Chronic Granulomatous Disease: Epidemiology, Pathophysiology, and Genetic Basis of Disease

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Chronic Granulomatous Disease (CGD) is one of the classic primary immunodeficiencies of childhood. While the incidence and severity of bacterial and fungal infections have been greatly reduced in this patient population, much remains to be learned about the pathophysiology of the disease, particularly for autoinflammatory manifestations. In this review, we examine the epidemiology, pathophysiology, and genetic basis for CGD.

EPIDEMIOLOGY AND CLINICAL IMPACT

CGD is one of the classic primary immunodeficiencies of childhood; the disease is diagnosed in most children in the first 1 to 3 years of life. Although it varies according to ethnicity, the estimated incidence of CGD is 1 in ~200,000 live births [2]. Given the annual US birth cohort of nearly 4 million infants, approximately 20 children each year are born with CGD. By comparison, severe combined immunodeficiency occurs in approximately 40 live births per year in the United States. Estimates of the incidence of CGD in Europe and Asia are similar, although some populations are affected more often, including the Arab population of Israel, in which the incidence is estimated to be 1.5 per 100,000 live births [3]. As is discussed later, males are affected more commonly than females (2:1) because of the predominant mode of genetic transmission.

Children typically present with acute bacterial infection, such as suppurative lymphadenitis (classically caused by Serratia marcescens infection), recurrent staphylococcal infection, failure to thrive, or recurrent lower respiratory tract infection [4]. The disease also is diagnosed in a proportion of children as a result of noninfectious complications, such as very-early-onset inflammatory bowel disease. The incidences of infectious and noninfectious complications in patients with CGD are described elsewhere in this supplement. Survival rates have increased steadily over time. In the 1960s, the mortality rate was >60% by 7 years of age, whereas data published in the last 10 years suggest that at least 50% of these patients remain alive 225 years after diagnosis. The diagnostic and prophylactic measures that contribute to this increased survival rate are also described elsewhere in this supplement.

GENETIC FEATURES

Any pathologic mutation within the 5 genes that encode the subunits of the NADPH oxidase system (also known as NOX2) can result in CGD. The most common cause of CGD is a defect in the
CYBB gene (gp91phox), located on the short arm of the X chromosome (Xp21.1-p11.4). Multiple types of mutations of the CYBB gene (eg, deletion, frameshift, nonsense, missense, and splice-site mutations) can lead to either absent or reduced production of gp91phox. CYBB-related CGD is inherited in an X-linked recessive manner; however, it is estimated that 10% to 15% of gp91phox mutations are the result of new germline mutations [5].

Although the genetic basis for CGD in nearly two-thirds of patients with the disease lies in the CYBB locus, approximately 25% of patients in Europe and North America carry biallelic mutations (typically a premature stop codon) in neutrophil cytosolic factor 1 (NCF1, 7q11.23), which results in impairment of the p47phox component of the NADPH oxidase system [5]. Genetic variability in NCF1 has been found also in patients with an unrelated autoimmune process such as systemic lupus erythematosus or Sjogren syndrome [6]. NCF1-related CGD is inherited in an autosomal recessive fashion; in this scenario, typically both parents are asymptomatic carriers of a single NCF1 mutation [5].

Mutations in CYBB (a membrane-bound component of cytochrome b558) and NCF1 (a cytosolic factor) account for 90% of CGD cases in Europe and North America. The remaining cases, also inherited in an autosomal recessive manner, include genetic defects in any of a second membrane-bound component, p22phox (CYBA, 16q24.3), or in 2 additional cytosolic components, p67phox (NCF2) and p40phox (NCF4), which represent <5% of CGD cases [5].

Males with 1 mutation in CYBB or biallelic mutations in CYBA or NCF1, NCF2, or NCF4 result in clinical manifestations of CGD. In addition, female carriers of a single CYBB mutation have impaired NADPH oxidase activity in a portion of phagocytes, most commonly from 20% to 80%, as a result of variation in inactivation of the X chromosome (lyonization) that possesses the CYBB mutation [7]. This active carrier state can lead to a range of symptoms, including aphthous ulcers, arthralgias, and cutaneous photosensitivity [4]. In a recent study of 162 X-linked CGD carriers, Marciano et al [8] found that infectious complications were highly likely in those with <10% dihydrorhodamine 123 (DHR) oxidase activity. Even patients with 10% to 20% DHR activity remained at a substantially higher risk of infection. For this reason, it might be prudent to manage CGD carriers in much the same way as those with CGD when their DHR activity falls below 20% [8].

Additional complications can arise when the Xp21.1 gene (CYBB) region possesses a more substantial gene deletion. Deletions large enough to span both the CYBB gene and the Kell locus (XK gene) lead to a contiguous gene-deletion syndrome [9]. Mutations in this region are associated with McLeod syndrome, an X-linked recessive disorder characterized by abnormalities in both the neuromuscular and hematopoietic systems, which lead to hemolytic anemia with acanthocytosis and chorea (ie, neuroacanthocytosis). In addition, the gene loci for Duchenne muscular dystrophy and retinitis pigmentosa are near the CYBB locus [9]; therefore, mutations in this region should prompt clinicians to consider CGD, as a result of a contiguous gene defect, particularly if clinical symptoms referable to CGD are present (eg, colitis or frequent infections).

**PATHOPHYSIOLOGY: THE NADPH OXIDASE SYSTEM**

The term “respiratory burst” was coined initially by Baldridge and Gerrard in 1933 [10] after discovering that neutrophils consume large quantities of oxygen during phagocytosis. Initially thought to be related to the mitochondrial oxidative phosphorylation pathway, the mechanism of respiratory burst was characterized distinctly when classic mitochondrial inhibitors (eg, cyanide) left the process unaffected [11, 12]. Additional insights came when neutrophils were found to have the capacity to engulf bacteria but had impaired killing ability in the absence of oxygen [12]. The critical underlying molecular component responsible for respiratory burst was finally discovered to be a membrane-bound flavocytochrome with an absorbance maximum near 558 nm, and thus it was named “flavocytochrome b558” [13, 14]. Flavocytochrome b558 is a heterodimer that stoichiometrically comprises 1 p22phox and 1 gp91phox molecule (Figure 1) [15, 16]. This component is situated primarily within the membranes of specific granules (~85%), and a smaller percentage is localized to the cell’s plasma and secretory granule membranes [17, 18].

The full NADPH oxidase system comprises the membrane-bound heterodimer (p22phox + p91phox) and 3 cytosolic subunits, p47phox (NCF1), p67phox (NCF2), and p40phox (NCF4) (Figure 1). After activation, cytosolic members translocate to the membrane-bound portion and assemble a functional 5-component oxidase complex [19, 20]. Activated NADPH oxidase forms

![Figure 1. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system. Ninety percent of patients with chronic granulomatous disease carry mutations in either CYBB (gp91phox) or NCF1 (p47phox). Mutations in either the membrane or cytosolic domain lead to a disruption of respiratory burst in phagocytes.](https://academic.oup.com/jpids/article-abstract/7/suppl_1/S2/4994080/5524894480)
a unique electron-transfer chain that can generate microbicidal superoxide anion and other killing agents, such as hypochlorous acid, after electron transfer from NADPH to 2 molecules of O₂ [21, 22]. A fully competent NADPH oxidase complex also depends on association of the neutrophil cytosol-derived small G-protein Rac2, which binds guanosine triphosphate and enables oxidase activation and superoxide formation [23].

The deficiency in NADPH oxidase function results in a surprisingly narrow spectrum of microbial susceptibility [24]. This biochemical perturbation relates, in part, to impaired killing of specific catalase-positive organisms with superoxide anion and other reactive oxygen species by neutrophils, monocytes, macrophages, and eosinophils. Catalase-positive organisms (eg, *Staphylococcus aureus*, *Pseudomonas*, *Aspergillus fumigatus*, *Candida albicans*, and *Enterobacteriaceae* such as *Klebsiella* spp and *Serratia* spp) can degrade host-produced hydrogen peroxide before its ultimate conversion to hypochlorous acid by myeloperoxidase. A striking example of disparate susceptibilities in different types of CGD was demonstrated by Kaplan et al [25]. In their elegant description, primary cells of patients with CGD did not kill *S aureus*, but killing of *Streptococcus* strains was preserved. Patients with CGD also experience a wide range of impairment in their ability to generate phagocyte-derived reactive oxygen intermediates (ROIs), typically ranging from undetectable (0%) to <30%. As a result, patients with a less severe mutation in NADPH oxidase–encoded genes have greater residual ROI production and less severe disease [26]. An additional important insight about the pathophysiology of this disease came when Quie et al [27] found that phagocytosis and intracellular killing are uncoupled in patients with CGD; only intracellular killing is affected. Although many aerobic organisms produce catalase, a relatively small group of catalase-positive organisms play a significant role in CGD morbidity and mortality. However, catalase is not the only critical virulence factor for organisms that disproportionately affect patients with CGD; the genetic deletion of catalase in either *S aureus* or *Aspergillus nidulans* does not appear to alter virulence in vitro or in vivo [28, 29].

In addition to having direct cytotoxic effects, the production of reactive oxygen species seems to have important consequences for other innate immune functions. For example, patients with CGD display reduced expression and function of Toll-like receptors, complement receptors, and chemokine receptors that correlate with disease severity [30]. In addition, neutrophils also use extracellular means of microbicidal activity through the use of neutrophil extracellular traps [31]. This mechanism depends on normal ROI production by NADPH oxidase and is dysfunctional in patients with CGD [31, 32]. Moreover, recent work by Marzaioi et al [33] found that monocyte–dendritic cell differentiation and maturation are also affected by disturbances in the NADPH oxidase system. NOX5 and p22phox, but not gp91phox/NOX2, seem to regulate the differentiation of monocytes into dendritic cells.

### HYPERINFLAMMATION

Inflammatory manifestations are common in patients with CGD and are noted most frequently in the gastrointestinal tract, urogenital tract, lungs, and eyes [34–36]. Because these complications are common and can precede the onset of infectious susceptibilities, it is important to consider the diagnosis of CGD in patients with atypical inflammatory disease or multi-system inflammation (eg, Behçet disease). Important to note is that patients with X-linked CGD are twice as likely to develop inflammatory disease than those with autosomal recessive disease [37].

Granuloma formation is a classic inflammatory finding in patients with CGD. Distinct from those related to other diseases, CGD-related granulomas typically are noncaseating, nestled within fibrotic tissues in the setting of acute or chronic inflammation [38]. In patients with CGD, granulomas have a propensity to affect hollow viscera, most notably the colon, stomach, and bladder. They do not seem to be related to infection, because microorganisms usually are not found in CGD granulomas, and the lesions generally respond to treatment with steroids or other immunomodulators such as cyclosporine [39–41].

Dysfunctional CGD neutrophils also can promote hyperinflammation because of impairment of normal inflammation-resolution mechanisms. In addition to host defense, neutrophils are important for phagocytic clearance of apoptotic cells, which are identified by external presentation of phosphatidylserine residues on their cell surface [42, 43]. This process, termed “efferocytosis,” is critical for quenching ongoing inflammation and tissue necrosis. Efferocytosis (“to take to the grave or bury”) prevents cytotoxic damage from dying cells and results in production of the antiinflammatory cytokine transforming growth factor β by macrophages [44–47]. Evidence for impaired efferocytosis exists in a murine model of CGD [48–50]. It is interesting to note that Fernandez-Byanapalli et al found that interferon γ improved both the host-defense mechanism and clearance of apoptotic cells in a mouse model of CGD [48].

### CONCLUSIONS

Although the molecular defects responsible for CGD are quite straightforward, the immunologic and inflammatory consequences of reduced NADPH oxidase activity are profound and can lead to life-threatening infections and life-altering auto-inflammatory symptoms, even in some asymptomatic carriers. Future work focused on the pathophysiology of CGD will most certainly benefit from the use of novel tools, such as the CRISPR-Cas9 genome-editing tool [51], to explore additional immunologic manifestations of NADPH oxidase defects and the development of new therapeutic targets.
Notes

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