

CONTENTS

Aspergillus Infections in CGD and CF

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Immunopathology of *Aspergillus* Infections in Children With Chronic Granulomatous Disease and Cystic Fibrosis

Adilia Warris, MD, PhD

Key Words: chronic granulomatous disease, cystic fibrosis, *Aspergillus*, aspergillosis, inflammation

Children with chronic granulomatous disease (CGD) and cystic fibrosis (CF) share some important clinical characteristics, which are the consequence of their intrinsic susceptibility to recurrent and opportunistic infections and exaggerated inflammation. Although the clinical phenotypes of *Aspergillus* disease differ substantially between those 2 non-neutropenic patient groups, the underlying pathophysiology shows remarkable commonalities with excessive inflammation being a hallmark of *Aspergillus* disease. The occurrence of *Aspergillus* infections in these 2 patient populations is associated with decreased quality of life and premature death.

Our knowledge of the pathophysiology of *Aspergillus* infections is mainly derived from the infections caused by a single species – *Aspergillus fumigatus* – in the

neutropenic host. The insights obtained in this setting do not translate well to the non-neutropenic host. Recently, more insight has been obtained in the underlying molecular mechanisms driving the excessive inflammation during *Aspergillus* infection and disease in those 2 patient groups and is summarized here.

CHRONIC GRANULOMATOUS DISEASE

CGD is a rare primary immunodeficiency (prevalence 1:200,000) characterized by a defective NADPH-oxidase in phagocytic cells. Patients with CGD have the highest lifetime risk of invasive aspergillosis (IA; incidences 26%-45%) and, despite the availability of antifungal prophylaxis and targeted antifungal therapy, IA remains the most common infectious complication and the most frequent cause of death in CGD.¹ *Aspergillus*-specific mortality in CGD appears to be decreasing over time, from around 30% to below 20% in the most recent case series. Curative options to treat CGD, for example, hematopoietic stem cell transplantation, are available, although not each patient is benefited from this treatment. Impaired production of reactive oxygen species (ROS) underlies the clinical phenotype of CGD. Lack of the direct microbicidal effects of ROS renders children with CGD susceptible to IA, while the lack of a functional NADPH oxidase leads to dysregulated inflammatory responses explaining the pathophysiology of IA as observed in CGD. IA in CGD is characterized by a subacute infection,

non-angio-invasive, with excessive granuloma formation in the affected tissue.

There is a clear association between the NADPH-oxidase mutation, the extent to which superoxide production is impaired, and the occurrence and severity of IA in CGD. X-linked CGD patients have both a higher incidence and worse outcome of IA compared with those with autosomal recessive CGD. IA in CGD is caused predominantly by 2 different *Aspergillus* species, *A. fumigatus* and *A. nidulans*. Interestingly, *A. nidulans* infection is found almost exclusively in patients with X-linked CGD, and seldom other (non-CGD) patient groups, indicating a unique unsolved interaction in the clinical setting.¹ Both in vitro and murine studies have shown that infection with *A. nidulans* and *A. fumigatus* induce a hyperinflammatory response driven by an exaggerated interleukin-1 (IL-1) production.^{2,3}

CYSTIC FIBROSIS

CF is caused by a mutation in the gene that encodes the CF transmembrane conductance regulator (CFTR) protein, an ion channel, expressed in epithelial cells and immune cells. This mutation accounts for the most common fatal genetically inherited disease in humans (prevalence 1:13,500). Median age of death is still a mere 29 years of age and progressive lung damage caused by inflammation and infection is the major cause of this fatal outcome. Up to 60% of patients with CF will be infected with *Aspergillus* and it has been suggested that persistent infection is associated with progressive lung function

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decline.⁴ A small proportion of those patients will develop allergic responses to *Aspergillus* (eg, allergic bronchopulmonary aspergillosis or fungal sensitization), while others will develop airway-IA (eg, *Aspergillus* bronchitis). Studies reporting on the relative number of *A. fumigatus* versus non-*fumigatus* *Aspergillus* species have shown that between 36% and 58% of colonization is caused by *A. fumigatus*.⁵ Although infection with *Aspergillus* has long been considered as innocent colonization because of the entrapment of *Aspergillus* conidia by the sticky mucus in the CF lung, recent data suggest otherwise. Adhesion, phagocytosis and killing of *Aspergillus* conidia has been shown to be impaired in CF bronchial epithelial cells.⁶ In the same article, the authors demonstrated, by using an experimental murine model, impaired clearance of *Aspergillus* conidia and epithelial necrosis and fibrin deposition in CFTR-deficient but not wild-type mouse airways. CFTR protein has been found in cells of both the innate and the adaptive immune system and has shown to play a critical role for their normal function. The observation that CF patients have a higher risk of developing IA after lung transplantation than non-CF transplant patients additionally suggests impaired antifungal effector mechanisms of CF immune cells.⁴ We have recently shown that although human CF neutrophils are capable to efficiently phagocytose and kill *A. fumigatus*, this is at a cost of excessive ROS.⁷ This excessive ROS response was significantly correlated to disease severity in terms of clinical exacerbations and lung function and suggests that the hyperinflammatory response by CF neutrophils upon exposure to *A. fumigatus* is likely to contribute to progressive lung disease.⁷

IMMUNOPATHOLOGY

Aspergillus infection triggers an aberrant host immune response characterized by hyperinflammation in patients with CGD and CF, resulting in insufficient fungal control and tissue damage. Over the last few years, a number of studies have shown commonalities in the molecular drivers of the observed immunopathology in both patients groups.

Autophagy is the process of effective microbial killing and clearance after phagocytosis and phagolysosome maturation, and controls inflammasome activation. Defective autophagy has been reported in both CGD and CF immune cells.^{8,9} The link between autophagy and ROS remains poorly understood, although it is known that ROS are essential for effective autophagy. On the contrary, excessive ROS production inhibits the process of autophagy. Therefore, the observations that the process of autophagy is defective in both the CF (excessive ROS) as well

as the CGD (deficient in ROS) host suggest a common element to the immunopathology.

Rapamycin, a macrolide compound, was initially developed as an antifungal agent until its potent immunosuppressive properties were discovered. Rapamycin is a mammalian target of rapamycin (mTOR) inhibitor and is mainly been used in clinical care to prevent rejections of organ transplants and uncontrolled lymphoproliferation. A first study showing its potential use in patients with CGD demonstrated that rapamycin induced autophagy induction thereby inhibiting inflammasome activation in human CGD phagocytes.¹⁰ Inhibition of pro-inflammatory cytokines including TNF- α and IL-17, independently of caspase-1 inhibition, was observed as well. Anakinra, an IL-1-receptor antagonist, enhanced the inhibitory effect of rapamycin on IL-1 β secretion by phagocytes in the in-vitro model used, suggesting that this combination could have extended benefits for dampening the immunopathology in CGD. Comparable studies are not performed yet using human CF phagocytes.

Another component in the vicious perpetual cycle of fungal-induced inflammation is the activation of the inflammasome. Increased activation of the inflammasome has been shown under conditions in which either too much or too little ROS is induced. This pathway has been shown to be upregulated in both CGD and CF immune cells upon fungal stimulation and leads to increased release of IL-1.^{3,11} IL-1 inhibitors, such as IL-1-receptor antagonists, have shown to reduce inflammation in human immune cells and experimental murine models of CGD and CF.^{3,11} We have shown previously that chloroquine, an antimalarial drug exhibiting anti-inflammatory properties, inhibits IL-1 release by *Aspergillus*-infected human CGD phagocytes.¹² The mechanisms of the anti-inflammatory activity of chloroquine is poorly understood. Our recent observations show that prophylactic hydroxychloroquine can attenuate mortality, weight loss and pulmonary inflammation in gp91^{-/-} mice infected with *A. nidulans*. This beneficial effect is not observed in *A. fumigatus* infections. Chloroquine and hydroxychloroquine have similar pharmacologic properties, but hydroxychloroquine has a more favorable side effect profile, increasing its appeal for long-term clinical use.

Studies in murine models of pulmonary aspergillosis showed that a defective NADPH-oxidase complex and CFTR dysfunction leads to defective indoleamine 2,3-dioxygenase (IDO) activity.^{13,14} IDO is a rate-limiting enzyme for tryptophan degradation in the kynurenine pathway. Impaired tryptophan catabolism resulted in exaggerated inflammation and abnormalities in T cell polarization with exaggerated Th17

responses. Therapeutic modulation of this pathway to enhance IDO activity enabled resolution of excessive inflammation in those experimental models. However, subsequent studies have found preserved tryptophan/kynurenine metabolism and Th17 differentiation in CGD patients,¹⁵ suggesting that this effect is likely limited to the p47^{-/-} murine model. Studies assessing its relevance in patients with CF are lacking.

IMMUNOTHERAPY

Corticosteroids are used to dampen the allergic inflammation in allergic bronchopulmonary aspergillosis in CF patients and the inflammatory complications postinfection in CGD patients.¹² Owing to the well-known severe side-effects and its unspecific and broad mode of action, new and more targeted immunotherapies are needed. Interferon- γ has been carefully studied as an antifungal adjuvant immunotherapy. Interferon- γ enhances antifungal activity of macrophages and polymorphonuclear neutrophils, and its first successful clinical application was in CGD patients in whom substantial protection was seen against IA.¹ Currently, only sparse preclinical data are available to support the potential beneficial effect of novel additional immunomodulatory agents in the management of *Aspergillus* infection in children with CGD or CF.

The CFTR modulators are the first causative treatment options for CF patients and have achieved significant improvement in lung function and quality of life. The results of a recent clinical study looking at the effect of Ivacaftor on specific airway microbial colonization showed a 53% reduction in *Aspergillus* colonization.¹⁶ This effect may be related to a reduction in inflammation, an improved immune function of the epithelial cells or a direct effect on immune cells present in the lung environment, or a combination of both.

SUMMARY

Patients suffering from CGD or CF are 2 non-neutropenic patient populations characterized by an increased susceptibility to *Aspergillus* infections. To improve the outcome of *Aspergillus* infections in these particular patient groups, a better understanding of the host–fungus interaction is urgently required to develop targeted and individualized management strategies. Targeting either the primary immune defect, or the defective autophagy, or dampening the activity of the inflammasome has the potential to reduce the immunopathology caused by *Aspergillus* species and consequently to improve patient outcome. Yet, the translation of the exciting

preclinical data into carefully designed clinical trials is awaited.

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