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Chronic Granulomatous Disease

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Chronic granulomatous disease was first described in 1954 (1) and 1957 (2) as recurrent infections occurring in the setting of hypergammaglobulinemia, as opposed to the disease then recently recognized by Bruton, in which infections were associated with hypogammaglobulinemia (3). The disease was not well characterized until 1959 (4), when it was initially termed fatal granulomatous disease of childhood, but it is now simply referred to as chronic granulomatous disease (CGD). Originally thought to be only an X-linked disease, its recognition in girls in 1968 led to the determination of autosomal recessive forms, as well (5). Over almost 60 years CGD has evolved from a disease of early fatality to one of effective management with high survival (6). CGD is a paradigm for non-lymphoid primary immune defects, and has guided elucidation of oxygen metabolism in the phagocyte, the vasculature, and the brain (7). It has been in the forefront of the development of antimicrobial prophylaxis before the advent of advanced HIV and before its routine use in neutropenia (8). It has been an attractive target for gene therapy and bone marrow transplantation for non-malignant diseases. Therefore, CGD is worthy of our interest both for its historical interest as well as for the fact that this is a disease for which expert management is imperative.

Multiple separate proteins contribute to the intact NADPH oxidase, mutations in 5 of which lead to a single syndrome that is known as CGD. This enzyme catalyzes the transfer of an electron from cytoplasmic NADPH to molecular oxygen (6; OMIM# 306400, 233690, 233700, 233710, 601488), thereby oxidizing NADPH and leading to the name, NADPH oxidase. While impairments of the NADPH oxidase typically present as phagocyte defects, in fact only gp91^{phox} is very phagocyte specific, while the other autosomal components are expressed elsewhere, as well (7). The components are broken into membrane bound (cytochrome b558, comprised of gp91^{phox} and p22^{phox}) and cytosolic (p47^{phox}, p67^{phox}, and p40^{phox}) components. gp91^{phox} and p22^{phox} require each other for expression in the phagocyte; however, 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 the other tissues, which are other members of the *Nox* family of proteins. Therefore, individuals who have autosomal recessive forms of CGD may also have other subtle

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abnormalities, such as vascular disease and diabetes in p47^{phox} deficient CGD or perhaps inflammatory bowel disease in p40^{phox} deficient CGD (9).

Activation of the NADPH oxidase is a carefully choreographed process (6). On cellular activation, such as ingestion of bacteria or fungi, the cytosolic components p47^{phox} and p67^{phox} are phosphorylated and bind tightly together. The secondary (specific) granules, which contain the cytochrome complex (gp91^{phox} and p22^{phox}) fuse with the phagolysosome, followed by the primary (azurophilic) granules, which contain the antibacterial peptides neutrophil elastase and cathepsin G. This process embeds the cytochrome in the wall of the phagolysosome and the antibacterial peptides inside it. The cytoplasmic complex of p47^{phox} and p67^{phox} in association with p40^{phox} and rac combine with the cytochrome to form the intact NADPH oxidase, which is oriented into the internal aspect of the phagolysosome (this process can also take place on the plasma membrane focused outside the cell). An electron is then taken from cytoplasmic NADPH and donated to molecular oxygen inside the phagolysosome, 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 phagolysosome, is converted to bleach. While the metabolites of superoxide themselves can contribute to bacterial killing, the generation of superoxide has broader implications (10). With the generation of superoxide a charge is imparted to the phagolysosome that is rectified by the rapid influx of K⁺ (11). This potassium influx leads to activation of the now intraphagosomal peptides, which mediate microbial killing (10, 11). Therefore, reactive oxidants are working more as intracellular signaling molecules, leading to activation of other non-oxidative pathways, in addition to causing killing directly. There is therefore a spectrum of microbicidal activity that can be regulated by NADPH oxidase activity, rather than distinct oxidative and non-oxidative pathways and mechanisms.

In addition to activation of intracellular antimicrobial peptides, the NADPH oxidase is required to activate neutrophil extracellular traps (NETs), complex assemblies of DNA and antimicrobial peptides released from apoptotic neutrophils (12). Interestingly, repair of the NADPH oxidase system with gene therapy in a patient with X-CGD led to reconstitution of NET function, proving that NET formation is impaired in CGD and dependent on NADPH function (13).

Mutations in all of the 5 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 are divided between p67^{phox} and p22^{phox}; there is one case of p40^{phox} deficiency. There are no autosomal dominant cases of CGD. A large voluntary retrospective study in the United States and Europe suggested rates of CGD of around 1:200,000–1:250,000 live births (14, 15). Rates in other countries vary 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 (16). However, the relative rates of X-linked compared to recessive CGD are very distinct. In many countries with high rates of consanguineous marriage recessive CGD rates exceed X-linked rates (15, 16). Clinically, X-linked gp91^{phox} deficient CGD is more severe with earlier presentation and diagnosis, and more severe infections and earlier death than the p47^{phox} deficient form (14, 15).

Mechanistically, the level of residual superoxide production determines survival, at least in X-linked CGD (17). The rates of long term survival are higher in X-CGD patients with higher residual superoxide production and lower in those with lower rates. Molecularly, this is due to the ability of the mutant protein to support superoxide production. Thus, mutations that lead to no protein production (nonsense mutations, deletions, certain splice defects) support no residual superoxide production and have the lowest level of survival. In contrast,

those mutations that permit protein production and superoxide generation (missense mutations before amino acid 310 except histidine 222) are associated with higher survival rates. However, mutations at or beyond amino acid 310 are unable to support superoxide production, presumably because of the strict structural requirements for the intracellular domain of gp91^{phox} for the binding of NADPH and FAD (17).

Infections of the lung, skin, lymph nodes, and liver are the most frequent first manifestations of CGD (6, 15). In North America, the overwhelming majority of infections in CGD are due: *S. aureus*, *Burkholderia cepacia* complex, *Serratia marcescens*, *Nocardia* species, and *Aspergillus* species (6). In other parts of the world *Salmonella*, *Bacille Calmette Guerin* (BCG) and tuberculosis are also important (15, 16). CGD patients tend to develop severe localized BCG rather than disseminated BCGosis. 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 (6). Staphylococcal liver abscesses encountered in CGD are dense, caseous, and difficult to drain, and previously required surgery in almost all cases (18). However, recent studies have shown that the combination of corticosteroids and antibiotics alone are highly effective in CGD liver abscesses, allowing cure of liver abscesses without surgery (19, 20). Until recently fungal infections, typically due to *Aspergillus* species, were the leading cause of mortality in CGD (14, 15). The advent of highly active antifungal therapy with the orally active 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 mortality overall is diminished (21). However, the non-fumigatus *Aspergillus* species, as well as some species of fungi other than *Aspergillus* remain difficult to treat and important contributors to mortality (22, 23).

Overall survival in CGD is now thought to be around 90%, stretching well into adulthood (24). Patients diagnosed before the advent of antifungal azole agents had a very different outcomes, as reflected by the very poor survival of patients into their 30s and 40s in those series (14, 15). The introduction of itraconazole in the late 1990s, its proof in antifungal prophylaxis in 2003 (25), and the introduction of more active antifungals and antibacterials, mortality in CGD has plunged (24, 26). Access to expert care is clearly important as shown by a Japanese study with a 90% survival rate for patients followed at single center (27). Similarly, Muoy (28) found an 8-year survival rate of 70.5% for children born before 1978 but a 92.9% survival rate for those born later. Before the introduction of oral antifungals Winkelstein (14) reported X-linked CGD mortality of about 5% per year, compared to 2% per year for the autosomal recessive varieties. Van den Berg found a 23% mortality in X-linked CGD and a 15% rate of mortality in autosomal recessive CGD over almost 50 years of European survey (15). Therefore, overall mortality from infection in CGD has been significant but will continue to improve with better therapies.

The morbidity of recurrent infections and inflammation with their associated end-organ damage as well as their impact on the child and family are major issues. Several large studies found similar rates of infection of around 0.3 per year (24, 26). That is, most patients are still experiencing at least one severe infection every 3–4 years, whether bacterial or fungal. The persistence of this rate may reflect a minimum inescapable environmental exposure, or the complexity of maintaining long-term prophylaxis over a lifetime with a disease that is only intermittently reinforced.

CGD is remarkable for its very narrow but profound spectrum of infection susceptibility. *Burkholderia cepacia* complex organisms are common causes of pneumonia and infrequently sepsis (29). The closely related *Burkholderia gladioli* has also been described in

CGD (30). *Chromobacterium violaceum* is found in brackish waters, such as those around the Gulf of Mexico in the United States and causes sepsis in CGD (31). *Francisella philomiragia* causes sepsis in CGD and is also found in brackish waters, such as the Chesapeake Bay, Long Island Sound and around Nova Scotia (32). *Granulibacter bethesdensis* is a novel Gram negative rod that causes chronic necrotizing lymphadenitis and can cause sepsis in CGD (33). It can have latent and active phases, similar to tuberculosis and has been identified from the United States, Panama, and Spain, suggesting wide distribution. Interestingly, while the rate of seropositivity for this organism is about 50% in CGD patients, the majority of whom have not had recognized infections, the rate of seropositivity is around 25% in normals, suggesting broad exposure and the possibility of a clinical syndrome yet to be identified (34).

Fungal infections are critically important to recognize in CGD. There are several that are characteristic of CGD and virtually never encountered in other diseases: *Aspergillus nidulans*, *Paecilomyces variotti* and *Paecilomyces lilacinus*, and *Neosartorya udagawae*. These organisms are highly pathogenic in CGD but not in any other patient group, including transplant recipients (35). In contrast to these filamentous molds that are virtually pathognomonic for CGD, the endemic dimorphic mold infections histoplasmosis, blastomycosis, and coccidioidomycosis do not occur in CGD; nor does cryptococcosis. Mucormycosis occurs in CGD but only in the setting of significant immunosuppression (36). The molecular identification of infection should be vigorously pursued in CGD patients, especially for fungal infections, since the identification of a non-*fumigatus Aspergillus* infection should prompt early consideration of therapeutic surgery (37).

Fungal elements elicit an exuberant inflammatory response in CGD regardless of whether they are live or dead (38). "Mulch pneumonitis" refers to acute inhalational exposure to aerosolized decayed organic matter, such as mulch, hay or dead leaves (39). The clinical presentation is stereotypic and dramatic: a previously well child or adult spreads mulch, turns compost, or clears moldy leaves, inhaling numerous fungal spores and hyphae; 1–10 days later a syndrome similar to hypersensitivity pneumonitis 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. The most successful treatments of this syndrome have been with simultaneous antifungals for the infection and steroids for the inflammation (39). We typically institute meropenem, voriconazole and prednisone for this syndrome, since steroids appear to be crucial for reducing inflammation and allowing independent ventilation. This syndrome should be considered in all cases of *Aspergillus* pneumonitis, especially with acute onset and hypoxia, and therefore implies CGD as the underlying diagnosis. This syndrome occurs both in known CGD patients and may be the presentation for disease, even in adults.

Inflammation in CGD is 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 (40, 41). Functional gastric outlet obstruction may be the initial presentation of CGD. In a large survey of CGD patients followed at the NIH, 43% of those with X-linked CGD had symptomatic, biopsy-proven inflammatory bowel disease (IBD), compared to only 11% of p47^{phox} deficient patients (40). However, growth rates were equally diminished below the mean in both IBD-affected and unaffected 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, since biopsies were only done in symptomatic patients. Interestingly, growth and growth rates in CGD recovered following bone marrow transplantation, regardless of

antecedent IBD (42). Although IBD may involve any part of the gastrointestinal tract in CGD patients, perirectal disease is especially common (41).

Treatment of CGD IBD is often long-term, difficult, and prone to relapse. Steroids are effective but may cause growth retardation, osteoporosis, and infection risk. However, at the doses typically used in CGD for maintenance, infections are rarely an issue. In contrast, while TNF-alpha blocking agents are highly effective and rapidly suppress bowel symptoms, they carry a very high risk of infection and death and should be carefully avoided in CGD (43). 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/d for one to two weeks and then slowly taper to 0.1–0.25 mg/kg/d over one to two months. Sometimes children can be taken off prednisone, but the relapse rate is very high, and retreatment typically requires re-initiation 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 are also highly effective.

Liver involvement in CGD is pronounced and important. Liver abscesses occur in around 35% of patients and have until recently been difficult to treat without surgery (18). With surgery, cure of liver abscess is common, but unfortunately so is reinfection, typically with a different organism than the previous one. It remains unclear whether certain CGD patients are simply predisposed to liver abscesses, or that having had one liver abscess alters hepatic architecture and blood flow in a way that makes subsequent infection more likely. High rates of portal venopathy and nodular regenerative hyperplasia may contribute to portal hypertension, splenomegaly, and splenic sequestration (44). This latter point is noteworthy, because the decline in platelet count is linked to splenomegaly and is also a strong predictor of mortality in CGD (45). Chronic drug effects, liver enzyme elevations, and recurrent infections are obvious risks for liver dysfunction. In this regard, it is unclear whether the predilection for recurrent liver abscesses is in part caused by surgery with its hepatic scarring and altered blood flow. However, it seems prudent to try to avoid surgery when possible, and the recent demonstration of the effectiveness of steroids and antibiotics alone in the resolution of liver abscess offers an alternative treatment (20).

Genitourinary manifestations of CGD are also common and include bladder granulomata, ureteral obstruction, and urinary tract infection, typically in those with gp91^{phox} and p22^{phox} deficiencies (46). Eosinophilic cystitis has also been described in CGD (47). Genitourinary complications are also highly steroid responsive. It is important to keep in mind that inflammatory masses in CGD can mimic tumors and should be considered (48).

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, its sensitivity to even very low numbers of functional neutrophils, and its utility in predicting the residual superoxide activity of the patient's neutrophils (49, 17). The two conditions known to give a falsely abnormal DHR are myeloperoxidase deficiency (50) and SAPHO syndrome (51). In myeloperoxidase deficiency the DHR tracing can look like that of X-linked CGD, while the NBT and ferricytochrome c results are normal. This is attributed to intracellular (DHR) compared to extracellular (NBT) superoxide release and dye activation. Glucose 6-phosphate dehydrogenase deficiency may also lead to a decreased respiratory burst and increased susceptibility to bacterial infections (52). However, G6PD deficiency is most often associated with some degree of hemolytic anemia, while CGD is not.

Female carriers of X-linked CGD 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 infrequent unless the normal neutrophils are <10%, and even then uncommon. 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 (53). 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 (53). 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 (54–6).

Immunoblot and flow cytometry can be used to infer the specific genotype, but molecular determination of specific mutations is necessary for prenatal diagnosis. A robust genotype/phenotype correlation has been shown for mutations that permit residual superoxide production compared to those that do not (17). 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, as it is a strong predictor of survival. Autosomal recessive p47^{phox} deficient CGD has a significantly better prognosis than X-linked disease (14, 15, 17).

Effective management of CGD is predicated on prophylactic antibiotics and antifungals and interferon gamma, along with management of acute infections as they occur. Prophylactic trimethoprim-sulfamethoxazole (5 mg/kg/d trimethoprim divided twice daily) reduces the frequency of major infections from about once every year to once every 3.5 years, reducing staphylococcal and skin infections without increasing in the frequency of serious fungal infections in CGD (57). Itraconazole prophylaxis prevents fungal infections in CGD (100 mg daily for patients < 13 y or < 50 kg; 200 mg daily for those ≥ 13 y or ≥ 50 kg) (58). IFN-γ has been shown in a large, multinational, multicenter, placebo-controlled study to reduce the number and severity of infections in CGD by 70% compared to placebo regardless of inheritance pattern, sex, or use of prophylactic antibiotics (59). Systemic IFN-γ also augmented neutrophil activity against *Aspergillus* conidia in vitro (60). Further, in a study of interferon gamma in CGD mice, infections were reduced (61). However, a retrospective Italian study detected no benefit to the addition of interferon gamma beyond that attributed to antibacterial and antifungals alone (62). Long-term follow up of the large prospective trials suggests sustained benefit (26). We use trimethoprim/sulfamethoxazole, itraconazole, and interferon gamma (50 μg/m²) in CGD.

Bone marrow transplantation can lead to stable remission of CGD. Regimens ranging from full myeloablation to non-myeloablative conditioning lead to cure of CGD (42, 63, 64). Even in the setting of refractory fungal infection bone marrow transplantation has been effective (65). Nonmyeloablative transplants in CGD have been more successful in children than adults (66). Although bone marrow transplantation is an attractive option for the definitive cure of CGD, survival without bone marrow transplantation is roughly comparable, but attended by continuing CGD morbidities like like boiwel disease and mildly reduced growth.

CGD is a single gene defect that can be reconstituted in vitro and does not require complete correction to be effective as proven by the normal lives of many X-linked carriers, as well as by the stable chimeras generated in some transplant protocols (67). Unlike the case with SCID, corrected CGD cells do not have a growth or survival advantage in the marrow or in the tissue. Therefore, selection and augmentation of those cells is difficult.

The NADPH oxidase is active outside the neutrophil, such as in NF κ B signaling, liver damage from carcinogens, and the arterial vasculature (68–70). NADPH oxidase somatic and hematopoietic activity is involved in strokes and in pulmonary vascular permeability (71). NADPH contributes to long-term potentiation of memory (72) and may be related to IQ (73). 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 just infections and bone marrow transplants alone.

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