Invasive Aspergillosis in Chronic Granulomatous Disease

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**Chronic granulomatous disease**

Chronic granulomatous disease is an inherited disorder of the NADPH oxidase complex in which phagocytes are defective in generating the microbicidal reactive oxidant superoxide anion and its metabolites, hydrogen peroxide, hydroxyl anion, and hypohalous acid. As a result of the defect in this key host defense pathway, CGD patients suffer from recurrent life-threatening bacterial and fungal infections. CGD is also characterized by abnormally exuberant inflammatory responses leading to granuloma formation, such as granulomatous enteritis, genitourinary obstruction, poor wound healing, and dehiscence. CGD affects approximately 1 in 200,000 persons.

The phagocyte NADPH oxidase functions to rapidly generate superoxide anion by transferring electrons from NADPH to molecular O₂ in response to physiologic stimuli such as phagocytosis. The cytochrome, composed of gp91<sub>phox</sub> (phox, phagocyte oxidase) and p22<sub>phox</sub>, is embedded in membranes. In neutrophils, approximately 85% of the cytochrome is in the membranes of specific granules or gelatinase-containing granules, and the remainder is present in the plasma membrane and in secretory granules [1, 2]. The NADPH binding site is located on the cytoplasmic side of membranes. Upon activation of the oxidase, the cytoplasmic subunits p47<sub>phox</sub>, p67<sub>phox</sub>, and p40<sub>phox</sub> appear to translocate en-bloc to the membrane-bound cytochrome. Activation of rac, a member of the low molecular weight GTP-binding proteins, and translocation of rac to the membrane-bound cytochrome are also critical for NADPH oxidase activation [3, 4]. NADPH is oxidized to NADP⁺, and electrons are transported down a reducing potential gradient to FAD and then possibly to 2 non-identical heme groups. On the vacuolar or extracellular side of the membrane, the final step in the electron transport chain occurs when oxygen accepts an electron and is converted to superoxide anion. The net equation involves the reduction of 2 molecules of O₂ to 2 molecules of superoxide anion (O⁻²) at the expense of 1 molecule of NADPH.
Superoxide, a relatively weak microbicidal oxidant, is metabolized to the more toxic hydrogen peroxide by superoxide dismutase. Hydrogen peroxide can in turn be converted to hypohalous acid (bleach in the neutrophil) by myeloperoxidase, and to hydroxyl anion. Possible molecular targets of these species include genomic DNA, electron transport, and sulfhydryl groups of proteins and nonproteins.

Recently, Reeves et al. [5] shed new light on the mechanism by which NADPH oxidase-derived reactive oxidants mediate host defense. Activation of the NADPH oxidase in neutrophils leads to an influx of reactive oxidant species into the endocytic vacuole, resulting in an accumulation of anionic charge. To maintain electrogenic neutrality, K+ ions cross the membrane in a pH-dependent manner. The rise in ionic strength leads to the release of cationic granule proteins, including elastase and cathepsin G, which are bound to the anionic proteoglycan matrix in the inactivated state. Knockout mice deficient in the cationic granule proteins recapitulate the CGD phenotype in susceptibility to experimental bacterial and fungal infections [5, 6]. Taken together, activation of preformed granular proteases is likely to be principally responsible for NADPH oxidase-mediated destruction of pathogens.

Chronic granulomatous disease results from disabling mutations in genes encoding gp91<sup>phox</sup>, p22<sup>phox</sup>, p47<sup>phox</sup>, or p67<sup>phox</sup>. X-linked CGD results from defects in gp91<sup>phox</sup>, and accounts for ~65% of cases of CGD. Female X-CGD carriers usually have a dual population of circulating neutrophils based on random X-chromosome inactivation (lyonization), in which one population has normal NADPH oxidase activity and the second is defective. X-CGD carriers usually have no increased susceptibility to infection, with the exception of cases of extreme skewing in favor of NADPH oxidase-defective cells, such that the proportion of normal circulating neutrophils is below 10% [7, 8].
p22\textsuperscript{phox} (5%), p47\textsuperscript{phox} (25%), and p67\textsuperscript{phox} (5%) forms of CGD are inherited in an autosomal recessive pattern.

Laboratory methods used to diagnose CGD rely on demonstration of defective NADPH oxidase function. In the nitroblue tetrazolium test (NBT), superoxide anion reduces the tetrazolium compound to a formozan precipitate, which is detected microscopically. NBT tests may yield false normal results in cases of p47\textsuperscript{phox/-} and variant X-CGD, in which low levels of reactive oxidants are produced [9, 10]. Such patients generally have a less severe phenotype than patients with X-CGD, in whom the flavocytochrome is not detectable and phagocyte oxidant production is almost nil. Thus, in circumstances of strong suspicion, a normal NBT test should be followed by more sensitive quantitative methods for evaluating oxidase activity, including the measurement of superoxide production by the ferricytochrome c reduction assay and the use of probes whose chemiluminescent or fluorescent properties are altered by their reaction with reactive oxidants. We use fluorescent cytometry employing the dihydrorhodamine 123 probe, an ultrasensitive method for detecting intracellular H\textsubscript{2}O\textsubscript{2} production [11].

CGD patients have recurrent infections with a subset of catalase producing pathogens [12-14]. A national database describing the spectrum of infections in 368 patients with CGD has been created [15]. The most common pathogens include: *Aspergillus* species (pneumonia, osteomyelitis), *Staphylococcus aureus* (suppurative adenitis, subcutaneous infections, liver abscess), *Serratia* species (osteomyelitis, pneumonia), *Nocardia* species, and *Burkholderia cepacia* (pneumonia and sepsis).

**Invasive aspergillosis in CGD**

Invasive aspergillosis is the most important cause of mortality in CGD [16, 17]. CGD patients are susceptible to a broad spectrum of opportunistic filamentous fungi, but *Aspergillus* infection is by far the most common. Despite the routine use of interferon-gamma prophylaxis (see below), fungal infections have remained a persistent problem
with an incidence of 0.1 fungal infections per patient year [15] . Patients with X-linked CGD appear to be at increased risk for invasive aspergillosis compared with the autosomal recessive forms [18, 19] . Invasive aspergillosis may be the first manifestation of CGD. Infection usually manifests within the first 2 decades of life [18] . Invasive aspergillosis in CGD patients during the neonatal period is occasionally observed [20, 21] . Very rarely, invasive aspergillosis may occur in a female carrier of X-linked CGD in whom the random process of lyonization (X-chromosome inactivation) has led to a skewing of the circulating normal neutrophil population to less than 10% oxidant competent [7] .

*Aspergillus fumigatus* and *Aspergillus nidulans* are the most common *Aspergillus* species in CGD. *A. nidulans* is a rare pathogen in most patient populations with quantitative or qualitative neutrophil defects. We reviewed all cases in which *A. nidulans* was isolated from patients at the National Institutes of Health (Bethesda, MD) between 1976 and 1997 [18] . *A. nidulans* infection occurred in 6 patients with CGD, but was not a pathogen in any other patient group. *A. fumigatus* was a more common pathogen in CGD (n = 17 cases), but *A. nidulans* was more virulent. *A. nidulans* was significantly more likely to result in death compared with *A. fumigatus* (3 of 6 versus 1 of 17 cases, respectively), to involve adjacent bone, and to cause disseminated disease (Figure 1).
Fig. 1. The NADPH oxidase. Cellular activation leads to the assembly of the cytochrome (gp91phox and p22phox) with the cytosolic factors (p47phox, p67phox, rac2, and p40phox). This complex leads to the donation of an electron from NADPH to molecular oxygen yielding superoxide anion. This is dismutated by superoxide dysmutase (SOD) to hydrogen peroxide. H2O2 is in turn converted into hypohalous acid (bleach in the neutrophil) by myeloperoxidase (MPO). Deficiency of gp91phox, p22phox, p47phox, or p67phox leads to chronic granulomatous disease.
Patients with *A. nidulans* received longer courses of amphotericin B therapy than patients with *A. fumigatus*, and were treated with surgery more often. In contrast to *A. fumigatus*, *A. nidulans* was generally refractory to intensive antifungal therapy, suggesting that early surgery may be important. However, the need for early resection of pulmonary lesions will need to be reevaluated with the availability of 2nd generation antifungal triazoles (see below).

CGD patients often do not have typical symptoms and signs of infection [12]. Even in the setting of life threatening infections, CGD patients may be asymptomatic or have mild non-specific symptoms. Fever and leukocytosis may be absent, and an elevated sedimentation rate may be the only abnormal laboratory test [12]. In a review of aspergillosis in CGD patients at the NIH, one third of patients were asymptomatic at diagnosis and only ~20% were febrile [18]. In many of these patients, a pulmonary infiltrate on routine screening chest x-ray or CT scan was the first indication of an infection. The white blood cell count was \( \leq 10,000/\mu l \) in 13/23 cases and the sedimentation rate was \( \leq 40 \) mm/hr in 9/20 cases.

Patients with CGD may have concurrent bacterial and fungal infections [22]. It is therefore essential to establish a culture diagnosis when feasible prior to initiating antimicrobial therapy. Biopsy material should be submitted for pathology as well as bacterial and fungal culture, including *Nocardia*. In addition, recrudescent fungal infection can occur after initial control of infection had been achieved — a feature that is common with *A. nidulans* [18]. Thus, a high level of vigilance in searching for infection is necessary in caring for CGD patients. Frequent radiographic evaluation (e.g., chest radiographs during routine clinic visits and CT scans in patients with fever or focal signs) is critical to making early diagnoses.

Pulmonary infection is the most common site of invasive aspergillosis in CGD. The clinical presentation is variable, but well-defined syndromes exist. The radiographic
appearance is usually an infiltrate, mass, or nodule, and multiple lesions may be present. In contrast to patients with chemotherapy-induced neutropenia, hyphal angioinvasion usually does not occur in CGD. Thus, a halo sign (reflecting angioinvasion with surrounding tissue ischemia), cavitated lesions, and pulmonary infarction are not typical features of CGD. In early disease, an acute neutrophilic response is observed. As shown in a lung biopsy of fungal pneumonia (Figure 2), neutrophils surround and adhere to the hyphal element, but the hyphal element remains intact, reflecting the impaired microbicidal killing.

**Fig 2.** (Top) *Aspergillus nidulans* hyphal element surrounded by not killed by neutrophils from a patient with CGD.

(Bottom) Chest CT from the patient with the fungus shown above. Note the extensive left hemithoracic involvement with bony destruction. Extension to sinuses and brain were fatal.
Rarely, pulmonary *Aspergillus* infection presents as an acute and sometimes life-threatening miliary pneumonitis due to acute massive inhalation (e.g., during mulching) that requires combination therapy with an antifungal agent and corticosteroids (Figure 3).

**Fig 3.** (Top) Chest CT of subacute pulmonary aspergillosis due to inhalation of a small inoculum of fungal spores.

(Bottom) Chest CT of a patient with massive inhalation of fungal spores leading to acute hypoxia, fever and requiring intubation and mechanical ventilation (Pending).

Invasive pulmonary aspergillosis typically causes a chronic indolent infection in CGD. The terms, “chronic septic granulomatosis” and “microgranulomatous aspergillosis” have been used to describe these lesions [23]. A chronic diffuse nodular pneumonia may also occur [24]. Lesions typically contain a mixed inflammatory cell response consisting of neutrophils, histiocytes, and lymphocytes (Figure 4).
**Fig 4.** *Aspergillus nidulans* pneumonia in p47phox-/- mice. A) Numerous focal pyogranulomatous lung lesions in p47phox-/- mice 4 days after intranasal challenge with *A. nidulans* (105 conidia per mouse) (H&E x25). B) Higher power magnification (H&E x 630) showing a granulomatous lesion composed mostly of neutrophils. The arrow shows a septated hyphal element. C) Invasive hyphae shown on GMS (x200). D) Mixed neutrophilic and histiocytic granuloma with giant cell formation 15 days after challenge with a sub-lethal inoculum of *A. nidulans* (104 conidia per mouse) (H&E x 400). E) Persistent granulomatous lesions at 10 weeks with chronic inflammation and fibrosis (H&E x 200).

Hyphae may be observed within these lesions. Hotchi et al. [25] described a different type of granuloma due to *Aspergillus* sp. in Japanese patients with CGD, consisting of either multinucleated giant cells alone, or giant cells surrounding a caseous center.
Experiments in CGD mice suggest that this chronic inflammatory response may not result solely from unresolved infection. Intratracheal challenge with heat killed *A. fumigatus* hyphae led to an exuberant inflammatory response in X-linked CGD mice, but only a modest self-limited inflammatory response in wildtype controls [26]. Within 24h of challenge, an acute alveolar neutrophilic response occurred in CGD mice, accompanied by an increase in proinflammatory cytokines. Within 1 week, pyogranulomatous lesions developed which persisted for at least 6 weeks. These observations indicate that there is an intrinsic defect in regulation of inflammatory responses in CGD independent of the defect in microbicidal killing.

Pulmonary invasive aspergillosis may spread locally to involve the vertebrae and chest wall structures. This is particularly common with *A. nidulans* infection [18]. Fungal infection may be limited to the bone or soft tissue, and such infections typically respond to surgical debridement and antifungal therapy [16]. Disseminated infection can involve several sites, including the central nervous system, internal organs, and the skin. Cutaneous infection may represent primary localized infection or disseminated disease [27].

**Therapy of invasive aspergillosis**

Standard therapy for invasive aspergillosis has been high dose conventional amphotericin B (1-1.5 mg/kg daily). In patients with CGD, a prolonged duration of antifungal therapy was typically required to control filamentous fungal infection, which often entails significant nephrotoxicity. Lipid formulations of amphotericin B appear to be more effective with less nephrotoxicity and infusion-related toxicity than conventional amphotericin B, based on non-comparative and compassionate use studies.

Voriconazole, posaconazole (SCH 56592), and ravuconazole (BMS 207147) are second generation triazoles which are currently being evaluated in clinical trials. These agents are active against *Candida* species (including most isolates that are resistant to
fluconazole), *Aspergillus* species, dimorphic fungi, *C. neoformans*, and several resistant fungal pathogens, including *Scedosporium* species, dark-walled moulds, *Trichosporon* species, and *Fusarium* species.

Voriconazole has been the most extensively studied of this group of azoles. In a non-comparative study of 116 patients with invasive aspergillosis for whom voriconazole was given either as initial (52%) or salvage (48%) therapy, a complete or partial response occurred in 48%, with a more favorable prognosis in the initial therapy group [28]. Voriconazole was also compared with conventional amphotericin B (1.0 to 1.5 mg/kg daily) as initial therapy in an open, randomized study of patients with invasive aspergillosis [29]. A total of 144 patients in the voriconazole arms and 133 patients in the amphotericin B arm with definite or probable aspergillosis received at least 1 dose of therapy. Voriconazole was more effective than amphotericin B (53% with complete or partial response vs. 32%, respectively) and had improved survival at 12 weeks (71% vs. 58%, respectively). Voriconazole had a superior response rate in both neutropenic and non-neutropenic patients [29]. Most of the patients in this study had a hematologic malignancy or were hematopoietic transplant recipients.

These studies established voriconazole as a new standard of care for invasive aspergillosis. The database of voriconazole and other 2\textsuperscript{nd} generation triazoles specifically in patients with primary immunodeficiencies is limited. Walsh et al. [30] evaluated compassionate use voriconazole in 58 children with invasive fungal infection, 13 of whom had CGD. Eight (62%) of the CGD patients had a successful outcome. In our practice, we now use and recommend voriconazole as initial therapy for invasive aspergillosis in CGD (manuscript in preparation).

The echinocandin, caspofungin, is approved for the treatment of invasive aspergillosis refractory to, and in patients intolerant of, conventional and lipid formulations of amphotericin B or itraconazole. This approval was based on a compassionate use study of 63 patients with invasive aspergillosis (53 refractory, 10
intolerant). Caspofungin led to a successful outcome (complete or partial response) in 41% of cases [31], which compares favorably with carefully matched historical controls. Echinocandins have not been evaluated as initial therapy for invasive filamentous fungal infections. There is growing interest in evaluating echinocandins in combination with other classes of antifungal agents in patients with invasive aspergillosis.

In addition to systemic antifungal therapy, debridement or excision of localized fungal infection is advised when such sites are surgically accessible. In particular with A. nidulans, multiple debridements may be required when chest wall structures and vertebrae are involved [18]. Adjunctive therapy with granulocyte transfusions is discussed below.

**Prophylaxis against fungal infections**

The routine use of antibacterial prophylaxis, usually with trimethoprim-sulfamethoxazole, has significantly reduced the frequency of severe bacterial infections in CGD. However, fungal infections have remained a persistent problem with an incidence of 0.1 fungal infections per patient year [15]. In a long-term follow-up of 39 patients with CGD, Liese et al. [19] reported an increase in the frequency of fungal infections under antibiotic prophylaxis from a mean incidence of 0.2 to 1.9 serious infections per 100 patient months. However, Margolis et al. [32] found no change in the rate of fungal infections with antibacterial prophylaxis. These data underscore the need for effective antifungal prophylaxis.

In a European prospective open label study of itraconazole prophylaxis, the rate of *Aspergillus* infections was reduced compared with historical controls, and the drug was well tolerated [17]. A randomized, double-blind, placebo-controlled cross over study of itraconazole prophylaxis has recently been completed at the NIH [33]. Thirty-nine patients over 5 years of age were enrolled. One serious fungal infection occurred in
itraconazole recipients versus 7 cases in the placebo group. No significant toxicity was observed. This study supports the routine use of itraconazole prophylaxis in CGD.

This study employed itraconazole capsules, which have erratic bioavailability. In approximately half of the plasma samples drawn during itraconazole therapy, the level was in the range (< 0.3 ug/ml) that is considered sub-therapeutic. The cyclodextrin (oral suspension) formulation of itraconazole has enhanced and more reliable bioavailability. Regardless of which oral formulation is used, monitoring of plasma levels is advised based on the experience using itraconazole prophylaxis in patients with hematologic malignancies and hematopoietic transplantation in which therapeutic levels of itraconazole were protective [34, 35].

**Interferon-gamma**

Interferon-gamma (IFN-g) is a macrophage activating factor critical in host defense against intracellular infections such as *Leishmania* and *Mycobacteria* species in humans and mice [36]. In clinical trials in patients with cancer [37] and with lepromatous leprosy [38], IFN-g increased H2O2 generation in circulating monocytes, providing a rationale for evaluating IFN-g in CGD. In preliminary studies, addition of recombinant IFN-g in vitro to phagocytes from some, but not all, CGD patients augmented superoxide production [39, 40]. Augmentation of superoxide production was observed in phagocytes from cytochrome positive X-linked and the majority of autosomal recessive CGD patients, as well as in ~30% of X-linked cytochrome negative patients. In a study of patients with cytochrome positive X-linked CGD, two consecutive subcutaneous injections of IFN-g resulted in a 5- to 10-fold increase in superoxide production from granulocytes and monocytes [41]. The effect lasted for more than two weeks and was associated with an increase in bactericidal activity. Rex et al. [42] showed that administration of IFN-g to CGD patients augmented the in vitro ability of CGD neutrophils to damage *Aspergillus* hyphae.
Given these promising results, a multicenter, randomized, double blinded, placebo-controlled study of prophylactic IFN-g (50 ug/m² subcutaneously thrice weekly) was conducted [43]. One hundred and twenty eight CGD patients (67% X-linked and 33% autosomal recessive) were enrolled. Prophylactic IFN-g reduced the number of serious infections by over 70%, and was beneficial in both the X-linked and autosomal recessive forms of CGD. In contrast to earlier studies, no significant differences occurred between the IFN-g and placebo groups with regard to reactive oxidant generation, cytochrome b expression, or in vitro bacterial killing. Subsequent studies in CGD patients [44, 45] and CGD mice [46] confirmed that IFN-g did not improve NADPH oxidase function or increase levels of its constituent proteins.

The benefit of IFN-g prophylaxis in CGD likely occurs through augmentation of oxidant-independent antimicrobial pathways. IFN-g increases TNF-alpha production, tryptophan metabolism, expression of Fc-gammaR1 receptors on phagocytes, B2-integrin expression on monocytes, granule protein synthesis, and MHC-expression [36, 47].

A 10-year follow-up phase IV study of CGD patients receiving IFN-g (50 ug/m²) at NIH suggests that the drug is, in general, well-tolerated and the benefits appear sustained over and above that achieved with antibacterial prophylaxis alone.

The optimal dose and frequency of IFN-g remain to be determined. Ahlin et al. [48] studied a small group of patients with either X-linked or autosomal CGD, receiving IFN-g 100 ug/m². They found enhancement of ex vivo neutrophil functions and killing of \textit{A. fumigatus} hyphae compared to CGD patients receiving the standard 50 ug/m² dose.

We do not know whether the therapeutic use of IFN-g after the onset of fungal infection is beneficial. Use of IFN-g as adjunctive therapy for invasive aspergillosis in CGD is limited to case reports [49]. A randomized, placebo controlled study of IFN-gamma as adjunctive therapy in patients with invasive aspergillosis (not restricted to CGD) has been initiated.
Granulocyte transfusions

Granulocyte transfusion in CGD is supported by the principle that a small number of normal phagocytes may be able to complement the oxidative defect in a large number of CGD phagocytes. In vitro, a small proportion of normal neutrophils mixed with CGD neutrophils kill *A. fumigatus* hyphae as well as a population consisting entirely of normal neutrophils [50]. The likely explanation is that H$_2$O$_2$ generated by the minority of normal neutrophils can diffuse into CGD neutrophils and provide the necessary reagent to generate hypohalous acid and hydroxyl anion [50, 51]. Transfused granulocytes retain respiratory burst activity and appear to traffic normally based on their recovery from sites of infection (e.g. wound drainage sites from liver abscesses), from mouth rinse preparations, from bronchoalveolar lavage, and from neutrophil exudate in experimental skin windows [52-54].

Over the past 20 years, we have used granulocyte transfusions in CGD in life threatening infections and in infections refractory to antimicrobial and surgical treatment. Granulocyte transfusions are generally well tolerated, but adverse effects include fevers, development of leukoagglutinins leading to rapid loss of transfused granulocytes, and rarely, pulmonary leukostasis. The likelihood of pulmonary leukostasis may be increased if amphotericin B and granulocytes are administered concomitantly [55]; therefore granulocyte transfusions and amphotericin B should be administered several hours apart. One concern regarding granulocyte transfusions is alloimmunization, which is of theoretical concern for patients under consideration for bone marrow transplantation.

Bone marrow transplantation

Because CGD results from a defect in hematopoietic stem cells, bone marrow transplantation (BMT) is a rational option to establish a stable population of normal myeloid progenitors. Myeloablative BMT has been reported in at least 10 CGD patients...
and was curative in all 6 reported cases since 1984 [56-60]. Most cured patients had 100% circulating donor myeloid cells. In one patient, a stable chimeric population of circulating granulocytes consisting of 10-15% normal cells conferred protection against infections, similar to X-CGD carriers [58-60].

The morbidity and mortality associated with BMT have militated against its routine use in CGD. Horwitz et al. [61] evaluated peripheral stem cell transplantation using a non-myeloablative conditioning regimen in 10 patients with CGD. A matched related T-cell depleted allograft was used to reduce the risk of severe graft versus host disease (GVHD). After a median follow-up of 17 months the proportion of donor neutrophils in the circulation in 8 of the 10 patients was 33 to 100 percent, a level that can be expected to be protective. Significant GVHD occurred in 3 of 4 adults with engraftment, but in none of the 5 children. Three of 10 patients died. Given the risks of this procedure, this non-myeloablative transplant regimen should probably be reserved outside of research protocols to CGD patients who have had recurrent serious infections despite antibiotic, antifungal, and IFN-g prophylaxis and who have HLA-matched donor siblings.

Hematopoietic transplantation has been successfully employed as a therapeutic measure in a few published cases of CGD patients with active refractory *Aspergillus* infection [62, 63]. The rationale is that a stable population of circulating neutrophils achieved through transplantation may cure the fungal infection. This option should only be considered as a last resort given that life-threatening fungal infections may progress during neutropenia and in the setting of GVHD following transplantation. In general, transplantation should only be performed in the absence of active infection.

**Gene therapy**

CGD is an ideal candidate disease for hematopoietic stem cell gene therapy for the following reasons [64]: (1) Engraftment of a stable population of normal myeloid
stem cells is curative in CGD, as illustrated by successful bone marrow transplantation; (2) The genes encoding the subunits of NADPH oxidase have been identified, cloned, and sequenced; (3) Transfer of the relevant gene corrected the NADPH oxidase defect in myeloid and EBV-transformed B cell lines [65-70] and in primary monocytes [71] from CGD patients, confirming that expression of the missing protein was sufficient to restore oxidase function in intact cells; (4) Reconstitution of phagocyte NADPH oxidase activity has been achieved in the X-linked and autosomal recessive forms of CGD by transduction of bone marrow [72, 73] and peripheral blood stem cell progenitors [74-76] with retroviral vectors containing the relevant gene; (5) In the X-linked [77] and p47phox-/- [78] mouse models of CGD, genetic correction of NADPH oxidase has been achieved in vivo with stem cell gene therapy and gene-corrected mice have increased resistance to experimental infection; (6) Based on the experience in X-linked CGD carriers, only a small proportion of normal phagocytes are required for normal host defense.

The goal of gene therapy in CGD is to achieve a stable population of gene corrected myeloid precursors, which give rise to a biologically significant number of peripheral phagocytes. In a pilot study, gene therapy in patients with p47phox-/- CGD led to detectable, albeit miniscule, circulating oxidant producing neutrophils for as long as 6 months [79]. In the short term, it is conceivable that infusions of gene corrected progenitors that persist for a few months may be beneficial as adjunctive therapy for serious infections [79]. Successful gene therapy in CGD and in other hematopoietic disorders is limited by the transduction efficiency of myeloid precursors ex vivo and by the ability of corrected cells to replace uncorrected cells in vivo.

In X-linked CGD mice, correction of the host defect either by bone marrow transplantation or retroviral-mediated gene transfer led to protection against challenge by Aspergillus and bacterial pathogens [77, 80]. Host defense was improved even with a limited proportion of gene-corrected neutrophils. Therefore, maintenance of a chimeric
state in which there is a small population of circulating gene-corrected neutrophils may be of benefit in invasive aspergillosis in CGD patients.

Summary
Invasive aspergillosis is the most important cause of mortality in CGD CGD patients are susceptible to a broad spectrum of opportunistic filamentous fungi, but Aspergillus infection is by far the most common. Current approaches should include prophylaxis with IFN-g and itraconazole. Vigilance should be maintained in terms of radiographic screening even in the absence of symptoms and aggressively pursuing microbiologic diagnosis of suspicious lesions. Voriconazole is our initial drug of choice for invasive aspergillosis. For such infections, granulocyte transfusions may be helpful. Bone marrow transplantation and gene therapy are promising methods for immune augmentation.
References


