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REVIEW

# Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase

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**Abstract** Chronic granulomatous disease (CGD) is an immunodeficiency caused by the lack of the superoxide-producing phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. However, CGD patients not only suffer from recurrent infections, but also present with inflammatory, non-infectious conditions. Among the latter, granulomas figure prominently, which gave the name to the disease, and colitis, which is frequent and leads to a substantial morbidity. In this paper, we systematically review the inflammatory lesions in different organs of CGD patients and compare them to observations in CGD mouse models. In addition to the more classical inflammatory lesions, CGD patients and their relatives have increased frequency of autoimmune diseases, and CGD mice are arthritis-prone. Possible mechanisms involved in CGD hyperinflammation include decreased degradation of phagocytosed material, redox-dependent termination of proinflammatory mediators and/or signaling, as well as redox-dependent cross-talk between phagocytes and lymphocytes (e.g. defective tryptophan catabolism). As a conclusion from this review, we propose the existence of $ROS^{high}$ and $ROS^{low}$ inflammatory responses, which are triggered as a function of the level of reactive oxygen species and have specific characteristics in terms of physiology and pathophysiology.

**Keywords** Chronic granulomatous disease · Phagocyte NADPH oxidase · Inflammation · Reactive oxygen species

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**Abbreviations**

| | |
|---|---|
| CGD | Chronic granulomatous disease |
| ROS | Reactive oxygen species |
| NADPH | Nicotinamide adenine dinucleotide phosphate-oxidase |
| NOX | NADPH oxidase |
| DUOX | dual oxidase |
| OR | Odds ratio |
| iNOS | inducible nitric oxide synthase |
| MOG | myelin oligodendrocyte glycoprotein |
| IQ | intellectual quotient |
| SNP | single nucleotide polymorphism |
| IDO | indol 2,3 dioxidase |

## Introduction

A fatal childhood disease characterized by the occurrence of granulomas and recurrent infections was first described

in 1954 by Janeway [1], and 1957 by Good [2]. About 10 years later, Paul Quie [3, 4] linked the disease to a deficiency in bactericidal activity of the phagocytes. After stimulation, the neutrophils of these patients failed to increase oxygen consumption and to generate reactive oxygen species (ROS) [3], the so-called "respiratory burst" [4, 5]. The disease, which was renamed chronic granulomatous disease (CGD), was first thought to affect only males [3], but with time female patients were also reported [6, 7]. Finally, phagocyte nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase deficiency was identified as the cause of CGD [8–10].

Today, the catalatic subunit of the phagocyte NADPH oxidase enzyme is called NOX2 (formerly $gp91^{phox}$) and despite the fact that is has never been crystallized, there is some knowledge about its structure. NOX2 contains six transmembrane domains, cytosolic NADPH and FAD binding sites, and two intramembranous haemes that are necessary for catalysing the reduction of molecular $O_2$ to generate the superoxide anion in the phagosome or the extracellular space. The NOX2 protein is associated with another transmembrane protein, $p22^{phox}$, which acts to stabilise the complex and to dock the cytosolic partner $p47^{phox}$. Generation of superoxide anion requires a phosphorylation-dependent activation step, allowing the recruitment of $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ and the GTPase Rac, all of which associate to the membrane-bound complex to form the functional NADPH oxidase [11]. NOX2 is one member of a multi-gene NOX family of ROS-generating NADPH oxidases comprising seven members (NOX1–NOX5, DUOX1 and DUOX2). In this review, we will focus exclusively on the deficiency of the NOX2 isoform.

One of the key pathognomonic features of CGD patients is recurrent infection. Tendency towards infection is usually evident during the first years of life. The sites of infection involve either epithelial surfaces, including skin, lungs and gut, or the reticuloendothelial system including liver, spleen and lymph nodes. The bactericidal defect is not absolute, but is rather quite specific for a subset of pathogens, causing pneumonia, soft tissue infections, sepsis, liver abscesses and osteomyelitis, to name the most common. There is a marked overrepresentation of certain bacterial pathogens such as *Staphylococcus aureus*, *Pseudomonas*, *Serratia marcescens* and *Nocardia* and for certain fungal pathogens, in particular *Aspergillus* [12, 13]. The United States national registry states that the most commonly found infections in CGD patients are pneumonia, subcutaneous and liver abscesses, osteomyelitis and septicaemia. These findings are corroborated by other large cohort studies [13] and by imaging studies [14]. Pneumonia is mostly caused by *Aspergillus*, abscesses by *Staphylococcus* spp, osteomyelitis by *Serratia* while septicaemias are mostly due to Salmonella. New germs are also emerging: *Burkholderia cepacia* (formerly referred to as *Pseudomonas cepacia*), which was absent in the first series, now represents the second most prevalent organism isolated from patients with pneumonia or bacteraemia [15]. NOX2 deficiency has been seen predominantly as a decrease in host defence, with an inability to mount an inflammatory response. However, there is increasing evidence for hyperinflammatory, non-infectious complications of CGD. Indeed, the disease took its name from the exuberant chronic granuloma formation, which in most instances occurs without an infectious agent. It seems counterintuitive that a genetic defect associated with immune deficiency also causes an amplified inflammatory response. However, presently available data suggest that both increased and decreased NOX2 activity may lead to inflammatory complications. The situation is most puzzling for arthritis and inflammatory bowel disease, which have been classified as diseases caused by increased NOX activity by some, but as diseases associated with a lack of ROS generation by others [16–19]. In general, the association of increased NOX2 activity with inflammation has been widely discussed (e.g. Bedard [20] and Lambeth [18]) and will not be discussed here. In contrast, the relationship between decreased NOX2 activity and inflammation remains poorly understood and will be the focus of this review.

The mortality in CGD patients is high and usually occurs in the first two decades of life [13, 18], with about 50% of patients surviving into their third decade [21]. Only isolated patients survive into the fifth and sixth decades. In the US, the overall mortality is estimated at 2–5% per year [15]. Deaths in CGD patients are mainly due to overwhelming infections, mostly pneumonia and sepsis. The most common germs are *Aspergillus*, accounting for one third of all deaths, followed by *Pseudomonas* and *Candida*. The emerging *Burkholderia cepacia* causes nearly 20% of the deaths alone [15]. Analysis of survival of CGD patients suggests that recent advances in treatment have improved survival of patients in the first two decades of life, but there does not appear to be increased survival at later ages [13]. The improved survival in the first two decades is mainly due to early diagnosis along with aggressive management, including the use of prophylactic and therapeutic antibiotics, as well as prophylactic interferon $\gamma$.

The genetic aspects of CGD are specifically addressed by MJ Stasia and Li, in this issue [22]. Therefore, we will simply provide a small reminder of elements necessary for the comprehension of the topic discussed here. The inheritance of CGD may be either X-linked or autosomal recessive. The X-linked trait results in a defect of CYBB gene on the X chromosome in position p21.1 [23], which accounts for two thirds of CGD cases. The CYBB gene codes for the NOX2 subunit of the phagocyte NADPH

oxidase. The autosomal recessive disorder accounts for the remaining third and is primarily due to mutations of the genes coding for $p47^{phox}$ (20%), $p22^{phox}$ (5%), or $p67^{phox}$ (5%) [9, 24–26]. To date, no mutation of the gene coding for the $p40^{phox}$ subunit has been identified. One needs to be aware that not all CGD cases are inherited, but that they may occur also through de novo mutations [27]. The small GTPase Rac2 is important for NOX2 activation. A patient with impaired superoxide production due to a point mutation (D57N) in Rac2 has been reported. He presented with severe bacterial infections and poor wound healing. However, the symptoms of his immunodeficiency were different from classical CGD [28, 29]. Autosomal recessive patients suffer a less severe disease than X-linked patients with a lower morbidity and mortality, a greater percentage surviving past the second decade (42% vs 22%) [15]. Most patients are male and white although there could be an underdiagnosis bias in other races [15].

A carrier state was recognised early on in the mothers and sisters of X-linked patients. These carrier females exhibit an abnormal tetrazolium dye-phagocytosis histochemical test [30], due to random inactivation of one of the X chromosomes. This phenomenon of X chromosome inactivation, called lyonization, is known to normally favour cells expressing the nonmutated X chromosomes in X-linked diseases [31]. Yet this is not the case in CGD, where X-linked carriers show a random mosaic population of two leukocyte populations, oxidase-positive and -negative neutrophils. It is not surprising that X-CGD carriers present a phenotype of CGD symptoms that are directly correlated with the amount of superoxide production [32]. Not only might they present with clinical evidence of host defence defect [33, 34], but also with increased frequency of inflammatory diseases, especially skin lesions. These lesions will be discussed in more detail in the appropriate chapters. Carriers also have been found to possess autoantibodies more frequently than non-carrier relatives (95% vs 10%) [35].

## Increased inflammation in chronic granulomatous disease

Patients with CGD suffer from a variety of inflammatory conditions [15, 36], also classified as “complications not obviously caused by infection” [37]. This terminology summarizes the poor knowledge of the mechanisms underlying these inflammatory CGD manifestations. In some instances, inflammatory disorders are the first clinical manifestation of CGD [38]. One of the most typical inflammatory responses in CGD is granuloma formation. Granuloma formation can affect various organs, with a preference for hollow viscera, such as colon, stomach, and bladder. A number of observations argue in favour of a non-infectious origin of CGD granulomas:

1) in many instances, no microbes can be recovered from the lesions [39–41].
2) the lesions respond to numerous immunomodulators, such as steroids [38, 40, 42, 43], salazopyrine [19, 44], or even cyclosporine A or azathioprine [45, 46], but not to antibiotics [19, 40].
3) hyperinflammatory reactions are readily induced by sterile fungal cell wall preparations in NOX2-deficient mice [47–49].

However, while the inflammatory, non-infectious nature of many CGD manifestations is now firmly established, the primary mechanism of the increased inflammatory response remains poorly understood. The histopathological findings show mostly non-specific persistent inflammation (Fig. 1; the histology shown in this figure is taken from mouse models, which strongly resemble histological findings in CGD patients). The most commonly described feature is an acute and/or chronic inflammation with fibrosis containing non-caseous granulomas. Only in particular tissues such as the intestinal tract, liver and lymph nodes do the lesions show particular features. In these organs, active chronic inflammation is described, with a relative paucity of neutrophils, increased number of eosinophils, eosinophilic crypt abscesses (intestinal tract), abundant nuclear debris and pigmented macrophages [50]. These features may allow an experienced pathologist to differentiate hyperinflammatory CGD complications from other granuloma-forming diseases such as tuberculosis or Crohn’s disease [41]. Granulomas may be of microscopic or macroscopic size (up to several centimeters). Microscopic granulomas are typically part of a diffuse inflammatory process, such as colitis, while macroscopic granulomas usually cause a localized pathology through mechanical disturbance, such as gastric outlet obstruction. The distinction between diffuse hyperinflammation and pathology due to large granuloma is usually not addressed in the literature. Therefore, we will specify these aspects in the specific subchapters below, wherever the information is available.

### Digestive tract and associated organs

*Digestive tract* Gut involvement is now reported as the most common hyperinflammatory symptom in CGD patients [13]. It was already recognised as a possible complication in the early descriptions of the disease [51]. The real prevalence is unknown, but the reported prevalence reaches up to 33%, with 70% of these patients identified within the first decade of life [38]. The X-linked NOX2-deficient patients are more affected by gastrointestinal symptoms than the autosomal patients lacking cyto-

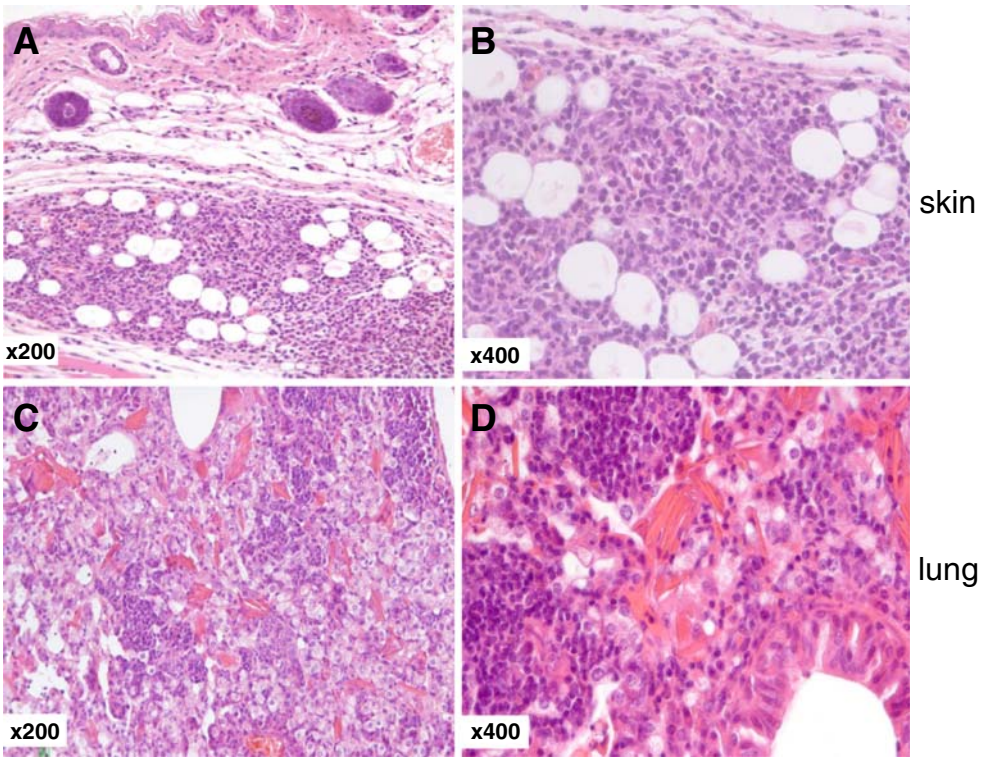


**Fig. 1** Hyperinflammatory responses in skin and lung of NOX2-deficient mice. Panels **A** and **B** show HE-stained histological sections from mice 7 days after intradermal injection of sterile *Aspergillus* cell wall preparations. A massive accumulation of inflammatory cells, in particular neutrophils, can be observed. In wild-type mice, virtually no inflammation would be observed at this point (not shown). Panels **C** and **D** show spontaneously occurring lung lesions (in particular eosinophilic crystals) in NOX2-deficient mice at the age of 6 months; such lesion is also observed in small fraction of wild-type mice, but in virtually 100% of CGD mice

plasmic subunits [38]. Thus, the genetic variant of the disease that causes the most severe infectious problems also causes the most frequent hyperinflammation. Note that, as opposed to infection, gastrointestinal hyperinflammation does not lead to an obvious increase in mortality of the patients. It has, however, become an important cause of morbidity in CGD patients [38].

Hyperinflammatory CGD lesions may affect any part of the gastrointestinal tract, from mouth to anus, as shown in the Table 1. Symptoms depend on the site and the pathology (diffuse vs localized) and range from abdominal manifestations, such as vomiting, to more systemic problems, such as weight loss or anaemia. An overview of gut-specific symptoms is listed in Table 2. The following clinical signs are commonly observed: growth failure, anaemia and failure to thrive, abdominal pain, diarrhoea, with or without blood (39%), nausea and vomiting (24%), and constipation (2%) [19, 38]. In CGD patients such symptoms are almost always stereotypically attributed to infection or to side effects of antibiotics. It is, however, important to include hyperinflammation in the differential diagnosis.

**Table 1** Characteristics of the gastrointestinal histology in CGD patients

| Gastrointestinal Histology | | Ament 1973[51] *N*–8 (%) | Schäppi 2001[19] *N*–7 (%) | Marciano 2004[38] *N*–15 (%) | Levine 2005[41] *N*–20 (%) |
|---|---|---|---|---|---|
| Upper GI | Involvement | / | / | / | 42 |
| | Pigmented macrophages | / | / | / | 4 |
| | Granuloma | / | / | / | 5 |
| Ileum | Involvement | 88 | / | 32 | – |
| | Pigmented macrophages | – | / | – | – |
| | Granuloma | 0 | / | – | – |
| Colon | Involvement | 100 | 100 | 67 | 100 |
| | Pigmented macrophages | – | 71 | – | 69 |
| | Granuloma | 63 | 29 | 33 | 41 |
| **Total GI involvement** | | / | / | / | 71 |

The table compares incidence in different studies for gut localizations, along with the typical histological features present in gastrointestinal biopsies of CGD lesions.
*GI* gastrointestinal, "/" this localisation was not studied by the authors, "–" data not provided by the authors

**Table 2** Description of gastrointestinal symptoms in CGD patients

| Gastrointestinal Symptoms | Ament 1973[51] *N*=9 (%) | Schäppi 2001[19] *N*=7 (%) | Marciano 2004[38] *N*=140 (%) |
|---|---|---|---|
| Abdominal pain | 11 | 86 | 100 |
| Diarrhoea | 55 | 71 | 33 |
| Bloody diarrhoea | 11 | 71 | 6 |
| Nausea, vomiting | 22 | 14 | 24 |
| Failure to thrive | – | 71 | 11 |
| Constipation | – | 29 | 2 |
| Height <5 DS | 11 | 43 | 32 |
| Weight <5 DS | 22 | 29 | 22 |

"–" data not provided by the authors

Inflammatory gut involvement in CGD presents itself in two ways: focal obstructive lesions and diffuse inflammation.

1) Focal obstructive lesions are observed in up to 35% of patients [38]. Although obstructive lesions can develop anywhere along the gastrointestinal tract, the most commonly affected region is the distal stomach. This leads to gastric outlet obstruction and affects a substantial fraction of CGD patients (15-50%) [15, 36, 37, 52]. It is more often present in the X-linked than in the autosomal patients [15]. Obstructive lesions can also be present in the oesophagus [36, 38], or the duodenum [38]. In general, it appears that obstructive CGD complications manifest themselves later in life than infectious complications [53]. For example, the mean age of CGD patients presenting with gastric outlet obstruction is 44 months [54], while at the age of 24 months most CGD patients have already gone through infectious complications.
2) Diffuse inflammation is observed in the oesophagus, the small bowel, and the colon. Colitis and enteritis are relatively common in CGD patients, being increasingly diagnosed during the last decade [15]. As seen for focal obstructive lesions, diffuse colitis is more prevalent in patients with the X-linked disease than in patients with the autosomal recessive form [36, 46, 55] (19% vs 13%) [15], (89% vs 11%; OR:6.07 [38]) and is more severe [19]. It might also start earlier in life [56]. The endoscopical lesions are a chronic active colitis, with patchy friability, pseudopolyps, petechial haemorrhages, strictures, fissures and ulcers [19, 46, 53, 57]. On histology, the characteristics are a focal infiltrate of polymorphonuclear cells causing cryptitis and crypt abscesses, with increased infiltration of eosinophils and macrophages, but paucity of neutrophils as compared to other inflammatory bowel diseases. The granulomas are well defined, due to aggregates of epithelioid histiocytes surrounded by a cuff of dense lymphocytic inflammation [50]. This is in contrast with the granulomas seen in Crohn's disease, which are poorly formed, less prominent [19, 38], and which contain periodic-Schiff reagent positive granules [45]. CGD granulomas are more frequent in colon biopsies than in small bowel biopsies [51]. Signs of chronic colitis, such as Paneth cell metaplasia and crypt shortening [38], are more rarely described. The architecture of the colon is disorganised with a reduction in the gland number [51]. A high-level expression of inflammatory markers is observed: human leukocyte antigen-DR expression is increased in the epithelium and vascular endothelium, along with an increased expression of adhesion molecules—vascular adhesion molecule-1 and intracellular adhesion molecule-1 specifically in the lamina propria, E-selectin in small vessels [50]. CGD colitis is commonly mistaken for Crohn's disease [57, 58], although CGD colitis is more patchy in its distribution [38]. Some typical features, such as the presence of nuclear debris, large pigmented macrophages with brown cytoplasm [17, 41, 51, 59], and eosinophilic cytoplasmic inclusions [19] can help to differentiate CGD colitis from other inflammatory bowel diseases, even in a blind fashion [60]. Taking all of these features into account will allow the careful examiner to distinguish it from Crohn's disease, avoiding the mistaken diagnosis [45, 58, 61]. Abnormal histology is found even in non-symptomatic patients [51]. Colitis in CGD patients is invariably culture-negative and responds to immunosuppression rather than to antibiotics [19, 40, 50].

The choice of treatment depends on the type of gut involvement. In general, immunomodulators are used. In obstructive complications, the first line of treatment is the use of steroids [38, 40, 46, 62, 63]. Recurrence of the symptoms is high, with 71% of relapse after reduction or cessation of the therapy [38]. Other therapeutical choices are drugs used in inflammatory bowel diseases, such as sulfasalazine and infliximab [19, 38, 45]. Remission induced by recombinant human granulocyte colony stimulating factor has been reported in a case of enteritis [64] and impaired wound healing [65]. The efficacy of hydroxychloroquine, a drug used in the treatment of malaria and

inflammatory disorders, has been recently reported in one case with severe gastric involvement [66]. Surgery is sometimes needed [38], with an ileostomy being raised in case of severe colitis. Gastric outlet obstruction is probably a contraindication for a surgical approach as it bears a risk of recurrent fistulae [67].

There are also a number of rarer forms of gut involvement. Granulomatous stomatitis, oral ulcers and dental abscesses have been described [68]. Oesophagitis is not frequent, but can lead to severe symptoms, such as progressive dysphagia, delayed emptying and either organ dilatation or stricture [53, 69, 70].

*Liver* Liver biopsies are performed in CGD patients in case of suspected liver abscess or organomegaly. When systematically reviewing such tissue samples, Levine did not detect the presence of microorganisms, but occasionally found non-specific inflammation, pigmented macrophages, or granuloma [41].

*Pancreas, spleen* No lesions other than the presence of scattered pigment-containing macrophages in these organs have been reported in the literature [71].

## Urogenital tract

The reported incidence of inflammatory lesions within the urogenital tract in CGD patients is around 40% [72, 73]. The most frequently reported lesions are urinary obstruction due to granuloma, and cystitis without apparent infection, which may be accompanied by focal or diffuse thickening of the bladder wall [43, 72, 74]. CGD lesions of the urinary tract can lead to decreased renal function [72]. The genital tract can also be affected with granulomatous orchitis and peniscrotal granulomas [43].

Obstruction of the urinary tract [13, 75] due to granulomas is frequent, being reported in 3.8% to 12% of patients [37, 72]. First noted by Kontras [76], chronic cystitis is one of the most frequently observed lesions. It presents as haematuria, sometimes leading to hydronephrosis [77] and even renal insufficiency [78]. This syndrome overlaps with the syndrome referred to as eosinophilic cystitis, which has been described in children [43, 76, 79] presenting as suprapubic pain, dysuria, urinary retention, frequency and haematuria. The ultrasound may reveal thickening of the bladder wall or a mass [43]. Eosinophilic bladder lesions can also be found in asymptomatic CGD patients [77]. Inflammatory bladder lesions are often associated with urinary tract infection [76, 77]. At this point it is not clear whether the chronic inflammatory cystitis is the consequence or possibly the cause of the infections.

The common treatment for these conditions are steroids, although an anti-allergic medication, ketotifen, has been reported to be efficient in one case [40, 43, 63].

Lesions of the urogenital tract are more frequently found in X-linked disease as compared to autosomal recessive [15, 36, 72].

## Brain

A retrospective study found that the prevalence of cognitive deficits in the X-linked CGD population was high, with 23% of patients having an IQ of 70 or below, indicative of cognitive deficits. They suggest chronic illness and frequent hospitalizations to be causal by affecting growth and development as well as social and educational opportunities [80]. NOX2 is expressed in the brain: at high levels in microglia, which are the main phagocyte of the central nervous system [81] and—probably at lower levels—in neurons [82].

The cause for cognitive deficit in CGD patients is not known, although three basic possibilities can be considered. They can be due to the fact that NOX2 has a role in neuronal development and/or brain function. However, it might also be to due the frequent infections suffered by CGD patients or to the dysregulation of inflammatory processes in the brain. Thus, lack of NOX2 function leads to cognitive deficits. However, it should be noted that there is also increasing evidence that enhanced NOX2 activity can lead to dementia, in particular in Alzheimer's disease [83, 84].

Non-infectious brain lesions have been rarely reported. They mainly consist in granuloma and infiltrate of pigmented, lipid-laden histiocytes [85]. One autopsy in a young patient with neurological deficit revealed extended brain involvement with the characteristic pigmented macrophages in the perivascular spaces and leptomeninges, focal white matter lesions with demyelinisation, intense sclerosis and lesions of the centrum ovale [86]. The authors hypothesise that the unexplained white matter destruction could originate from macrophage activity, previous infections or post-infectious encephalomyelitis.

## Skin

Skin histology taken in the context of non-specific skin alterations from patients with CGD showed granulomatous (7/18) or non-specific inflammation [41]. The typical pigmented macrophages are also found in skin biopsies [87]. Poor wound healing has also been reported as a feature of the skin of CGD patients [88]; however it is not clear whether this is linked to an infection problem, or whether this also belongs to the non-infectious complications of CGD. Discoid and systemic lupus erythematosus

[13, 75] is reported in up to 3.8% of CGD patients [36]. There are also case reports of other autoimmune diseases such as juvenile rheumatoid arthritis, immune-mediated thrombocytopenia [36], and erythema nodosus [13].

Cutaneous lesions similar to discoid lupus are the most common phenotype in X-CGD carriers (26%) and kindreds (12%) [13, 89-95]. Other carriers had recurrent aphthous-like stomatitis [89, 90, 92]. Their histology cannot be differentiated from classical discoid lupus erythematosus [96]. The occurrence of discoid lupus erythematosus-like lesions and aphtous stomatitis is closely related to the degree of reduction in superoxide production [96]. There is also a high incidence of lupus erythematosus in family members of CGD patients, up to 9% in the US registry. Conversely, when females with discoid lupus erythematosus were screened for CGD carriage, no cases were found [96]; however, only a small number of women were tested in this trial. Thus, while CGD carriage strongly augments the risk of discoid lupus erythematosus, it does not appear to be a major cause of the disease globally. Photosensitivity, together with other cutaneous symptoms such as rash, is a symptom reported in carrier mothers [89]. These symptoms typically precede the development of discoid lupus erythematosus-like lesions [96]. Photosensitivity and rash are reported by up to 58% of carriers, but the incidence of discoid lupus is only 12% [95].

## Joints and arthritis

Arthritic lesions can have numerous causes, and it seems that increased ROS are present at the site of inflammation where they are expected to oxidize membranes and components of the matrix and to contribute to tissue damage and enhanced inflammation [97]. Evidence indicates that this increase in ROS is due to the activity of NOX2. Circulating neutrophils and monocytes have increased NOX2 activity in patients suffering from rheumatoid arthritis. In arthritis, a large number of neutrophils infiltrate the inflamed joints and several studies show that neutrophils isolated from synovial fluid of rheumatoid arthritis patients generate more ROS than circulating resting neutrophils [98–100]. This increased ROS generation is therefore considered to participate in tissue destruction.

Nevertheless, there is an emerging concept that increased ROS production could also be beneficial, in particular, in cases of autoimmune disorders such as rheumatoid arthritis and lupus. In humans, it has been reported that 37% of mothers carrying the X-linked CGD mutation reported joint pain, that improves under lupus treatment [95] and that a patient with CGD presented signs of polyarthritis resembling juvenile rheumatoid arthritis [16]. It is not yet known whether this reduced capacity to produce ROS is a significant factor in human rheumatoid arthritis, but, on an interesting note, there is a strong association between a single nucleotide polymorphism (SNP) in NCf4 ($p40^{phox}$) and rheumatoid arthritis in rheumatoid factor-negative men [101]. This supports the importance of decreased reactive oxygen species production at least for a subgroup of patients with rheumatoid arthritis.

The possibility that decreased NOX2 activity leads to arthritis is of major interest. It is supported by an increasing body of evidence in animal data that will be discussed in detail later in this review in the section regarding animal models of arthritis.

Note that in rare cases CGD patients infected by *Aspergillus* spp (fumigatus and nidulans) have developed arthritic lesions [102, 103]. These cases were due to direct infection and successfully treated with antifungal compounds.

## Eyes

Chorioretinitis has been described in CGD patients [13, 75]. The observed lesions are well circumscribed, with chorioretinal scars lying next to the major retinal vessels [104, 105]. Their incidence is 30% of X-linked, with no case in autosomal recessive patients [104]. They are associated with punched-out like atrophic areas of the choroid, retinal pigment epithelium and retina [71, 104], sparing the macula [105]. These lesions affect visual acuity only when they are extensive [104] and can otherwise be asymptomatic [104, 105]. It has been hypothesised that the underlying pathomechanism is an abnormal degradation of phagocytosed cellular debris; this might point towards an expression of the NOX2 in retinal pigment epithelium, which is generally thought to be the phagocyte of the retina [20, 104]. The same typical lesions are present in 10% of CGD carriers, although in a less extensive form [104].

Rare cases of oculomucocutaneous syndrome (Behçet syndrome) [75], chronic uveitis [106], as well as peripheral ulcerative keratitis [107] have been reported in CGD patients, although a coincidental association cannot be excluded.

## Lungs

Given the high frequency of pulmonary infection in CGD patients, there is relatively little data available on non-infectious complications. In our opinion, however, such lesions are most likely very frequent, clinically important, but unfortunately generally overlooked. Globally, histological studies demonstrated a high incidence of chronic active inflammation and granuloma in the lungs (50%) and in the pulmonary lymph nodes (83%) [14, 41]. However, the question to which extent these lesions were non-infectious, inflammatory lesions remains unclear. Mouse models strongly argue in favour of inflammatory lung lesions (see below).

Other organs

Bone marrow biopsies are seldom performed in CGD patients and no notable abnormalities have been reported [41].

## Animal models of increased inflammation in NOX2 deficiency

To study the CGD phenotype, different components of the NADPH oxidase complex have been deleted by targeted homologous recombination. The first mouse model of CGD was a knock-out mouse generated in the laboratory of Mary Dinauer [108] by targeting the gene encoding the 91-kD NOX2 subunit, creating a null allele of the gene involved in X-linked CGD. A second model was generated by Jackson [109] by disruption of the $p47^{phox}$ gene. Recently, a third model, the $p40^{phox}$ deficient mouse, was produced [110]. Although no $p40^{phox}$ mutation leading to a CGD phenotype has been described in humans yet, $p40^{phox-/-}$ neutrophils exhibit a low oxidative burst and a severe deficiency in bacterial killing in vitro.

Naturally occurring mutations in the Ncf1 gene ($p47^{phox}$) affecting the oxidative burst have been identified in rat [111] and in mouse [112]. These mutations are responsible for both a decrease in oxidative burst and a susceptibility to arthritis.

All of these mouse models lack phagocyte superoxide production, which manifests as an increased susceptibility to infection. Spontaneous phenotype of the NOX2 mouse model is characterized by severe infections with pathogens such as *Aspergillus*, *Candida*, *Staphylococcus* or *Pseudomonas* [113].

Rac2-deficient mice reproduce many characteristics of CGD mice. In particular, Rac2 deficient phagocytic cells have a reduced oxidative burst, decreased microbial killing, and increased mortality after invasive aspergillosis [114]. However, Rac2 has other functions besides NOX activation. In particular, it is involved in the organisation of the cytoskeleton. In addition to the reduced NOX2 activity, other abnormalities include defects in F-actin polymerization, chemotaxis, and exocytosis of primary granules in response to chemoattractants as well as decreased L-selectin-mediated adhesion [115, 116]. Thus, alterations observed in Rac2-deficient mice cannot be unequivocally attributed to a CGD phenotype.

Nevertheless, an interesting phenotype was observed in Rac1- and Rac2-deficient mice in an arthritis model using the infectious agent *Chlamydia* [117]. A dual role of Rac was observed: (1) Rac-deficient neutrophils showed delayed migration into the joints, which resulted in less joint inflammation; (2) in the chronic phase, however, Rac serves to alleviate arthritis, as Rac deficiency resulted in more severe arthritis. The reduced bactericidal oxidative activity of Rac-deficient mice results in a lack of host clearance of *Chlamydia*, which probably leads to chronic joint inflammation.

Digestive tract

Although a high percentage of patients suffer from gut involvement, no spontaneous phenotype has been described in NOX2-deficient mice. However, studies using *Helicobacter pylori* have yielded unexpected results that argue in favour of an involvement of CGD hyperinflammation. NOX2-deficient mice have a stronger inflammatory response, but a decreased bacterial load in the *Helicobacter* gastritis model [118, 119].

Brain

There is significant evidence showing a role of NOX2 in neuronal injury during neuroinflammatory processes, including Alzheimer's disease [120], Parkinson's disease, as well as stroke, brain trauma and meningitis [20, 84]. Microglia is the resident macrophage in the brain and the key cell involved in brain inflammation. During neuroinflammation, microglia can enter an overactivated state and release ROS by NOX2 and reactive nitrogen species by inducible nitric oxide synthase (iNOS) that cause neurotoxicity [121, 122].

However, in a model of autoimmune multiple sclerosis, in vivo data on the role of the phagocyte NADPH oxidase system in myelin oligodendrocyte glycoprotein (MOG)-induced autoimmune encephalomyelitis yielded conflicting results: injection of MOG peptides showed protection from autoimmune encephalomyelitis for $p47^{phox}$-deficient mice or in mice carrying SNPs in the Ncf1 ($p47^{phox}$) gene [123, 124], while after injection of whole length MOG, which causes a more chronic and relapsing disease, $p47^{phox}$ mutant mice developed a more severe autoimmune encephalomyelitis [124]. Thus, the exact role of NADPH oxidase in autoimmune encephalitis remains unclear.

However, a role of NOX2 does not appear to be limited to pathologies of the central nervous system. Studies on different CGD mouse models demonstrate that NADPH-dependant ROS generation is required for long-term potentiation and normal memory, two hippocampus-dependent roles [125]. Moreover, NOX2-deficient mice show also a spatial memory defect. These results could provide some insight into the cognitive dysfunction in CGD patients (see above).

Skin

Intradermal injection of heat-inactivated *Aspergillus fumigatus* cell wall causes severe hyperinflammation in CGD

mice [47]. Indeed, intradermal injection of *Aspergillus fumigatus* extracts causes maximal inflammation at 72 h persisting up to 4 weeks in the CGD mice, while it resolved within 10 days in wild type mice. However, CGD skin hyperinflammation is only observed with defined stimuli, in particular, fungal cell wall components. For bacterial cell wall components, there is no difference in the inflammatory response between NOX2-deficient and wild-type mice [49]. Under certain pathological circumstances, inflammation is dampened in CGD mice; models include antibody-mediated autoimmune epidermolysis bullosa acquisita [126] and sunburn [127].

## Joints and arthritis

The participation of NOX2 in joint inflammation has been extensively studied. However, the precise role of NOX-generated ROS in arthritis is still controversial. On one hand, there is a longstanding concept that NOX-derived ROS are involved in the pathogenesis of arthritis [98, 128, 129]. However, many of these results are based on studies using rather non-specific NOX inhibitors and should therefore be taken with caution [20, 130–136]. On the other hand, the anti-inflammatory role of NOX in arthritis is strongly supported by studies using mutant rodents with a defective oxidative burst. In NOX2 and $p47^{phox}$ knock-out mice there is a more severe arthritis induced by zymosan and poly-L-lysine coupled lysozyme. Deficient mice show granulomatous synovitis and increased matrix destruction as well as enhanced expression of inflammatory mediators [137].

As Ncf1 ($p47^{phox}$) was identified as a gene that regulates arthritis severity in rats, the group of Rikard Holmdahl has provided a large amount of information supporting the anti-inflammatory action of ROS generated by NOX2. These comprehensive studies are described in detail elsewhere [138]; however, we will briefly outline the important findings.

In a search for genes associated with arthritis, linkage analysis in rat strains differing in arthritis susceptibility led to the identification of SNPs in the Ncf1 ($p47^{phox}$) gene. A coding non-synonymous SNP in the arthritis-prone Dark Agouti rat was responsible for a decrease (low, but not absent) in oxidative burst and an increase in arthritis severity. These results were confirmed in another species, the mouse. A spontaneous null mutation in the mouse Ncf1 ($p47^{phox}$) was isolated by multiple back-crossing. These $p47^{phox}$ mutant mice showed a reduced oxidative burst and developed severe and chronic collagen-induced arthritis.

The alkane compound phytol has been shown to be effective in the treatment of arthritic inflammation by restoring the oxidative burst in arthritis-prone Dark Agouti rats and in other models of arthritis, such as collagen-induced arthritis, anti-collagen II antibody-induced arthritis and non-oil collagen-induced arthritis [139]. Similar effects were observed in rats with normal oxidative burst capacity.

Thus, the evidence that decreased ROS generation plays a role in arthritis development is strong. Globally, however, the role of ROS in autoimmune diseases like rheumatoid arthritis is very complex and can be destructive or anti-inflammatory depending on where, when and to what extent they are generated. Indeed, a recent study showed that a single injection of IFN-gamma in the joint increased the symptoms in $47^{phox}$ deficient mice while stable over-expression of adenoviral IFN-gamma in the knee joint decreased bone destruction [140].

## Eyes

So far, there are no reports on retinal disorders in CGD mice.

## Lungs

Acidophilic macrophage pneumonia is a non-infectious condition found in aging mice. Its incidence depends on the mouse strain (3–30%) [141, 142]. The cause of this lung inflammation is poorly understood. However, in our opinion, it might be related to a problem of degradation of phagocytosed material. Acidophilic macrophage pneumonia is found in 100% of CGD mice as young as 2.5 months, both NOX2-deficient and $p47^{phox}$-deficient [113, 143]. On histology eosinophilic, non-birefringent crystals are seen either extracellularly or in macrophages and giant cells. Also observed in CGD patients, particularly in the colon, the provenance has not yet been clearly established. Proposed physiopathology will be discussed in the next chapter.

Models of non-infectious lung inflammation using heat-inactivated *Aspergillus fumigatus* wall caused an infiltration of neutrophils with tightly clustered foci within 24 h. The lesions in the CGD mice included five times more neutrophils than in the wild type, and more mononuclear cells but the same amount of leukocytes. At day 7, the lesion constitutes a distinctive pneumonia with neutrophil microabscesses surrounded by large mononuclear cells. At day 21, granuloma-like structures were observed and lasted up to 6 weeks [47].

However, increased inflammation in CGD may be beneficial under certain circumstances. Indeed, recent data obtained with CGD mice suggest that the increased inflammatory response is protective in pulmonary infection due to influenza virus [144], pneumococci [145] and cryptococci [146]. Note, however, that CGD mice are immune-deficient with respect to pulmonary infections with *S. aureus* [47] and *Escherichia coli* [147].

## Characteristics and mechanisms of the inflammatory process in NOX2 deficiency

As ROS are usually associated with inflammation, the increasingly well-documented observation that the absence of ROS generation by NOX2 leads to enhanced inflammation represents a change in paradigm and requires investigation into underlying mechanisms. Inflammation is a highly regulated pathway, which is mediated by pro- and anti-inflammatory biochemical signals. In particular, there is emerging evidence that the resolution of inflammation is an active process requiring the activation of endogenous programs (for review, see [148]). In the light of the various hyperinflammatory states observed in CGD, ROS production by NOX2 is likely to play an active role in this resolution. Degradation of phagocytosed material is one of the obvious roles of ROS in the resolution of inflammation. However, ROS could also contribute to the termination of inflammation through suppression of pro-inflammatory signals or through impaired survival of pro-inflammatory cells. Defects in any of these processes can lead to aggravated inflammation.

The mechanistic investigations into these processes are in their initial phase and the possibilities remain wide open. Basically the following mechanisms have to be considered (Fig. 2):

### 1) decreased degradation of phagocytosed material

The initially proposed mechanism of CGD hyperinflammation is a decreased degradation of phagocytosed material due to deficient generation in CGD phagocytes. Phagocytosed material could accumulate in NOX2-deficient phagocytes leading to persistent cell activation [49, 149]. Deficient degradation could implicate either the remaining phagocytosed microbial material [49] or phagocytosed apoptotic neutrophils by macrophages. The pathognomonic eosinophilic crystals described in both CGD patients and mice might be residues of poorly degraded apoptotic neutrophils. In fact, the proteins within these crystals are, at least in part derived from neutrophils [143].

### 2) NOX2 signalling in myeloid cells

a) Calcium and ion channels

Reactive oxygen species (ROS)-dependent attenuation of $Ca^{2+}$ signalling [150, 151] may be impaired in CGD, contributing to enhanced inflammation. This might occur

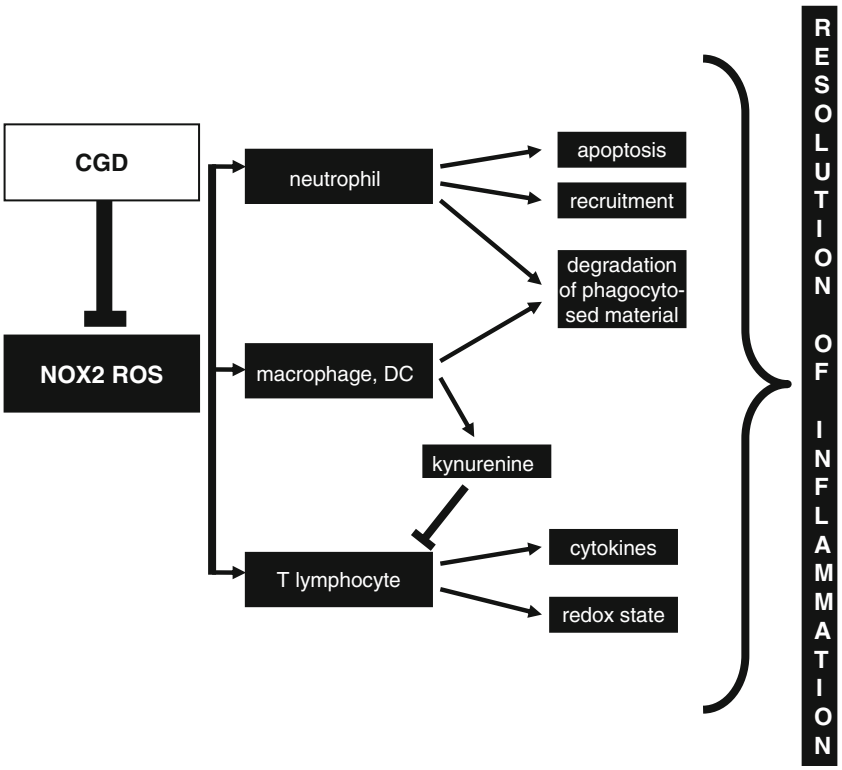


**Fig. 2** Mechanisms implicated in the ROS-dependent resolution of inflammation in chronic granulomatous disease. NOX2-derived ROS might exert their anti-inflammatory activity on the level of neutrophils, macrophages and dendritic cells, or on the level of lymphocytes. NOX2-derived ROS might enhance neutrophil apoptosis and limit neutrophil recruitment. A role of NOX2 in degradation of phagocytosed material (microbial material, apoptotic cells) is likely. NOX2-dependent kynurenine generation dampens T lymphocyte activation. NOX2 might also be expressed at low levels in T lymphocyte and regulate cell surface redox state and cytokine release. Thus, a multitude of mechanisms involved in the resolution of inflammation are lacking in NOX2-deficient cells

through regulation of membrane potential in CGD granulocytes, showing a more negative membrane potential, which allows increased $Ca^{2+}$ influx and thereby an enhanced inflammatory response [150]. Also, a direct regulation of $Ca^{2+}$ channels by the redox potential via thioredoxin has been suggested recently [152].

b) Altered intracellular signalling

ROS are increasingly implicated in the regulation of intracellular signalling, particularly through the oxidation of cysteine residues in phosphatases and in transcription factors [20]. Thus, it is possible that the absence of NOX2-derived ROS in CGD leukocytes creates signalling alterations which favour proinflammatory responses. Indeed, there are numerous publications suggesting that the inflammatory response can be more pronounced in CGD phagocytes with higher release of TNF-α and IL-8 [150, 153–155]. On the other hand, human CGD phagocytes have an impaired ability to produce anti-inflammatory mediators, such as TGF-β and prostanglandine 2 [156]. The stimulus is also an important factor. In our studies, the fungal wall component β-glucan, but not bacterial cell wall components, induced hyperinflammation. This raises the possibility that ROS provide a feedback inhibition to inflammatory signalling through β-glucan receptors [49]. Also, human CGD leukocytes stimulated by sterile *Aspergillus* cell wall extracts release either pro- or anti-inflammatory cytokines, depending on the source of the extract: conidial stimulation tips the balance towards proinflammatory cytokines, such as TNF-α and interleukine 6, while hyphal stimulation leads to higher levels of Th2 regulatory cytokines, such as IL-10 [157].

c) Apoptosis

Apoptosis of inflammatory cells is a potential mechanism to limit inflammation. There is abundant evidence suggesting that ROS can induce neutrophil apoptosis [158–164]. Consequently, it has been suggested that decreased apoptosis of neutrophils is one of the mechanisms of CGD hyperinflammation [137, 156, 160, 165, 166]. Constitutive apoptosis seems to be abnormal in both human and murine CGD neutrophils due to diminished/delayed phosphatidyl serine exposure [167]. The recognition of exposed phosphatidyl serines is essential for the uptake of the apoptotic cells. The failure to ingest apoptotic cells is hypothesised to cause immunisation to self-antigens, leading, for example, to higher lupus prevalence in CGD patients. It should, however, be noted that in a skin model of CGD hyperinflammation, increased, rather than decreased, neutrophil apoptosis was observed [49]. Thus, there is no strong in vivo data for the NOX2-dependent apoptotic mechanism.

d) Immune receptor expression

Recently, impaired expression and function of innate immune receptors has been described in neutrophils of CGD patients [168]. A decreased expression of specific receptors (TLR5, TLR9, complement receptors and CXCR1) results in impairment of the various neutrophil functions such as pathogen recognition, phagocytosis and chemotaxis [168]. On the other hand, there is an increased cell surface expression of other immune receptors, such as TLR5 and CD18, in CGD patients. It has been suggested that this upregulation has a protective role concerning the development of lymphadenitis and pneumonia [168]. It also appears that CD35 expression is increased in immune cells from CGD patients, which might be linked to the increased frequency of autoimmune pathologies [168].

e) Inflammatory mediators

The inability of CGD immune cells to inactivate inflammatory mediators is another potential explanation for hyperinflammation. Indeed, it has been suggested that impaired oxidative inactivation of proinflammatory mediators may prolong the inflammatory response [169]. In vitro catabolism of inflammatory mediators such as leukotrienes [170–172] and S100 proteins has been shown to be ROS production dependent [169].

### 3) NOX2 signalling in lymphocytes

The role of ROS in the activation of T-cells has been mostly studied in the context of arthritis. Intracellular ROS are increased in synovial T cells from patients with rheumatoid arthritis, but this increase is not NOX2 dependent as it is not inhibited by DPI [173]. However, it appears that the severity of arthritis is regulated by the redox levels at the surface of T cells [174]. Lack of reactive oxygen species breaks T-cell tolerance to collagen type II and allows development of arthritis in mice [175]. NOX2 is also expressed in EBV-transformed B-cells, but the physiological role of this expression is only poorly understood [20].

The question whether NOX2 is indeed expressed in T lymphocytes or whether NOX2 regulates redox-dependent processes in lymphocytes exclusively through a paracrine interaction (i.e. $H_2O_2$ diffusion) between phagocyte and T lymphocytes remains open. The fact that adoptive transfer of CD4+ T cells from mice with Ncf1 ($p47^{phox}$) polymorphism transfers their arthritogenic potential would argue in favour of a direct role of NOX2 in T lymphocytes [174]. On the other hand, determination of ROS generation in T cells alone has suggested that they have little or no oxidative burst capacity [138].

### 4) Crosstalk between lymphocytes and NOX2 from myeloid cells

a) Redox status

As discussed above, macrophages and their ability to generate ROS is thought to be involved in T cell responses and arthritis development in mice [138].

A recent paper shows that hyperinflammation in NOX2-deficient mice might—at least in part—be due to a dysfunctional kynurenine pathway [176]. Kynurenine is produced from tryptophan by indol 2,3 dioxidase (IDO). It favours T-cell tolerance. Superoxide is a required cofactor for tryptophan oxidation by IDO. Thus, lack of ROS precludes kynurenine generation and therefore the development of immune tolerance. It is interesting to note that a recent study suggested a reversal of the hyperinflammatory phenotype in CGD mice by replacement therapy with kynurenine.

### 5) Modifier genes

The severity of CGD hyperinflammation may also be a function of modifier genes. Indeed, the risk to develop granulomatous complications appears to be influenced by genotypes of myeloperoxidase and Fcγ receptors, while the risk to develop a rheumatologic disorder is modified by the presence of variant alleles of mannose binding lectin or FcγRIIa [36]. Thus, subtle genetic differences in molecules of innate immunity seem to contribute to interindividual differences in host inflammatory responses in CGD patients.

## Conclusions: $ROS^{high}$ and $ROS^{low}$ inflammatory responses

This review summarises the currently available information about NOX2 and inflammation and aims at deciphering the seemingly heterogeneous responses in both CGD patients and animal models. In particular, we highlight the apparently counterintuitive findings that NOX2 deficiency leads to a hyperinflammatory response. To clarify this emerging concept, we proposed the distinction between a "$ROS^{high}$ inflammatory response" and a "$ROS^{low}$ inflammatory response" (Fig. 3).

*The $ROS^{high}$ inflammatory response* is characterized by phagocyte NADPH oxidase activation, activation of ROS-dependent killing mechanisms, but with a limitation in the influx of neutrophils and oxygen-independent killing mechanisms. It also may dampen the activation of the specific immune system through mechanisms including kynurenine generation. The $ROS^{high}$ inflammatory response appears to be superior for the host defence against staphylococci [108], *Aspergillus* [47], *E. coli* [147], *Mycobacterium tuberculosis* [177], as well as for the clearance of phagocytosed material. The $ROS^{high}$ inflammatory responses are typically associated with chronic lung disorders [18, 178], and cardiovascular and neurodegenerative diseases [20].

*The $ROS^{low}$ inflammatory response* is characterized by a massive influx of neutrophils and a strong activation of oxygen-independent killing mechanisms. It allows a stron-

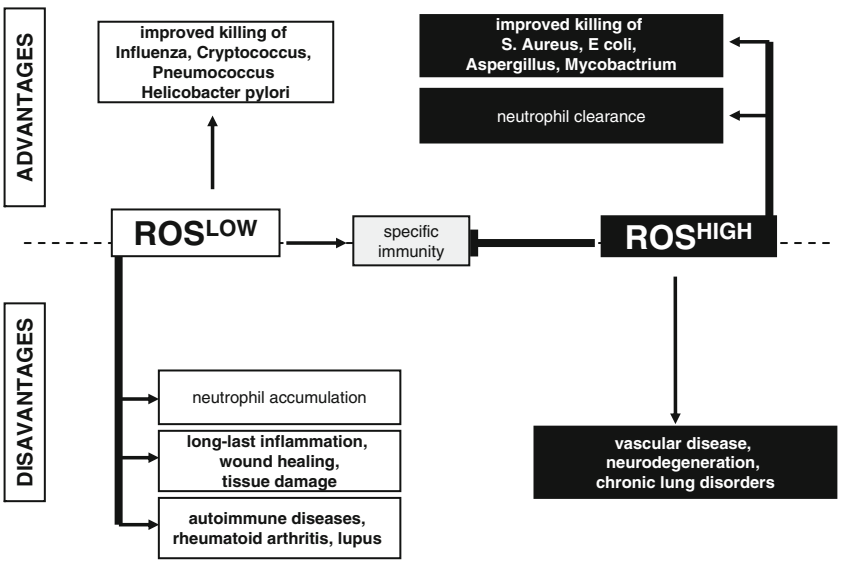


**Fig. 3** Concept of $ROS^{high}$ and $ROS^{low}$ inflammatory responses. The level of ROS production determines the type of inflammatory response and thereby the killing of specific microorganisms. $ROS^{high}$ response improves killing of pathogens typically encountered in CGD patients, e.g. *S. aureus, Aspergillus*, while $ROS^{low}$ response improves the defence against *Influenza*, *Cryptococcus*, etc. These advantages are counterbalanced by disadvantages: $ROS^{high}$ response is associated with increased ROS-dependent tissue damage, including vascular disease, neurodegeneration, and chronic lung disorders. On the other hand, long-lasting inflammation and increased incidence of autoimmune disorders are seen in case of $ROS^{low}$ response

ger activation of the specific immune system. The $ROS^{low}$ inflammatory response seems to be superior for the host defence in many situations, including pneumococcal [145], *Influenza* [144], and cryptococcal pneumonia [146], *Helicobacter* gastritis [118, 119]. $ROS^{low}$ inflammatory responses are histologically more severe and tend to lead to more tissue damage. Also the $ROS^{low}$ inflammatory response is inefficient in removing phagocytosed material, as evidenced by the pigmented macrophages, which are consistently observed in CGD patients and in CGD mice. Finally, the $ROS^{low}$ inflammatory response is associated with autoimmune disease, in particular lupus and arthritis.

Are $ROS^{low}$ inflammatory responses an oddity of CGD patients and their relatives, or is this a more widely applicable concept? In our experience, the amount of ROS generation by phagocytes varies greatly from one individual to the other (unpublished observation). Also, there is increasing evidence of high or low levels of ROS production in patient cohorts with defined diseases [18, 84] such as lupus, Alzheimer's disease, amyotrophic lateral sclerosis [179], osteopetrosis [180], osteoporosis [181]. Thus, there appear to be genetic variations in ROS generation.

However, there might also be variations in ROS generation independent of genetic factors, which would favour a $ROS^{high}$ and $ROS^{low}$ inflammatory response, respectively. Such putative factors include the oxygen tension in a given tissue, nutritional uptake of prooxidants and antioxidants, as well as the hormonal and cytokine environment.

In summary, we propose that the study of the inflammatory response in CGD patients and mice, as discussed in this review, opens a new avenue for an improved understanding of inflammation and immune balance in general.

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