

Chronic granulomatous disease: why an inflammatory disease?

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Abstract

Chronic granulomatous disease is a primary immunodeficiency caused by mutations in the genes encoding subunits of the phagocytic NADPH oxidase system. Patients can present with severe, recurrent infections and noninfectious conditions. Among the latter, inflammatory manifestations are predominant, especially granulomas and colitis. In this article, we systematically review the possible mechanisms of hyperinflammation in this rare primary immunodeficiency condition and their correlations with clinical aspects.

Key words: Chronic granulomatous disease; Inflammation; Infection; Granulomas; NADPH oxidase

Introduction

Chronic granulomatous disease (CGD) is a fatal primary immunodeficiency caused by mutations in the genes encoding subunits of the NADPH oxidase system, which consists of five subunits. Therefore, this disease has five genetic variants. Mutations in the *CYBB* gene are responsible for the X-linked form of the disease that encodes gp91phox, also called NOX2, accounting for two-thirds of CGD cases. Mutations in the other four components account for the remaining one-third of CGD cases and are inherited in an autosomal recessive manner. These are *CYBA*, encoding p22phox; *NCF2*, encoding p67phox; *NCF1*, encoding p47phox; and *NCF4*, encoding p40phox, with the latter being the most recently described (1-6). These defects lead to a failure of phagocytes to catalytically convert oxygen to superoxide and other reactive oxygen species (ROS) that play a key role in killing intracellular pathogens. Usually, patients with X-linked CGD manifest a more severe clinical phenotype with greater morbidity and mortality than patients with autosomal recessive forms (7-9).

The prevalence of CGD at birth has been estimated to be 1 per 120,000-250,000 people (10). CGD patients suffer from early and recurrent severe bacterial and fungal infections (11) that primarily affect the natural barriers of the human body, such as the skin, lymph nodes, and the respiratory tract. Bones and the liver, spleen, and brain can also be affected by infections (12).

The main causes of infections in CGD patients are *Staphylococcus aureus*, *Burkholderia cepacia*, *Pseudomonas*, *Serratia marcescens*, *Aspergillus*, and *Nocardia* species (13). *Salmonella* sp. are also an important cause of sepsis and mortality and can affect 18% of CGD patients (14). Olfactomedin 4 (OLFM4) is a neutrophil granule protein that negatively regulates host defenses against bacterial infection. Recently, Liu et al. (15) evaluated the impact of OLFM4 deletion on host defenses against *S. aureus* and *Aspergillus fumigatus* in a murine gp91phox-deficiency CGD model. The authors demonstrated that resistance to *S. aureus* sepsis, as well as intracellular killing and *in vivo* clearance of these bacteria, were significantly increased in gp91phox and OLFM4 double-deficient mice compared with CGD mice. However, the authors did not observe enhanced defenses against *A. fumigatus* in OLFM4-deficient mice. These results suggest that OLFM4 may be an important tool in CGD patients to increase host defenses against bacterial infection.

Inflammation in CGD

NADPH oxidase consists of a family of seven members: NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1, and DUOX2 (16). The NADPH oxidase complex 2 is mainly expressed by phagocytes and endothelial cells (17,18). The membrane complex is formed by two

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transmembrane subunits (p22phox and gp91phox, also called NOX2), which are able to couple with the cytosolic subunits (p47phox, p40phox, and p67phox) and small GTPases Rac1 or Rac2. These GTPases are essential for coupling of p67phox to gp91phox, and the subunits p47phox and p40phox are phosphorylated close to gp91phox on the cell membrane when stimulated. This sequence of events allows the transfer of electrons from NADPH to molecular oxygen and produces ROS (19,20). Deficiency of any subunit of NADPH oxidase 2 results in CGD.

Inflammation is frequent in patients with CGD, and in some instances it can be the first clinical manifestation. The disease is so named for the exuberant chronic granuloma formation observed in patients. Current data indicate that either increased or decreased NOX2 activity may lead to inflammatory complications (21,22). The association of increased NOX2 activity with inflammation has been widely discussed. The transcription nuclear factor kappa B (NF-kappa B) controls the expression of several genes involved in the immune-inflammatory response, and is a key activator of innate immunity (23). However, studies evaluating the role of NOX2 in NF-kappa B-induced transcription and, consequently, activation or maintenance of the inflammatory response are controversial. Weissmann et al. (24) demonstrated that deficiency of endothelial NOX2, induced by ischemia-reperfusion in mice, prevented lung edema. Reinforcing these findings, Gandhirajan et al. (25) showed that NOX2-deficient mice had reduced inflammatory release mediators in a model of acute lung injury compared to wild-type controls. On the other hand, some evidence points to a protective role of endothelial NOX2 in lung inflammation. Whitmore et al. (26) used a murine model of sterile generalized inflammation and observed dramatically increased mortality of NOX2-deficient mice compared to wild-type mice. The recruited NOX2-deficient neutrophils demonstrated an enhanced inflammatory phenotype and induced a hemorrhagic inflammatory response in the lungs with rapid and persistent recruitment of neutrophils to the alveolar space.

NADPH oxidase deficiency has been seen predominantly as an immunodeficiency, characterized by an inability to mount an inflammatory response. However, there is current evidence for hyperinflammatory complications of CGD.

The main manifestations are represented prominently by granulomas, as well as colitis, which is frequent and leads to substantial morbidity. Defects of microbicidal activity can facilitate the persistence of pathogens and increase the inflammatory response in some patients (27,28).

Symptomatic disease can include colitis or enteritis, usually caused by microscopic granulomas, or mechanical obstruction of either the digestive or urinary tract, especially by macroscopic granulomas (29). Research

conducted at the National Institutes of Health in the United States showed inflammatory involvement of the gastrointestinal tract in 32.8% of 140 patients with CGD, 89% of whom had X-linked inheritance (30). Granuloma formation can affect various organs, with a preference for hollow viscera, such as the colon, stomach, esophagus, and bladder (22).

Granulomas can occur in most instances without an infectious pathogen. This fact can be verified by the absence of microbes inside the lesions and because the lesions often respond to several immunomodulators, such as steroids, cyclosporine A, or azathioprine (22). Nevertheless, the primary mechanism of the increased inflammatory response remains poorly understood.

Nonspecific persistent inflammation is a frequent histopathological finding, and the most commonly described feature is acute and/or chronic inflammation with fibrosis containing noncaseous granulomas. However, the lesions can exhibit particular features in some tissues such as the liver, lymph nodes, and intestinal tract. With active chronic inflammation, one can observe increased numbers of eosinophils, eosinophilic crypt abscesses (intestinal tract), abundant nuclear debris and pigmented macrophages, and a relative paucity of neutrophils (31). This can help differentiate hyperinflammatory manifestations of CGD from other granulomatous diseases like Crohn's disease or tuberculosis (32).

Characteristics and possible mechanisms of inflammation in CGD

A high production of ROS is usually associated with hyperinflammation. Therefore, the absence or reduction of ROS generation by the NADPH oxidase system leading to enhanced inflammation represents a paradigm shift and requires investigation into underlying mechanisms. It is possible that ROS can modulate the inflammatory response, and the NADPH oxidase system is likely to play an important role in this resolution (22). Some properties of ROS may justify a regulatory action in inflammatory processes, like suppression of proinflammatory mediators, degradation of phagocytosed particles, and impaired survival of proinflammatory cells. The following sections describe possible mechanisms.

NOX2 signaling in myeloid cells

Disruption of calcium channels. Ca^{2+} signaling may be impaired in patients with CGD, contributing to hyperinflammation. This can be explained by a more negative membrane potential of CGD granulocytes, which contributes to increased Ca^{2+} influx and thereby an enhanced inflammatory response (33).

Impaired intracellular signaling and an imbalance of inflammatory mediators. The absence of ROS in CGD leukocytes creates signaling alterations that favor

proinflammatory responses. This can be explained by ROS involvement in the regulation of intracellular signaling, especially the oxidation of cysteine residues in phosphatases and transcription factors (34). CGD phagocytes produce high levels of tumor necrosis factor- α and interleukin (IL)-8, probably through hyperactivation of NF- κ B, contributing to the inflammatory response (33,35). Moreover, there is some evidence that NOX2 deficiency can reduce the activation of indoleamine dioxygenase, leading to higher gamma-delta T cell activity and IL-17 production (3). Myeloid-related proteins (MRP), also called S100A8 (MRP8) and S100A9 (MRP14), are two calcium-binding proteins linked to innate immunity, and they are expressed in neutrophils and monocytes. One of their main functions is to favor NADPH oxidase activation. Therefore, these proteins are important mediators of inflammatory disease (36). On the other hand, the production of anti-inflammatory mediators such as IL-10, transforming growth factor beta (TGF- β), and prostaglandin 2 by human CGD phagocytes is usually low (37).

It seems that CGD phagocytes are unable to inactivate inflammatory mediators. The catabolism of leukotrienes and S100 proteins is ROS production dependent *in vitro* (38).

Abnormal apoptosis. There is evidence suggesting that ROS can induce neutrophil apoptosis. Apoptosis of inflammatory cells represents an important physiological mechanism to avoid hyperinflammation and secondary necrosis (22). Apoptotic cells expose phosphatidyl serines (PS), which can be recognized by PS receptors in macrophages, allowing the uptake of the apoptotic cells (39). This process leads to TGF- β production by the macrophages, promoting control of inflammation (40). There is evidence that human and murine CGD neutrophils have diminished/delayed PS exposure. The failure to ingest apoptotic cells is hypothesized to cause immunization to self-antigens, leading, for example, to a higher prevalence of lupus in CGD patients (3,41). However, impaired phagocytosis of apoptotic cells by macrophages can be reversed by interferon- γ in a nitric oxide-dependent manner (42).

Dysregulation of toll-like-receptor (TLR) expression. The expression of some TLRs, like TLR-5 and TLR-9, can be low in CGD patient neutrophils. This can contribute to impaired pathogen recognition, phagocytosis, and chemotaxis, leading to increased disease severity. On the other hand, some innate immune receptors, such as

CD35, can be upregulated, which might be linked to the increased frequency of autoimmune diseases (43).

Reduced degradation of phagocytosed particles or apoptotic cells

A deficiency in the NADPH oxidase system in CGD patient phagocytes leads to reduced degradation of phagocytosed material or apoptotic cells, which implicates either the remaining phagocytosed pathogen or apoptotic neutrophils phagocytosed by macrophages, such as pathognomonic eosinophilic crystals. The main consequence is a persistent activation and hyperinflammation at the cellular level (44).

Genetic polymorphisms

The trend to develop granulomatous complications in CGD patients appears to be influenced by genetic modifiers, as demonstrated by Foster et al. (45). The authors studied a cohort of 129 CGD patients, and 7 candidate genes, each containing a physiologically relevant polymorphism predicted to influence the host inflammatory response, were selected for analysis. Patients with genotypes associated with myeloperoxidase, Fc γ RIIIb, and Fc γ RIIa receptors had the highest risk for chronic gastrointestinal complications. Thus, subtle genetic differences in molecules of innate immunity seem to contribute to specific inflammatory responses in CGD patients.

Final considerations

It seems that the amount of ROS generated by neutrophils varies considerably from one individual to the other, and this can be dependent on genetic variations and other factors, such as nutritional uptake of pro-oxidants and antioxidants, oxygen tension in a given tissue, and the hormonal and cytokine environment (46-48).

CGD patients usually exhibit a ROS^{low} inflammatory response, characterized by a strong activation of oxygen-independent killing mechanisms and an increase in neutrophil influx. This kind of response tends to cause more tissue damage and is inefficient in removing phagocytized material, as evidenced by pigmented macrophages. In addition, this kind of response can be associated with autoimmune disease, as is often observed in CGD patients.

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