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Chronic Granulomatous Disease

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Abstract Chronic granulomatous disease (CGD) was first described in the 1950s and has become a paradigm for genetic neutrophil diseases. It is characterized by recurrent infections with a narrow spectrum of bacteria and fungi as well as a common set of inflammatory complications most notably including inflammatory bowel disease. Over the last half century major advances in management have profoundly altered the major clinical issues and the life expectancy of CGD. With X-linked and autosomal recessive forms, it has been an important disease for the development of bone marrow transplantation and gene therapy. Some of the recent developments in infectious syndromes, inflammatory complications, and curative approaches are discussed in this review.

Keywords Chronic granulomatous disease (CGD) · X-linked disease · NADPH oxidase · Nitroblue tetrazolium (NBT) · Dihydrorhodamine oxidation (DHR) · Aspergillus · Granulibacter · Burkholderia

Chronic granulomatous disease (CGD) was first described in 1954 [1] and 1957 [2], but was not well-characterized until 1959 [3], when it was initially termed fatal granulomatous disease of childhood. The obvious limitations of that name became apparent and it is now simply referred to as CGD. Although originally thought to be only an X-linked disease that appeared exclusively in males, its recognition in girls in 1968 led to the determination of autosomal recessive forms as well [4]. Over the last 50 years, we have learned much about CGD, converting it from a disease of tragic and early complications to a disease of chronic management and high survival [5]. It has served as a paradigm for the primary immune defects that are not part of the severe combined immunodeficiency group and has guided us in understanding the importance of oxygen metabolism in the phagocyte, the vasculature, and the brain. Following its genetic determination, it has provided an important target for gene therapy and has been a leading disease for the development of bone marrow transplantation for nonmalignant diseases. Therefore, in the 50th year anniversary of its comprehensive description, it is fitting to take stock of the disease, its management, and its implications.

CGD is a single disease with four genetic etiologies, reflecting that all four proteins go into the composition of the single enzyme complex that catalyzes the transfer of an electron from cytoplasmic NADPH to molecular oxygen (5; OMIM# 306400, 233690, 233700, 233710). Since NADPH is oxidized by this electron harvest, the enzyme complex is known as the NADPH oxidase. Although this process takes place mostly in the phagocyte, it is not exclusively there; therefore, defects in this enzyme have subtle effects in other tissues as well. Furthermore, although the components of the NADPH oxidase are usually considered as phagocyte proteins, in fact only $gp91^{phox}$ is very phagocyte-specific, while the other autosomal components are expressed elsewhere, as well [6]. The components are broken into membrane bound (cytochrome b558, comprised of $gp91^{phox}$ and $p22^{phox}$) and cytosolic ($p47^{phox}$ and $p67^{phox}$) components. The cytochrome components $gp91^{phox}$ and $p22^{phox}$ require each other for expression in the phagocyte. One implication of this is that since $p22^{phox}$ is expressed in other tissues and $gp91^{phox}$ is not, there are other partners that $p22^{phox}$ and the other members of the NADPH oxidase join with in other tissues, which are other members of the *Nox* family of proteins. Therefore, individuals who have autosomal recessive forms of CGD may also have subtle abnormalities in tissues other than leukocytes where these proteins are expressed, such as vascular endothelium or renal epithelium.

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In addition to the structural components of the NADPH oxidase, there are the critical regulatory components $p40^{phox}$ and rac. On cellular activation, the cytosolic components $p47^{phox}$ and $p67^{phox}$ are phosphorylated and bind tightly together. In association with $p40^{phox}$ and rac, these proteins combine with the cytochrome complex ($gp91^{phox}$ and $p22^{phox}$) to form the intact NADPH oxidase. An electron is taken from NADPH and donated to molecular oxygen, leading to the formation of superoxide. In the presence of superoxide dismutase, this is converted to hydrogen peroxide, which, in the presence of myeloperoxidase and chlorine in the phagosome, is converted to bleach. Until recently, the metabolites of superoxide themselves were thought to be the critical mediators of bacterial killing. However, Reeves and coworkers [7] have shown that phagocyte production of reactive oxygen species leads to microbial killing through the activation of certain primary granule proteins inside the phagocytic vacuole. This new paradigm for NADPH oxidase-mediated killing suggests that reactive oxidants are working more as intracellular signaling molecules, leading to the activation of other nonoxidative pathways, rather than causing killing directly. One implication of this understanding is that, in the absence of NADPH oxidase activity, the same enzymes are present but hypofunctional, whereas, upon cellular stimulation, they are more highly activated. This suggests a spectrum of microbicidal activity that can be regulated to distinct degrees, rather than distinct types, such as oxidative and nonoxidative pathways and mechanisms [5].

Mutations in all of the four structural genes of the NADPH oxidase have been found to cause CGD. Mutations in $gp91^{phox}$ account for about 65% of cases, mutations in $p47^{phox}$ about 25%, and the remainder is divided between $p67^{phox}$ and $p22^{phox}$; there are no autosomal dominant cases of CGD. Estimates of frequency are hard to verify, but a large voluntary retrospective study in the United States suggested rates of around 1:200,000 live births. Rates in other countries are roughly similar but vary somewhat depending on the ethnic practices and degrees of intermarriage: Sweden 1/450,000; Japan 1/300,000; Israeli Jews 1/218,000; Israeli Arabs 1/111,000 (reviewed in [8]). Clinically, CGD is quite variable, but the X-linked $gp91^{phox}$-deficient form appears more severe with earlier presentation and diagnosis and more severe infections and earlier death than the $p47^{phox}$-deficient form [5, 8]. Large series of $p22^{phox}$ and $p67^{phox}$ patients are unavailable, so it is not entirely clear which form they are more similar to. The majority of patients are diagnosed as toddlers and young children [5, 8].

Infections and granulomatous lesions are the usual first manifestations. The lung, skin, lymph nodes, and liver are the most frequent sites of infection. In North America, the overwhelming majority of infections in CGD are due to only five organisms: *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Nocardia*, and *Aspergillus*. In other parts of the world, *Salmonella*, Bacille Calmette-Guerin (BCG), and tuberculosis are also important [5, 8, 9]. In contrast to patients with severe combined immunodeficiency or defects in the interferon (IFN)-gamma receptor pathway, CGD patients develop severe localized BCG rather than disseminated infection. Trimethoprim/sulfamethoxazole prophylaxis has reduced the frequency of bacterial infections in general and staphylococcal infections in particular. On prophylaxis, staphylococcal infections are essentially confined to the liver and cervical lymph nodes [5]. Staphylococcal liver abscesses encountered in CGD are dense, caseous, and difficult to drain, requiring surgery in almost all cases [10]. Until recently, fungal infections, typically due to *Aspergillus* species, were the leading cause of mortality in CGD [9]. However, the advent of highly active antifungal therapy with the azole antifungals itraconazole, voriconazole, and posaconazole has changed the face of fungal infections in CGD. Mortality from *Aspergillus fumigatus* infection in CGD is now uncommon and, therefore, overall mortality is diminished.

Overall survival in CGD has changed remarkably over the last decade and is now around 90%, stretching well into adulthood. However, survival is heavily influenced by several distinct factors. Not surprisingly, the period in which the diagnosis was made is crucial to understanding the likelihood of survival. Patients diagnosed before the advent of antifungal azole agents had a very different trajectory of disease, as reflected by the very poor survival of patients into their 30s and 40s in those series [9, 11]. However, following the introduction of itraconazole in the late 1990s and its proof as a potent antifungal prophylactic in 2003 [12], as well as the introduction of more active agents, fungal mortality in particular and CGD mortality overall have plunged. This is easily appreciated in the examination of survival rates in several of the large cohorts reported [11, 13, 14]. Access to care and the expertise of caregivers are also clearly important. One Japanese study showed a 90% survival rate for patients followed up at single center [15]. Similarly, in a longitudinal analysis of 47 patients, Muoy et al. [16] found an 8-year survival rate of 70.5% for children born before 1978 but a 92.9% survival rate for those born later. Winkelstein et al. [9] found mortality for the X-linked form of the disease of about 5%/year, compared to 2%/year for the autosomal recessive varieties, both of which numbers preceded the introduction of oral antifungals. Therefore, it seems very likely that overall mortality from infection in CGD is relatively low and will improve.

In contrast to mortality, the morbidity of recurrent infections and their impact on the child, the family, and

end-organ function remain major issues. Several large studies have shown a relatively similar rate of infection of around 0.3/year [11, 13]. That is, most patients are still experiencing at least one severe infection every 3–4 years, whether bacterial or fungal. This rate has remained rather constant over the last 10 years, despite improved orally available antibiotics and antifungals and increasing familiarity with the disease. This may, therefore, reflect either a relative minimum rate driven by inescapable environmental exposures or the reality of maintaining long-term prophylaxis in the setting of childhood, adolescence, and young adulthood with a disease that is only intermittently intrusive. However, even though imperfect, it is clear that antibacterial prophylaxis is helpful and effective [17].

Several infections are distinctive to CGD and important to consider and recognize. Among gram-negative bacteria, *B. cepacia* complex organisms are common causes of pneumonia and infrequently sepsis [9]. The closely related *Burkholderia gladioli* has also been described in CGD [18]. Interestingly, these organisms are almost exclusively encountered in CGD and cystic fibrosis, but rarely in other forms of lung disease, suggesting a pathophysiologic link between these two diseases, although this has been elusive so far [19]. *Chromobacterium violaceum* causes sepsis in CGD and is found in brackish waters, such as those around the Gulf of Mexico in the United States [20]. *Francisella philomiragia* causes sepsis in CGD and is also found in brackish waters, such as the Chesapeake Bay in the United States [21]. Recently, a novel organism has been described exclusively in CGD, *Granulibacter bethesdensis* [22]. This organism causes necrotizing lymphadenitis and can cause sepsis [23]. It is unusual in that it has been documented over many months in CGD patients and can have latent and active phases, similar to tuberculosis. It has been found in CGD patients from the United States, Panama, and Spain, suggesting that it is widely distributed in the environment.

Among fungi, the list of unusual infections is even more lengthy and obscure, since there are so many species of fungi and so relatively few are medically important. The filamentous molds *Paecilomyces variotti* and *Paecilomyces lilacinus* are rare outside of CGD [24]. *Aspergillus nidulans* is highly pathogenic in CGD but not in any other patient group, including transplant recipients [25]. A newly recognized species, *Neosartorya udagawae*, has been shown to cause a characteristically chronic and progressively invasive infection in CGD, but is rarely encountered in other groups [26]. In contrast to these rare organisms that are virtually pathognomonic for CGD, other fungi are rarely encountered. The endemic dimorphic mold infections histoplasmosis, blastomycosis, and coccidioidomycosis do not occur in CGD, nor does cryptococcosis. Mucormycosis only seems to occur in CGD when significant immunosuppression, such as sustained high dose steroids, has been used [27]. Therefore, the microbiology of infection should be vigorously pursued and can be highly suggestive of CGD as the underlying cause.

Fungi elicit an exuberant inflammatory response in CGD lungs, which is independent of whether the fungi are alive or dead, and in mice, even boiled *Aspergillus* lead to striking pulmonary inflammation [28]. The recently recognized syndrome of "mulch pneumonitis" embodies these phenomena and can either complicate previously recognized CGD or be its initial presentation [29]. In humans, this syndrome is caused by exposure to aerosolized decayed organic matter, such as mulch, hay, or dead leaves. The clinical syndrome is very distinct: a previously well child or adult spreads mulch, turns compost, or clears moldy leaves, inhaling numerous fungal spores and hyphae; about 2 days later, a syndrome similar to pulmonary hypersensitivity begins with fever and dyspnea; chest radiographs show diffuse interstitial infiltrates; bronchoscopy is usually uninformative but may yield *Aspergillus*; lung biopsy shows acute inflammation with necrotizing granulomata and fungi. Successful treatment of this syndrome is with simultaneous antifungals for the infection and steroids for the inflammation [29]. Counterintuitive though it may seem, the use of steroids is crucial for maintaining ventilation and may be life-saving. This syndrome and, therefore, CGD as the underlying diagnosis, should be considered in all cases of otherwise unexplained diffuse infiltrates or *Aspergillus* pneumonitis, especially with acute onset and hypoxia.

The inflammatory complications of CGD are most prominent in the gastrointestinal and genitourinary tracts. Esophageal, jejunal, ileal, cecal, rectal, and perirectal involvement with granulomata mimicking Crohn's disease have been described [30, 31]. Gastric outlet obstruction is especially common and may be the initial presentation of CGD. In a large survey of CGD patients followed up at the NIH, Marciano et al. found that 43% of X-linked CGD patients had symptomatic biopsy-proven inflammatory bowel disease (IBD). In contrast, only 11% of $p47^{phox}$-deficient patients had IBD [30]. However, growth rates were equally diminished below the mean United States levels for both IBD-affected and IBD-unaffected patients. Since gastrointestinal endoscopy was only performed for cause on symptomatic patients, we do not know whether the mild growth retardation seen in most CGD patients was due to IBD in all cases or due to some other CGD-associated feature of the disease. Perirectal disease is especially prominent in CGD [31].

Treatment of CGD IBD is vexing. Steroids are effective but have obvious risks and complications including growth retardation, osteoporosis, and infection risk. However, at the doses typically used in CGD for maintenance, infections are rarely an issue. In contrast, the newer TNF-alpha

blocking agents, infliximab and adalimumab, are highly effective and rapidly suppress bowel symptoms, but carry a very significant risk of infection. In our experience, TNF-alpha inhibitors predispose to characteristic CGD pathogens, only more severe episodes. Our current practice is to initiate therapy for proven IBD in CGD with prednisone 1 mg/kg/day for 1 to 2 weeks and then slowly taper to 0.1–0.25 mg/kg/day over 1 to 2 months. Sometimes, children can be taken off prednisone, but the relapse rate is very high and retreatment typically requires reinitiation of the higher dose. Therefore, after the first recurrence or relapse, we usually add an antimetabolite such as imuran along with salicylic acid derivatives. Local treatments such as steroid enemas and rectal creams can also be highly effective.

In addition to the bowel involvement in CGD, liver involvement is pronounced and important. Liver abscess occurs in around 35% of patients and is difficult to treat successfully without surgery [10]. With surgery, cure of liver abscess is common, but unfortunately so is subsequent reinfection, suggesting that either there are CGD patients who are innately predisposed to liver abscess or that the fact of having had a liver abscess alters hepatic metabolism and architecture in a way that makes subsequent infection more likely. Further complicating liver function in CGD is the recent report of high rates of portal venopathy and nodular regenerative hyperplasia, both of which may contribute to portal hypertension, splenomegaly, and splenic sequestration [32]. This latter point is noteworthy because further study has shown that the decline in platelet count, which is linked to splenomegaly, is also a strong predictor of mortality in CGD [33]. The reasons why microvascular disease in the liver should be so common are unclear. However, chronic drug effects, liver enzyme elevations, and recurrent infections are obvious risks for liver dysfunction. Why platelet count declines are such strong harbingers of complications is unclear, but hematologic data are easy to acquire, well-performed almost everywhere, and easily tracked over time [33].

Genitourinary manifestations of CGD include bladder granulomata, ureteral obstruction, and urinary tract infection. All patients with granulomata of the bladder or stricture of the ureter in an early series had defects of the membrane component of the NADPH oxidase ($gp91^{phox}$ and $p22^{phox}$) [34]. Subsequent descriptions have confirmed the occurrence of pseudotumors of the bladder [35–37] and eosinophilic cystitis [38].

The diagnosis of CGD is usually made by direct measurement of superoxide production, ferricytochrome *c* reduction, chemiluminescence, nitroblue tetrazolium (NBT) reduction, or dihydrorhodamine oxidation (DHR). DHR is preferable because of its relative ease of use, its ability to distinguish X-linked from autosomal patterns of CGD on flow cytometry, and its sensitivity to even very low numbers of functional neutrophils [39]. However, one condition that can give a falsely abnormal DHR is myeloperoxidase deficiency [40]. In the case of myeloperoxidase deficiency, DHR activity can look like X-linked CGD, while NBT and ferricytochrome *c* testing are normal. This is attributed to intracellular (DHR) compared to extracellular (NBT) superoxide release and dye activation. The syndrome of synovitis, acne, pustulosis, hyperostosis, and osteitis has also been associated with abnormal intracellular generation of oxidants [41]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency may also lead to a decreased respiratory burst and increased susceptibility to bacterial infections [42]. However, G6PD deficiency is most often associated with some degree of hemolytic anemia, while CGD is not.

The X-linked carriers of $gp91^{phox}$ have two populations of phagocytes: one that produces superoxide and one that does not, giving carriers a characteristic mosaic pattern on oxidative testing. Infections are not usually seen in female carriers unless the normal neutrophils are below 5–10%. However, cases of severe skewing of X-inactivation have been reported in which females have virtually no detectable normal cells; these carriers are at risk for CGD type infections [43]. There are reports suggesting that the balance of wild-type to mutant cells may vary over time in the same woman, but this has not been rigorously proven yet, as likely as it may appear to be [43]. Discoid lupus erythematosus-like lesions, aphthous ulcers, and photosensitive rashes have been seen in $gp91^{phox}$ carriers. Similarly, screening of patients with discoid lupus erythematosus detected a significant number of previously unsuspected CGD carriers [44–46].

Immunoblot and flow cytometry can be used to infer the specific genotype. However, molecular determination of specific mutations, available from various research and commercial laboratories, is necessary for prenatal diagnosis. With further study, there may be genotype/phenotype correlations that predict outcomes and thereby might help in genetic counseling as well as in the consideration of bone marrow transplantation. Male sex, earlier age at presentation, and increased severity of disease suggest X-linked disease, but these are only rough guides. The precise gene defect should probably be determined in all cases, but at this point, it does not have a profound impact on management. Autosomal recessive $p47^{phox}$ CGD appears to have a significantly better prognosis than X-linked disease [9, 11].

Management of CGD is predominantly with prophylactic antibiotics and antifungals and IFN-gamma, along with acute management of infections as they occur. Prophylactic trimethoprim/sulfamethoxazole (5 mg/kg/day based on trimethoprim) reduces the frequency of major infections from about once every year to once every 3.5 years,

reducing staphylococcal and skin infections without increasing the frequency of serious fungal infections in CGD [17]. Itraconazole prophylaxis showed marked efficacy in the prevention of fungal infection in CGD (100 mg daily for patients <13 years or <50 kg; 200 mg daily for those ≥13 years or ≥50 kg) [12]. IFN-gamma was shown in a large, multinational, multicenter, placebo-controlled study to reduce the number and severity of infections in CGD by 70% compared to placebo. These benefits held true regardless of inheritance pattern of CGD, sex, or use of prophylactic antibiotics. Interestingly, no significant difference could be detected in terms of in vitro superoxide generation, bactericidal activity, or cytochrome *b* levels [47]. Systemic IFN-gamma also augmented neutrophil activity against *Aspergillus* conidia in vitro [48]. Furthermore, in a study of IFN-gamma in CGD mice, infections were reduced [49]. However, a retrospective Italian study detected no benefit to the addition of IFN-gamma beyond that attributed to antibacterial and antifungals alone [11]. Although the study of IFN-gamma preceded the widespread use of itraconazole, a long-term follow-up of the large prospective trial suggests sustained benefit [13]. Therefore, our current recommendation is to use prophylaxis with trimethoprim/sulfamethoxazole, itraconazole, and IFN-gamma (50 $\mu g/m^2$) in CGD.

The erythrocyte sedimentation rate remains a highly sensitive laboratory test for ongoing infection. Since the differential diagnosis for a given process in these patients includes bacteria, fungi, and granulomatous processes, a microbiologic diagnosis is critical. In severe infections, leukocyte transfusions have been used, although their efficacy is anecdotal. In CGD, irradiation of the granulocyte product is not necessary for graft versus host disease prevention and it does inhibit the bactericidal activity of the cells. However, recurrent leukocyte transfusions run the risk of alloimmunization, which is problematic if bone marrow transplantation is to be considered.

Bone marrow transplantation is an attractive technique because it can lead to stable remission of CGD. Several approaches are in use, ranging from full myeloablation resulting, when successful, in complete engraftment [50], to nonmyeloablative conditioning regimens, leading to stable hematopoietic chimerism [51]. Bone marrow transplantation has been performed in the setting of ongoing refractory fungal infection in several instances. While some have been successful, active fungal infections continue to pose a problem for elective bone marrow transplants [52]. Low-intensity nonablative transplants from HLA-identical siblings into CGD patients have had mixed success [53]. Success was greater in children than adults, but transplant-related toxicities, such as graft versus host disease, were problematic. Recent reports of matched sibling and matched unrelated donor bone marrow transplants from Newcastle on Tyne [14] show an overall long-term success rate of 90%. Importantly, these British investigators also showed a striking reconstitution of impaired pretransplant growth, suggesting that much of the growth retardation and delay seen in CGD is in fact due to various aspects of hematopoietic dysfunction, ranging from colitis to chronic inapparent infection. Although bone marrow transplantation is an attractive option for the definitive cure of CGD, it should be borne in mind that survival without bone marrow transplantation is roughly comparable, even though certain morbidities like IBD might be ameliorated or prevented by transplantation. However, not all patients have matched related or unrelated donors, effectively excluding a substantial number of patients from this option [54].

CGD is an attractive target for gene therapy for several reasons: it is a single gene defect, it can be reconstituted in vitro, and correction need not be complete in order to provide complete protection [55]. This latter point is proven by the normal lives of many X-linked carriers, as well as by the stable chimeras generated in some transplant protocols. Gene therapy for the paradigmatic disease X-linked severe combined immunodeficiency (SCID) has been effective but complicated by hematopoietic malignancies caused by unanticipated retroviral integration into active sites in the human genome that activated oncogenes [56]. In CGD, many years of study have shown marking of cells in the periphery for several months, but clinical benefit has been elusive, presumably due to the low numbers of corrected cells in the circulation (<0.01%) [57]. With the addition of bone marrow conditioning, marking rates have increased and persistence has as well. However, in studies in Germany using a novel vector and bone marrow conditioning, Ott et al. encountered clonal expansions of transduced cells; one patient subsequently died (reviewed in [58]). Unlike the case with SCID, corrected CGD cells do not have a growth or survival advantage in the marrow or in the tissue, as far as we know. Therefore, selection and augmentation of those cells is an area of effort.

Other roles for the NADPH oxidase outside the neutrophil include signaling for NFκB activation in the setting of liver activation, either by ethanol or by carcinogens [59, 60]. CGD mice appear to be protected from aortic fatty deposits when crossed to the $ApoE^{-/-}$ model of hypercholesterolemia [61]. Interestingly, that was not the case in the coronary arteries, suggesting that the factors that regulate NADPH activity in the coronaries and the aorta are distinct [62]. NADPH contributes to long-term potentiation, the in vitro brain slice equivalent to memory [63]. In support of those ex vivo mouse data, CGD mice had trouble with certain learning tasks compared to normal mice. In addition, CGD patients had a higher rate of lower intelligence quotient and learning issues than disease controls [64]. NADPH oxidase somatic and hematopoietic

activity is involved in strokes and in pulmonary vascular permeability [65]. Therefore, it is clear that the NADPH oxidase is active in many more sites than just phagocytes, suggesting that CGD is more complex and has more to teach than about infections and bone marrow transplants alone.

Despite its advanced age and relative rarity, CGD is a disease that is rapidly changing in its diagnosis, management, science, and outlook. It spans the frontiers of basic science, medicine, genetics, infectious diseases, transplantation, and gene therapy.

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