

Role of Fungi in the Pathophysiology of Chronic Rhinosinusitis: An Update

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Abstract The pathogenesis of chronic rhinosinusitis (CRS) is multi-factorial with an infectious process likely at least partly involved. While bacteria have been proposed to play a critical role in CRS, fungi have also been implicated by some investigators, although the pathogenesis of fungi in CRS represents a significant controversy among rhinologists. Fungal-associated factors believed to be involved in CRS include the ability of fungi to induce significant inflammatory reactions by different means through inducing localized cytokine production in the sinonasal tract. Despite these observations, randomized, controlled studies on CRS patients using antifungal therapy have not resulted in significant improvement in CRS patients. The role of fungi in the pathogenesis of CRS remains controversial.

Keywords Chronic rhinosinusitis · Fungi · Allergic fungal rhinosinusitis · Eosinophilic mucin · Th2 · Eosinophils · Pathogenesis · Pathophysiology · Antifungal therapy · Treatment

Introduction

Chronic sinusitis is a multi-spectrum hypertrophic, inflammatory process of the sinonasal mucosa characterized by symptoms of nasal blockage/obstruction, congestion, facial pain/swelling, and discharge for greater than 3 months duration combined with the clinical finding of mucosal swelling and mucopurulent discharge by nasal endoscopy and

characteristic imaging findings [1•, 2, 3••]. It is overwhelmingly the most common type of sinusitis afflicting thousands of adults and resulting in millions of dollars in healthcare costs annually. The pathogenesis of CRS is multi-factorial with infectious, genetic, and environmental exposures all believed to be important factors [1•, 2, 3••, 4–9]. Histologically, CRS is characterized by mucosal thickening with goblet cell hyperplasia, submucosal fibrosis, and chronic inflammation composed of lymphocytes and plasma cells with or without polyps and/or eosinophils. Due to the inflammatory nature of CRS, it is not surprising that infectious etiologies have been implicated in the pathogenesis, though the response of CRS to antibiotics, while promising, has been inconsistent [7]. Other infectious etiologies of CRS including fungi have been widely debated. This review will concentrate on the role of fungi in the pathogenesis of CRS.

Fungi in Allergic Fungal Rhinosinusitis and CRS

The consideration of fungi as an etiologic role in CRS is not unexpected. It has been known for centuries that fungi are associated with chronic sinonasal disease processes and the association of fungi with allergic fungal rhinosinusitis (AFRS), often considered a form of CRS, has been known for decades. It has been more than 10 years since the controversial publication by Ponikau implicating fungi in the etiology of most, if not all, cases of CRS [10]. In fact, Ponikau's report has been one of the most debated topics in the rhinologic literature since its publication and has spurred several studies supporting and refuting its hypothesis [11•, 12, 13]. In the original prospective study of 210 patients from the Mayo Clinic, using ultra-sensitive culturing techniques which utilized enzymatic digestion techniques to degrade mucin, this group found that cultures of nearly all CRS patients grew fungal pathogens. The group concluded

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that most, if not all, CRS is actually AFRS and proposed that the best terminology for this entity was eosinophilic fungal rhinosinusitis (EFRS), since the eosinophilic inflammation, and not necessarily Type I hypersensitivity, were likely involved in the pathogenesis of the disease process. A major controversial issue in their study, however, was that their sensitive technique also cultured fungi from control subjects without CRS. More recent studies by Braun et al. and Bassiouny et al., using similar culturing techniques have been supportive of this initial work [14, 15•]. Importantly, these studies have indicated that, while most individuals have fungal organisms in their sinuses identifiable by culture and molecular techniques, (studies have shown an increase in sinonasal fungus with increasing age starting as early as 2 months of age [16]), there is often a larger burden identified in subjects with CRS compared with non-CRS subjects, and the etiology of CRS may be related not to type 1 or type 3 hypersensitivity but to the burden of fungal organisms and the inflammatory reaction that is caused by their presence [10]. In fact, the burden of fungus may be a force behind the potential pathogenesis of fungi in CRS. Ponikau showed an average of 2.7 organisms in CRS patients with a maximum of 8 fungi per subject with 40 different genera and 31 different species [10]. Control patients' cultures grew fewer organisms per patient with many fewer genera and species represented. Bassiouny et al. observed similar results in their 2011 study [15•]. Murr et al. showed by polymerase chain reaction (PCR) that multiple fungal organisms were detected in CRS patients and, following surgery, the number of fungal organisms were significantly reduced [17•]. However, Eyigor et al. observed fungi by PCR in <20 % of CRS patients [18]. Using conventional techniques, cultures from AFRS most commonly grow Dematiaceous fungi (*Alternaria* sp., *Bipolaris* sp., *Curvularia* sp., *Cladosporium* sp., etc.), and *Aspergillus* sp. and multiple different fungal organisms can be cultured from one patient [19•, 20]. Studies using sensitive culture methods have also commonly identified these same organisms [10, 14, 15•].

AFRS (EFRS) occurs in immunocompetent patients who initially were believed to develop a type 1 or type 3 hypersensitivity reaction to extra mucosal fungal antigens. More recently, the proposed inflammatory response is believed to be non-type 1-dependent. AFRS is seen worldwide, predominantly in warm, humid climates, particularly in the south and southeastern US, although large urban areas in the mid-west and northeastern US also report large numbers of cases [19•, 20–23]. The published diagnostic criteria for AFRS are variable and include: characteristic CT imaging findings, nasal polyps, the presence of eosinophilic (“allergic”) mucin (EM), and evidence of fungi by histology or culture [24–26]. Other criteria for diagnosis include an established type 1 sensitivity reaction to fungus, but recent

studies have indicated that the pathogenesis of AFRS is independent of hypersensitivity reactions and more in keeping with Th2 cytokine production (IL-4, IL-5, and IL-13) [27]. Microscopic examination of sinus contents shows the characteristic EM which contains mucin admixed with sloughed epithelial cells, eosinophils, Charcot-Leyden crystals, and other inflammatory cells arranged in a laminar pattern and associated with rare, scattered fungal hyphae. EM has also been described in patients without identifiable evidence of fungal infection by histology or conventional culture. Ferguson coined the term eosinophilic mucinous rhinosinusitis (EMRS) to describe patients with EM but without the presence of fungal organisms [28]. In Ferguson's experience, EMRS patients were clinically different from AFRS patients including presentation at an older age, an association with asthma, and a significantly higher risk of aspirin sensitivity syndrome. However, EMRS as a distinct entity has been called into question by those that feel that sensitive methods to detect fungus have not been applied fully to EMRS patients. As previously stated, in Ponikau's original study, sensitive culturing techniques resulted in the detection of fungal organisms in most CRS patients. Further studies have corroborated these data. More recent studies have set out to show that even negative standard histochemical stains on tissues may not be enough to indicate the absence of fungi in EM. Guo et al., using conventional Gomori methenamine silver stain (GMS) on EM, observed fungal organisms in only 27 % of EM specimens; however, with trypsin pre-digestion followed by GMS, the yield was increased to 91 % of specimens [29•]. In addition, using immunofluorescence targeting chitinase and *Alternaria* sp. antigens, they detected fungal organisms in >90 % of EM samples. Bassiouny et al. observed that 60 % of their EM specimens showed fungi by either PAS or GMS stains, but 100 % using antibodies targeting chitinase [15•]. Interestingly, using in situ hybridization, Healy et al. observed fungi in biofilms in patients they classified as having CRS and in greater numbers in patients classified as having EMRS [30]. Therefore, it is likely that using less sensitive histochemical and culturing techniques may lead to decreased diagnosis of AFRS. In addition, initial DNA microarray analyses have not observed significantly different expression profiles between AFRS and EMRS, although these results are preliminary [31]. However, in a recent study using PCR amplification to perform HLA DNA genotyping in AFRS, Class II HLA-DQB1*301 and *302 allelic variants were observed in AFRS but not EMRS patients [32]. An additional study using mass spectrometry on serum samples from patients with AFRS, in comparison to those with aspirin sensitivity syndrome and CRS with nasal polyps, has shown that the proteomic profile between the groups is different, and that analysis could distinguish among the three groups with high sensitivity and specificity [33],

indicating at least molecular differences between these groups of patients, at least in provisional studies.

Fungi and Inflammatory Response in CRS

One factor believed to be behind the pathogenesis of CRS is biofilms. Biofilms are collections of microorganisms which grow in a self-produced matrix which attaches to moist surfaces such as sinonasal mucosa [34]. Biofilms allow bacteria survival advantages by providing protection from antibiotics and host inflammatory responses [34]. Interestingly, recent studies have identified fungal organisms in addition to bacteria in biofilms in CRS patients [30]. While fungi may be just incidentally found in these biofilms and not associated with disease, it is also possible that bacterial/fungal interactions are key to the disease pathogenesis and that the fungal organisms themselves induce the intense inflammatory reactions observed in CRS. Fungi can induce the production of cytokines which in turn induce the influx of inflammatory cells including eosinophils which migrate through the barrier epithelial cells in response to the fungi present extramucosally. Shin et al. showed that exposure of peripheral blood mononuclear cell (PBMCs) isolated from CRS patients to *Alternaria alternata* extract led to non-IgE-mediated production of eosinophil attracting Th-2 cytokines (IL-4, IL-5, and IL-13)[27]. However, in a limited patient population, Orlandi et al. did not observe this same response universally in their CRS patients, and felt this was strong proof that, while fungi are important in the pathogenesis of some cases of CRS such as AFRS, not all CRS is related to fungi [35].

An additional factor believed to be associated with induction of marked inflammatory responses in CRS is fungal proteases. Fungal hyphae produce proteases which are hypothesized to activate protease-activated receptors (PARs) on sinonasal epithelial cells, which lead to the production and release of Th2 cytokines resulting in a pro-inflammatory response including attraction of eosinophils [3•]. Interestingly, Montone et al. observed that a marked eosinophilic sinonasal mucosal infiltrate preceded the observed presence of fungal organisms (either by histology or by culture) in over 30 % of their AFRS patients, and this marked infiltrate preceded that diagnosis by several years [19•].

Antifungal Therapy and Treatment of CRS

With the proposal that fungi are the prime etiologic agent in CRS, it is natural that studies utilizing antifungal therapies have been undertaken. To date, limited utility for antifungal therapy has been seen [36•, 37–46]. Sacks et al. recently did an extensive study of the literature using antifungals for

treating CRS and concentrated on those studies which were randomized, placebo-controlled evaluating the use of either topic or systemic antifungal therapy [36•]. They reported that the side effects from antifungal therapy far outweigh the benefits of the use of antifungal agents for treatment purposes. Interestingly, though, in a recent study by Murr et al. using quantitative PCR in subjects with and without CRS, they identified seven different fungi found at high concentrations of patients with CRS (*A. alternata*, *C. cladosporioides* 1 and 2, *C. herbarum*, *P. brevicompactum*, *P. crustosum*, and *P. chrysogenum* type 2) [17•]. They found there were subsets of CRS patients with high fungal loads and those without, and found that in vitro studies fail to demonstrate complete antifungal activity of a variety of antifungal agents for these particular species. They concluded that a variety of antifungal agents may be necessary to eradicate fungi in at least patients with high fungal loads, and since only a handful on antifungal agents have been utilized in these clinical trials to date, it is still possible that further studies with different anti-fungals may produce different results.

Conclusions

The etiology of fungi in the pathogenesis of CRS remains debatable. While the controversy may be waning, there are still preliminary studies which implicate fungi as having a role in CRS, particularly those that implicate fungi as a potential inducer of marked inflammatory reaction by inducing eosinophil attracting cytokine production. It will be interesting to continue to follow how the literature progresses on this topic over the next 10 years.

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- Of major importance

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