstone of CGD management is prevention. The recommended preventive regimen includes the use of antibiotics, namely trimethoprim-sulfamethoxazole (TMP-SMX), antifungal (itraconazole) and interferon gamma for immunomodulation. TMP-SMX is the prophylactic antibiotic of choice due to its concentration inside neutrophils. It should be administered at 5 mg/kg per day up to 320 mg in two divided doses. If the patient is allergic to sulfa drugs, then TMP, fluoroquinolones or a cephalosporin can be considered instead. Itraconazole should be administered as 100 mg per day for children under 13 years old or who weigh less than 50 kg, and 200 mg per day for those older than 13 years or who weigh more than 50 kg.

CGD is one of the first diseases that have used immunomodulatory cytokine therapy in its routine management. Interferon gamma has been shown in a multicenter international double-blind placebo-controlled study of 128 patients to reduce development of serious infections by 67 percent versus placebo. Secondary endpoints of the study also showed there was a 67 percent decrease in hospitalization days in the treatment group versus placebo. Interferon gamma should be administered subcutaneously at 50 mcg per meter-squared body surface area three times a week for those with body surface area greater than 0.5 meters-squared and weight-based dosing at 1.5 mcg/kg per dose when body surface area is less than 0.5 meters-squared.

Immune globulin therapy is not typically used to treat CGD. Historically, CGD was first described as recurrent infections in the setting of hypergammaglobulinemia. The defect is not in the adaptive immune system or the production of antibodies. There have been rare case reports of some CGD patients with hypogammaglobulinemia, but it is uncertain whether those cases really represent two different deficiencies in the same patient.

CGD patients should receive all routine vaccinations, including live virus vaccines because CGD does not reduce the body's immune response against viruses. However, CGD patients should never receive the BCG vaccine due to potential of developing life-threatening infection from BCG infection. This can be a concern in most countries around the world due to routine policies of BCG vaccination; however, it is generally not a concern in the U.S. because BCG vaccines are not generally administered.

The only approved form of curative therapy for CGD is hematopoietic stem cell transplantation. While transplant is a curative option, the majority of CGD patients have not been transplanted, and there are significant risks associated with stem cell transplant. Since each type of CGD arises from a single gene defect, there is significant research interest in the possibility of gene therapy as a potential cure.

For X-linked carriers, management should be long-term follow-up and education to raise awareness that they may become symptomatic, and to repeat DHR testing if either autoimmune or infectious complications arise.

Survival in CGD has been shown to correlate with residual oxidative ability of the neutrophils. Therefore, patients with a higher amount of residual oxidative capability will generally have better outcomes. This is consistent with the fact that autosomal recessive forms (higher residual oxidative capability) have better survival than the X-linked type. CGD is an example of the value of genotyping for predicting prognosis and management strategies. IG

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