

The Mold Conundrum in Chronic Hyperplastic Sinusitis

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The role of fungi in chronic rhinosinusitis (CRS) is not clear. Fungi can be detected in the nose and paranasal sinuses of virtually all CRS patients; however, they also appear to be present in healthy controls. Various theories attempt to explain the mechanisms by which fungi can exert an effect on sinus mucosa in susceptible individuals. Further studies are necessary to clarify the role of fungi in CRS, which fungal organisms (if any) are pathogenic, and what exactly characterizes the immunologic response to fungi that may result in the development of disease. However, in the absence of convincing immunologic data and evidence of clinical improvement of CRS after antifungal therapy, the case against the fungus remains unproven.

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses characterized by the presence of distinctive symptoms (nasal blockage, nasal discharge, facial pain, and/or reduced sense of smell) and endoscopic signs and/or CT changes characteristic of the disease for at least 12 weeks without complete resolution [1•,2]. Although bacteria have long been implicated in the pathogenesis of most forms of CRS, fungi are also implicated in some forms. Due to their ubiquitous nature, fungal spores are continuously inhaled and deposited on the airway mucosa. Although they rarely behave as pathogens in the airways of healthy individuals, they may occasionally be the cause of disease.

In 1983, Katzenstein et al. [3] identified noninvasive *Aspergillus* spp in thick, tenacious, dark-colored eosinophilic mucus (so-called eosinophilic or allergic mucin) obtained from the nose and paranasal sinuses of patients suffering from CRS with nasal polyps. They introduced

the term *allergic Aspergillus sinusitis* because of histopathologic similarities to allergic bronchopulmonary aspergillosis. The term *allergic fungal sinusitis* (AFS) was coined after other noninvasive fungi were shown to produce similar symptoms [4]. As clinical evidence of AFS accumulated, controversy regarding its definition (including the presence of allergy to fungi as a diagnostic criterion), prevalence, and pathogenesis emerged [5,6]. Ponikau et al. [7] demonstrated the presence of fungi and eosinophils in the nose and paranasal sinuses of nearly all CRS patients using novel collection, culturing, and histology techniques, thus suggesting that most CRS patients actually suffer from AFS. Their findings stimulated new discussions about the definition, prevalence, and disease mechanisms of AFS.

Prevalence and Microbiology of Fungi

Fungi are ubiquitous in CRS patients and healthy controls

An obvious diagnostic criterion for AFS is the presence of noninvasive fungi in the nose and paranasal sinuses. However, contradictory results have been published, with prevalence rates ranging from 0% to 100% in CRS patients and healthy controls [3,7–22,23•,24–28]. (An overview of all studies was recently published in *Rhinology* [29].) As suggested by Ponikau et al. [7], differences in collection and detection techniques may explain the observed differences in fungal yield. Of all collection techniques, the nasal lavage technique is considered the most accurate [8,17,19]. Although Ponikau et al. [7] described a prevalence rate of 100% upon culture in their study using novel collection and detection techniques, most authors agree that polymerase chain reaction is superior to both culture and Grocott methenamine silver stains in detecting fungal elements [9,10,12,17,22]. In a recent study, one or more fungal hyphae were detected in 100% of CRS mucus specimens using the fluorescein-labeled chitinase stain [15]. Although no other studies using this technique have been published to date, this interesting finding warrants further research.

No difference in fungal microbiology between CRS patients and healthy controls

The ubiquitous presence of fungi in the nose and paranasal sinuses of CRS patients and healthy controls could lead to the contention that it is not the presence

or absence of fungi, but rather a specific fungal species or fungal load that is relevant to disease development. Cultures collected via the novel technique described by Ponikau et al. [7] grew 37 to 40 different genera, with 2.7 to 3.2 species per CRS patient and 2.3 to 3.1 per healthy control [7,12,13,17]. *Aspergillus*, *Penicillium*, *Cladosporium*, *Candida*, *Aureobasidium*, and *Alternaria* spp appeared most frequently, with no significant differences between the two groups. Similarly, Scheuller et al. [11] assessed the amount of fungal DNA present in tissue specimens obtained from CRS patients and healthy controls and found no differences in fungal load between the two groups. Thus, it is unlikely that fungal species and fungal load play a role in disease development. Whether an increase in fungal allergen content is involved in CRS pathogenesis remains unclear.

Hypersensitivity to Fungi

A generally accepted concept has been that an IgE-mediated systemic fungal allergy drives the pathologic process behind AFS. Various authors have studied sensitization rates to fungi in CRS patients, demonstrating values ranging from 18% to 75% [7,15,30,31]. However, no significant differences were found between patients with and without fungi in their nose and paranasal sinuses [21]. Importantly, Ponikau et al. [7] reported sensitization rates in CRS patients that were no different from those in healthy controls, although others have reported higher levels of fungus-specific IgE in CRS patients with eosinophilic mucin (with or without fungi) [32]. Although levels of fungus-specific IgE were higher in CRS patients with eosinophilic mucin, no significant differences were observed between this group of patients and a group suffering from allergic rhinitis with proven allergy to fungi but without sinus involvement [32]. In addition, Shin et al. [33] recently showed that IgE levels to various common airborne fungi (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* spp) were similar in 18 CRS patients and 15 healthy controls, rendering it unlikely that an allergy to a specific fungus is involved. Interestingly, a recent study demonstrated that patients with CRS who do not have an allergy to the fungus present in their eosinophilic mucin may have elevated IgE levels to other fungi [32]. This leads to the obvious question of whether the presence of type I hypersensitivity to fungi is relevant to disease development. We believe that the presence of type I hypersensitivity most likely is not causative but rather represents concurrent fungal allergy in most CRS patients.

Local Host Defense Against Fungi

Fungi and eosinophils: a cause-and-effect relationship?

Cellular immune responses vary according to the fungal species, the morphotype encountered, and the anatomic site of interaction. Whereas yeasts and spores are often effectively phagocytosed, the larger size of hyphae precludes effective ingestion and requires interaction with

different inflammatory cells. Although eosinophils, neutrophils, macrophages, and monocytes are all important antifungal effector cells, most research in CRS has focused on the role of eosinophils in antifungal immune defense.

The concurrent presence of fungi and eosinophils in nearly all CRS tissue specimens has led to the suggestion of a cause-and-effect relationship [7,34,35]. Wei et al. [36] recently demonstrated a concentration-dependent increase in CRS eosinophil migration toward CRS nasal mucin and CRS nasal tissue extracts, suggesting that fungi may trigger inflammatory cells to initiate a complex localized eosinophilic reaction. However, most CRS patients in this study suffered from asthma (9 of 10 patients) and/or atopy (4 of 10). Eosinophils from individuals with allergic and nonallergic asthma are known to exhibit a primed phenotype that is likely the consequence of eosinophil interaction with cytokines in the peripheral blood, resulting in increased eosinophil migration, adhesion, and degranulation capacities. Thus, the presence of asthma and/or atopy rather than fungi may explain the observed concentration-dependent increase in eosinophil migration [37,38]. This hypothesis is supported by recent data in sheep, in which primed eosinophils were demonstrated to be more effective than unprimed eosinophils in immobilizing and killing gastrointestinal parasites in the presence of specific antiparasite antibodies [39].

Cytokines and chemokines:

are they involved in antifungal immune defense?

Cytokines and chemokines are low molecular weight proteins with growth, differentiation, and activation functions that regulate and determine the nature of innate and adaptive immune responses [40]. Various cytokines and chemokines have been implicated in CRS pathogenesis [1•,2]. Striking differences were recently observed between CRS and healthy control peripheral blood mononuclear cell (PBMC) cytokine responses when they were cultured with extracts from four common airborne fungi (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* spp). When cultured with *Alternaria* extract, PBMCs of CRS patients produced significantly more interleukin (IL)-5 and interferon- γ compared with healthy control PBMCs. In addition, some PBMCs of CRS patients produced more IL-5 in response to *Aspergillus* and *Cladosporium* extracts. PBMCs from all CRS patients produced IL-13 upon culture with all tested fungal extracts [33]. Together, these data suggest that fungi may induce PBMC cytokine production (mainly T-helper type 2 cytokines). However, comorbid illnesses may have played a confounding role, as 61% of patients demonstrated increased IgE levels to common aeroallergens, and 78% suffered from bronchial asthma. Because PBMCs from asthmatic patients (allergic and nonallergic) are known to produce more IL-5 in response to allergen compared with allergic rhinitic patients and healthy controls [41] and because a second study (in which the frequency of atopy was equally distributed) showed only minimal changes in IL-5 and

interferon- γ expression upon culture with *Aspergillus* and *Alternaria* extracts [42], the role of fungi in inducing a T-helper type 2 cytokine response requires further study.

Antimicrobial peptides: decreased levels may be involved in CRS pathogenesis

Cathelicidins and defensins are two major families of cationic antimicrobial peptides involved in innate immunity at mucosal surfaces. Recently, Ooi et al. [43•] demonstrated that LL-37, the free C-terminal peptide of human cathelicidin hCAP-18 (human cationic antimicrobial peptide 18kDa), is significantly upregulated in a dose-response effect at the mRNA and protein level in CRS patients without eosinophilic mucin in response to *Aspergillus fumigatus* and *Alternaria tenuis*. However, in CRS patients with eosinophilic mucin (but without fungal presence), no significant increase in LL-37 was observed at the mRNA or protein level in response to *Aspergillus* challenge. Likewise, no increase in expression in tissue and secreted LL-37 was observed upon *Alternaria* challenge [43•]. Although the idea is interesting, as neither CRS patients with eosinophilic mucin and fungal presence nor a control group were included in this study, the exact role of LL-37 in the CRS pathogenesis remains to be determined.

Besides cathelicidins and defensins, various other antimicrobial peptides—including lactoferrin, lysozyme, and secretory leukoprotease inhibitor—have been identified in nasal secretions [44] and sinus mucosa [45]. Lactoferrin has a variety of functions, including antibacterial, antifungal, and antiviral activities [46]. It recently was shown to possess antibiofilm properties as well [47]. Bacterial biofilms are present in most CRS patients, may contain large amounts of fungal elements [48], and have been suggested to play a significant role in CRS pathogenesis [49]. Downregulation of lactoferrin was recently observed in CRS patients with nasal polyps [50] and those with biofilms [51]. However, no difference was observed between CRS patients with and without eosinophilic mucin, those with and without fungal allergy, and those with and without fungi present [50,51].

Surfactant proteins: absence of surfactant protein D may result in failure to clear fungi from the nose and paranasal sinuses

Pulmonary surfactant is a mixture of phospholipids and proteins. Four different surfactant proteins (SPs) are known to exist: SP-A, SP-B, SP-C, and SP-D [52]. SP-D binds and agglutinates microorganisms and enhances phagocytosis, chemotaxis, and cytokine production. SP-D has been shown to play an important role in the immune response to *A. fumigatus* in the lung and is present in submucosal glands of CRS patients without eosinophilic mucin, CRS patients with eosinophilic mucin but without fungal allergy, and healthy controls. The highest levels are detected in healthy controls. However, in CRS patients with eosinophilic mucin and fungal allergy, SP-D protein remains below detection levels. In vitro studies have

demonstrated that *A. tenuis* upregulates SP-D mRNA in CRS patients with and without eosinophilic mucin. On the other hand, *A. fumigatus* increases SP-D mRNA expression in CRS patients without eosinophilic mucin only [53•]. Absence of SP-D protein may result in failure to clear fungi from the nose and paranasal sinuses and as a result may cause disease development.

Fungus Antihost Effects

Besides innate and adaptive antifungal immune responses that may contribute to disease development, fungus antihost effects may be involved in CRS pathogenesis. Ubiquitous airborne fungi (especially *Alternaria* and *Aspergillus* spp) are known to produce proteases that bind to protease-activated receptors expressed on epithelial cells, airway cells, leukocytes, and blood vessels, activating intracellular signaling pathways and giving rise to multiple responses, including the production and release of mediators involved in tissue damage [54–56]. In addition to an indirect effect, recent studies indicate that *Alternaria alternata* may activate eosinophils directly. *A. alternata*—but not IL-5—has been shown to induce eosinophil IL-8 synthesis and eosinophil surface expression of CD11b (a $\beta 2$ integrin used by eosinophils to adhere to β glucan, a major fungal cell wall component [57]) and CD63 (a component of eosinophil granule membranes) in healthy volunteers, patients with allergic rhinitis, and patients with bronchial asthma. Upon recognition of *A. alternata*, eosinophils released eosinophil-derived neurotoxin [58], and this response may play a pivotal role in CRS pathogenesis.

Therapy

Topical and oral antifungals: ineffective in the treatment of CRS

If CRS inflammation is caused by an immune reaction to fungi, reducing the presence of this inflammatory trigger could improve the disease course [7]. Ideally, treatment should eliminate the fungus without causing harm to the host. In 1996, 22 fungal cultures grown from 15 AFS patients were studied by Bent and Kuhn [59] for in vitro susceptibility to five common antifungal drugs. Ketoconazole and amphotericin B were most effective, independent of the fungal organism tested [59]. Despite the potential for clinical effectiveness, use of systemic antifungals is limited by adverse systemic reactions. Topical treatment may have the advantage in that high concentrations may be achieved locally without causing major systemic side effects. Although the injectable formulation of amphotericin B carries US Food and Drug Administration–approved labeling solely for intravenous administration, several alternative routes of administration that use the injectable formulation have been reported, including the administration of amphotericin B into the pleural cavity [60] and bladder [61]. Amphotericin B nasal lavages recently have been advocated in the treatment of CRS (Table 1). Although they are safe,

Table 1. Studies on topical and oral antifungals in patients with chronic rhinosinusitis

Reference	Active drug, n	Placebo, n	Drug name	Solvent	Dose	Duration	Method	Study design	Outcome
Ponikau et al. [30]	51	0	Amphotericin B	Sterile water	100 µg/mL (20 mL) twice daily in each nostril	3–17 mo	Nasal lavage	Non-placebo-controlled, single-center	Positive
Ricchetti et al. [62]	74	0	Amphotericin B	Sterile water	1:1000 (20 mL) twice daily in each nostril	4 wk	Nasal lavage	Non-placebo-controlled, single-center	Positive
Weschta et al. [14]	28	32	Amphotericin B	Glucose, 5%	3 mg/mL (200 µL) 4 times daily in each nostril	8 wk	Nasal spray	Randomized, placebo-controlled, double-blind, single-center	Negative
Ponikau et al. [18]	10	14	Amphotericin B	Sterile water	250 µg/mL (20 mL) twice daily in each nostril	6 mo	Nasal lavage	Randomized, placebo-controlled, double-blind, single-center	Positive (CT) and negative (symptoms)
Kennedy et al. [16]	25	28	Terbinafine	Not applicable	625 mg/d	6 wk	Oral	Randomized, placebo-controlled, double-blind, single-center	Negative
Helbling et al. [63]	21	0	Amphotericin B	Sterile water	1% (0.1 mL) 3 times daily in each nostril	3 mo	Nasal spray	Non-placebo-controlled, single-center	Negative
Ebbens et al. [31]	59	57	Amphotericin B	Glucose, 2.5%	100 µg/mL (20 mL) twice daily in each nostril	13 wk	Nasal lavage	Randomized, placebo-controlled, double-blind, multicenter	Negative
Liang et al. [23•]	32	32	Amphotericin B	Sterile water at dispersion; NaCl, 0.9%, just before administration	4 µg/mL (250 mL) once daily in each nostril	4 wk	Nasal lavage	Randomized, placebo-controlled	Negative

(Adapted from Ebbens et al. [72].)

and despite initial evidence of benefit of topical amphotericin B irrigations in two uncontrolled trials [30,62], one subsequent uncontrolled, prospective trial [63] and four subsequent double-blind, placebo-controlled studies investigating the effect of topical amphotericin B irrigations [14,18,23•,31] and nasal spray [14,63] either failed to show benefit [14,23•,31,63] or showed at best only modest radiologic benefit without symptomatic improvement [18] in CRS patients with and without nasal polyps. Since Weschta et al. [14] demonstrated that fungal eradication did not alleviate symptoms, it should be questioned whether fungal eradication is involved in disease resolution. Whether dosage, treatment time, and route of administration have an impact on treatment outcomes requires additional research. This is especially important because recent *in vitro* data suggest that amphotericin B nasal lavages are ineffective in killing fungi at concentrations of 100 µg/mL (dosage used by Ponikau et al. [30] and Ebbens et al. [31]) when used for 6 consecutive weeks [64]. Irrigations with concentrations of 200 and 300 µg/mL were shown to prevent fungal growth at 5 and 6 weeks, respectively [64]. Whether prolonged treatment with topical amphotericin B at a concentration of 100 µg/mL is equal to treatment with concentrations of 200 and 300 µg/mL for shorter periods of time remains unclear.

Although several uncontrolled reports have suggested that oral antifungal agents are effective in treating CRS [65], in a recent double-blind, placebo-controlled study, Kennedy et al. [16] treated 53 CRS patients with high-dose oral terbinafine (625 mg/d) for a period of 6 weeks and demonstrated no improvement in subjective or objective outcome measures, which is in line with previous results on topical antifungal treatment [14,23•,31]. Thus, the use of topical and oral antifungals to treat patients with CRS successfully has not been substantiated by most publications.

Topical amphotericin B: if it does work, how does it work?

Although the use of topical amphotericin B to treat CRS is not substantiated by most studies, dosage and duration of treatment may have influenced outcome. If we assume that topical amphotericin B is effective in treating CRS patients, various mechanisms may be involved. First and most likely, amphotericin B may reduce fungal load and, as a consequence, the inflammatory response in the nose and paranasal sinuses. Second, amphotericin B may have a direct (cytotoxic) effect on nasal polyp epithelial cells. Although amphotericin B is a sterol-binding agent with high affinity for ergosterol (the dominant fungal sterol) and low affinity for cholesterol (the mammalian sterol), recent evidence suggests that topical amphotericin B can modify cell membrane structure of nasal polyp epithelium but not of inferior turbinate epithelium by forming aqueous pores, resulting in increased membrane permeability and, as a consequence, disruption of nasal polyp epithelial cells [66]. The underlying mechanism remains unclear. Third, amphotericin B may have a direct anti-inflammatory effect on CRS mucosa. Although interesting, no existing data can confirm this hypothesis, and the

three studies published in this field report no differences in cytokine, chemokine, and growth factor responses between placebo and amphotericin B [67–69].

Immunotherapy: effective in CRS patients with concurrent fungal allergy

If CRS stems from a hypersensitivity to retained fungal elements (a conclusion that should be questioned, as mentioned previously [14]), the removal of fungal elements may minimize ongoing stimulation. However, if true, when the underlying hypersensitivity remains untreated, the disease would be expected to recur. Starting in 1994, Mabry et al. [70] prospectively treated 23 patients with AFS with antifungal immunotherapy after thorough exenteration of the involved sinuses. A decreased need for systemic and topical corticosteroids, a marked decrease in polyp recurrence, and a lessening of long-term nasal and sinus crusting were observed over a treatment period of 1 to 3 years in 11 patients [70]. Cessation of immunotherapy after 3 years did not result in recurrence of symptoms during 7 to 17 months of follow-up [71]. When interpreting these data, one should note that no placebo group was included, controls included patients who dropped out of the immunotherapy group, several patients were lost to follow-up, most patients were treated with immunotherapy to fungal and nonfungal antigens, and all patients were treated with nasal irrigations and topical steroids for a variable period of time postoperatively. Despite the many confounders present, the results of this study are intriguing. Even if one assumes that fungal allergy does not cause CRS, one may conclude that antifungal immunotherapy is effective in reducing signs and symptoms in CRS patients with concurrent fungal allergy. Future placebo-controlled studies are needed to reveal the true role of antifungal immunotherapy in the treatment of CRS patients.

Conclusions

The role of fungi in CRS remains unclear. Initial enthusiasm from studies demonstrating that fungi can be detected in the nose and paranasal sinuses of nearly all CRS patients was tempered when it was demonstrated that they are also present in healthy controls. We currently have more questions than answers concerning the role of fungi in the pathophysiology of CRS. Further studies are needed to clarify the role of fungi in CRS, which fungal organisms (if any) are pathogenic, and what exactly characterizes the immunologic response to fungi that may result in the development of disease. Presently, in the absence of convincing microbiologic and immunologic data or evidence of effectiveness of antifungal agents, the case against the fungus remains unproven, and the use of topical and oral antifungals to treat patients with CRS is not advocated.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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- Of importance
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