Fungus as the cause of chronic rhinosinusitis: the case remains unproven

Fenna A. Ebbens, Christos Georgalas and Wytske J. Fokkens

Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, The Netherlands

Correspondence to Fenna A. Ebbens, MD, MSc, Department of Otorhinolaryngology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
Tel: +31 20 5663789; fax: +31 20 5669573; e-mail: f.a.ebbens@amc.uva.nl

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Purpose of review
To review the literature on the role of fungi in chronic rhinosinusitis (CRS) pathogenesis and the effect of antifungal drug therapy and antifungal immunotherapy.
Recent findings
This paper reviews the most recent articles investigating the role of fungi in CRS pathogenesis. In addition to possible aberrant innate and adaptive antifungal immune responses and fungus antihost effects, which all may explain disease development, the effect of antifungal drug therapy and antifungal immunotherapy is reviewed.
Summary
Although fungi can be detected in the nose and paranasal sinuses of nearly all patients with CRS and are present in almost all healthy controls, various studies suggest that there may be mechanisms by which fungi exert an effect on sinus mucosa in susceptible individuals only. Future studies will have to clarify the role of fungi in CRS, which fungal organisms, if at all, may be pathogenic and what exactly characterizes the immunological response to fungi that potentially results in the development of disease. Presently, in the absence of convincing immunological data and evidence for clinical improvement of CRS upon therapy with antifungal agents, the case against the fungus remains unproven.

Keywords
allergic fungal sinusitis, antifungals, chronic rhinosinusitis, fungi, immunotherapy, nasal polyps, pathogenesis

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Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses that is present for at least 12 weeks without complete resolution and is characterized by the presence of distinctive symptoms (e.g. nasal blockage, nasal discharge, facial pain and/or reduced sense of smell) and either endoscopic signs or computed tomography (CT) changes characteristic of the disease [1••,2]. Although bacteria have long been implicated as pathogens in most forms of CRS, it has been recognized that fungi may be responsible for some forms. Fungal spores, owing to their ubiquitous nature, 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 some.

In 1983, Katzenstein *et al.* [3] identified noninvasive *Aspergillus* species in thick, tenacious, dark-coloured eosinophilic mucus (so-called eosinophilic or allergic mucin) obtained from the nose and paranasal sinuses of patients with CRS with nasal polyps and introduced the term 'allergic *Aspergillus* sinusitis' because of histopathological similarities with allergic bronchopulmonary aspergillosis. Later the disease name 'allergic fungal sinusitis' (AFS) was coined after other noninvasive fungi were demonstrated to produce similar symptoms [4]. As clinical evidence of AFS accumulated, controversy regarding its definition (should fungal allergy be present?), prevalence and disease mechanisms emerged [5,6]. When Ponikau *et al.* [7] demonstrated the presence of both fungi and eosinophils in the nose and paranasal sinuses of nearly all patients with CRS by using novel collection, culturing and histology techniques, thus suggesting that the majority of patients with CRS actually have AFS, discussions about the definition, prevalence and disease mechanisms of AFS increased.

Prevalence of fungi: ubiquitous in both patients with chronic rhinosinusitis and healthy controls

The presence of noninvasive fungi in the nose and paranasal sinuses is required for the diagnosis of AFS. Nevertheless, to prove their presence has been difficult.

For many years, contradictory results have been published with prevalence rates ranging from 0 to 100% in both patients with CRS and healthy controls [3,7–28] (a comprehensive overview of all studies was recently published in *Rhinology* [29]). As was suggested by Ponikau *et al.* [7], differences in collection and detection techniques may explain the observed differences in fungal yield. Of all the collection techniques, the nasal lavage technique is considered to be the best [8,17,19]. Although Ponikau *et al.* [7] describe a prevalence rate of 100% upon culture in their study using novel collection and detection techniques, most authors agree that PCR is superior to both culture and Grocott methanamine silver stains in detecting fungal elements [9,10,12,17,22]. Although no other studies using the fluorescein-labelled chitinase stain have been published to date, the detection of one or more fungal hyphae in 100% of CRS mucus specimens is striking and warrants future research [15].

Microbiology of fungi: no difference between patients with chronic rhinosinusitis and healthy controls

As fungi are ubiquitous in nature and equally present in the nose and paranasal sinuses of both patients with CRS and healthy controls, one could argue that it is not the presence or absence of fungi, but rather the fungal species or fungal load that is relevant for disease development. However, in cultures collected via the novel technique described by Ponikau *et al.* [7], 37–40 different genera grew with 2.7–3.2 species per patient with CRS and 2.3–3.1 per healthy control with the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Candida*, *Aureobasidium* and *Alternaria* appearing most frequently and with no significant differences between the two groups [7,12,13,17]. In addition, there were no differences in the amount of fungal DNA present in tissue specimens obtained from patients with CRS and healthy controls [11], rendering it 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.

Type I hypersensitivity to fungi most likely represents concurrent fungal allergy in the majority of patients with chronic rhinosinusitis

For many years, an immunoglobulin E (IgE)-mediated systemic fungal allergy has been thought to drive the pathological process characteristic of AFS. If fungal allergy is necessary to adequately diagnose AFS, one should be able to distinguish diseased patients from healthy controls based on elevated levels of fungus-specific IgE or a positive skin prick test to common airborne fungi. Various authors [7,15,30,31] have studied sensitization rates to fungi in patients with CRS, demonstrating values ranging from 18 to 75%, with no significant differences between the patients with fungi and the patients without fungi in their nose and paranasal sinuses [21]. Compared with healthy controls, some authors report similar sensitization rates in all patients with CRS [7], whereas others report higher levels of fungus-specific IgE in patients with CRS with eosinophilic mucin (with or without fungi) [32]. Although higher in patients with CRS with eosinophilic mucin, no significant differences were observed between this group of patients and a group of patients with 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*) were similar in 18 patients with CRS and 15 healthy controls, rendering it unlikely that an allergy to a specific fungus is involved. As Pant *et al.* [32] recently demonstrated that some patients with CRS do not have an allergy to the fungus that is present in their eosinophilic mucin but may have elevated IgE levels to other fungi, one should question whether the presence of type I hypersensitivity to fungi is relevant for disease development. Most likely, the presence of type I hypersensitivity to fungi represents concurrent fungal allergy in the majority of patients with CRS.

Nasal host defence against fungi

The nasal mucosa is the point of first contact for airborne particles including fungi and as such constitutes the interface between the external environment and the internal milieu of the nose [34]. Upon exposure, the innate immune system ensures the initial defence against infection and damage caused by microorganisms. In healthy individuals, its activation is followed by activation of the adaptive immune system. Various mechanisms are involved in innate immunity. These include mucociliary clearance (allowing physical removal of debris and inhaled microorganisms) [35], clearance of pathogens by local inflammatory cells, secretion of cytokines, antimicrobial peptides and surfactant proteins by epithelial cells, local inflammatory cells and submucosal glands [36,37] and interaction with the adaptive immune system. Failure of innate and adaptive immune responses may result in microbial colonization and recurrent/persistent infections.

Clearance of fungi by local inflammatory cells: mainly eosinophils?

Normal cellular immune responses vary with respect to the fungal species, the morphotype encountered and the anatomical 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 defence.

As fungi and eosinophils are colocalized in nearly all CRS tissue specimens, a cause–effect relationship between fungi and eosinophils has been suggested [7,38,39]. Although Wei *et al.* [40] recently demonstrated a concentration-dependent increase in (CRS) eosinophil migration towards both CRS nasal mucin and CRS nasal tissue extracts, suggesting that fungi may trigger inflammatory cells to initiate a complex localized eosinophilic reaction, one should note that most patients with CRS in this study were diagnosed with either asthma (9/10 patients) and/or atopy (4/10 patients). As eosinophils from patients with asthma (both allergic and nonallergic asthma) are known to exhibit a primed phenotype that is probably the consequence of eosinophil interaction with cytokines in the peripheral blood, resulting in increased eosinophil migration, adhesion and degranulation capacities, it may well be that the presence of asthma and/or atopy explains the observed concentration-dependent increase in eosinophil migration [41,42], a hypothesis supported by recent data in sheep. In sheep, primed eosinophils were shown to be more effective in immobilizing and killing gastrointestinal parasites in the presence of specific antiparasite antibodies in comparison with unprimed eosinophils [43].

Cytokines and chemokines: are they involved in antifungal immune defence?

Cytokines and chemokines are low molecular weight proteins with growth, differentiation, and activation functions that regulate and determine the nature of both innate and adaptive immune responses [44]. Various cytokines and chemokines have been implicated in CRS pathogenesis [1••,2]. Recently, striking differences were observed between CRS and healthy control peripheral blood mononuclear cell (PBMC) cytokine responses when cultured with extracts from four common airborne fungi (*Alternaria*, *Aspergilllus*, *Cladosporium* and *Penicillium*). When cultured with *Alternaria* extract, PBMCs of patients with CRS produced significantly more interleukin (IL)-5 and interferon (IFN)-γ than healthy control PBMCs. In addition, some PBMCs of patients with CRS produced more IL-5 in response to *Aspergillus* and *Cladosporium* extracts. PBMCs from all patients with CRS produced IL-13 upon culture with all tested fungal extracts [33]. Together, these data suggest that fungi may induce PBMC cytokine production (mainly T_H2 cytokines). Unfortunately, 61% of the patients demonstrated increased IgE levels to common aeroallergens and 78% had bronchial asthma. As PBMCs from asthmatic patients (both allergic and nonallergic) are known to produce more IL-5 in response to allergen than both allergic rhinitic patients and healthy controls [45] and as a second study (in which the frequency of atopy was equally distributed) showed only minimal changes in IL-5 and IFN-γ expression upon culture with *Aspergillus* and *Alternaria* extracts [46], the role of fungi in inducing a T_H2 cytokine response remains to be determined.

Antimicrobial peptides: decreased levels may be involved in chronic rhinosinusitis pathogenesis

Immunocompetent hosts, when exposed to fungi, increase their production in cationic antimicrobial peptides to protect themselves against fungal invasion. Cathelicidins and defensins are two major families of cationic antimicrobial peptides involved in innate immunity at mucosal surfaces. Recently, Ooi *et al.* [47•] demonstrated that LL-37, the free C-terminal peptide of human cathelicidin hCAP18 (human cationic antimicrobial peptide 18 kDa), is significantly upregulated in a dose–response effect at the mRNA and protein level in patients with CRS without eosinophilic mucin in response to *Aspergillus fumigatus* and *Alternaria tenuis*; however, in patients with CRS with eosinophilic mucin (but without fungal presence), no significant increase in LL-37 was observed at either the mRNA or the protein level in response to *Aspergillus* challenge. No increase in expression in both tissue and secreted LL-37 was observed upon *Alternaria* challenge. Although the idea is interesting, as neither patients with CRS with eosinophilic mucin and fungal presence nor a control group were included in this study, the exact role of LL-37 in CRS pathogenesis remains to be determined.

In addition to cathelicidins and defensins, various other antimicrobial peptides, including lactoferrin, lysozyme and secretory leukoprotease inhibitor, have been identified in nasal secretions [48] and sinus mucosa [49]. Lactoferrin possesses a variety of functions, including antibacterial, antifungal and antiviral activities [50]. More recently, this peptide has been shown to possess antibiofilm properties [51]. Bacterial biofilms are present in the majority of patients with CRS and may contain large amounts of fungal elements [52]. Downregulation of lactoferrin was recently observed in patients with CRS with nasal polyps [53•] and/or those with biofilms [54]. No difference was observed, however, between patients with CRS with or without eosinophilic mucin, those with or without fungal allergy and those with or without fungi present [53•,54].

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 are known to

exist: surfactant protein-A, surfactant protein-B, surfactant protein-C and surfactant protein-D [55]. Surfactant protein-D binds and agglutinates microorganisms and enhances phagocytosis, chemotaxis and cytokine production. Surfactant protein-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 patients with CRS without eosinophilic mucin, patients with CRS with eosinophilic mucin but without fungal allergy and healthy controls. The highest levels are detected in healthy controls. In patients with CRS with eosinophilic mucin and fungal allergy, however, surfactant protein-D protein remains below detection levels. In vitro studies demonstrate that *A. tenuis* upregulates surfactant protein-D mRNA in patients with CRS with eosinophilic mucin and those without eosinophilic mucin. *A. fumigatus*, on the contrary, increases surfactant protein-D mRNA expression in patients with CRS without eosinophilic mucin only [56•]. Absence of surfactant protein-D protein may result in failure to clear fungi from the nose and paranasal sinuses and, as a result, disease development.

Fungus antihost effects may be involved in chronic rhinosinusitis pathogenesis

In addition to 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*) are known to produce proteases that bind to protease-activated receptors (PARs) expressed on epithelial cells, airway cells, leukocytes and blood vessels, thereby activating intracellular signalling pathways that give rise to multiple responses, including the production and release of mediators involved in tissue damage [34,57,58]. 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 β_2-integrin that is used by eosinophils to adhere to β-glucan, a major fungal cell wall component [59]) 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 (EDN) [60] and this response may play a pivotal role in CRS pathogenesis.

Topical and oral antifungals: ineffective in the treatment of patients with chronic rhinosinusitis

If CRS inflammation is caused by an immune reaction to fungi, reducing the presence of this inflammatory trigger may improve the course of the disease [7]. Ideally, treatment should eliminate the fungus without causing harm to the host. In 1996, 22 fungal cultures grown from 15 patients with AFS were studied by Bent and Kuhn [61] for in vitro susceptibility to five common antifungal drugs. Ketoconazole and amphotericin B were shown to be the most effective, independent of fungal organism tested. Despite their clinical effectiveness, the use of systemic antifungals is limited by adverse systemic reactions. Topical treatment may have the advantage 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 labelling 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 [62] and bladder [63]. Recently, amphotericin B nasal lavages have been advocated in the treatment of CRS (Table 1). Although safe to use and despite initial evidence of benefit of topical amphotericin B irrigations in two uncontrolled trials [30,64], one subsequent uncontrolled prospective trial [65] and four subsequent double-blind placebo-controlled studies investigating the effect of topical amphotericin B irrigations [14,18,23•,31] and nasal spray [14,65] either failed to show benefit [14,23•,31,65] or showed, at best, only modest radiological benefit without symptomatic improvement [18] in patients with CRS with or without nasal polyps. As 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 true because recent in vitro data suggest that amphotericin B nasal lavages are ineffective in killing fungi at a concentration of 100 μg/ml (dosages used by both Ponikau *et al.* [30] and Ebbens *et al.* [31]) when used for 6 consecutive weeks [66]. Irrigations with concentrations of 200 and 300 μg/ml were shown to successfully prevent fungal growth at 5 and 6 weeks, respectively [66]. 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, to date, unclear.

Although several uncontrolled reports have suggested that oral antifungal agents are effective in the treatment of CRS [67], in a recent double-blind placebo-controlled study, Kennedy *et al.* [16] treated 53 patients with CRS with high-dose oral terbinafine (625 mg/day) for a period of 6 weeks and demonstrated no improvement in subjective and objective outcome measures, results in line with previous results on topical antifungal treatment [14,23•,31]. Thus, the use of both topical and oral

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

Author	Year	Country	Active drug (*n*)	Placebo (*n*)	Drug name	Solvent	Dose	Duration	Method	Study design	Outcome
Ponikau *et al.* [30]	2002	United States	51	0	Amphotericin B	Sterile water	100 μg/ml (20 ml) twice daily in each nostril	3–17 months	Nasal lavage	Nonplacebo-controlled single centre study	Positive
Ricchetti *et al.* [64]	2002	Switzerland	74	0	Amphotericin B	Sterile water	1 : 1000 (20 ml) twice daily in each nostril	4 weeks	Nasal lavage	Nonplacebo-controlled single centre study	Positive
Weschta *et al.* [14]	2004	Germany	28	32	Amphotericin B	Glucose 5%	3 mg/ml (200μl) four times daily in each nostril	8 weeks	Nasal spray	Randomized placebo-controlled double-blind single centre study	Negative
Ponikau *et al.* [18]	2005	United States	10	14	Amphotericin B	Sterile water	250 μg/ml (20 ml) twice daily in each nostril	6 months	Nasal lavage	Randomized placebo-controlled double-blind single centre study	Positive (computed tomography) and negative (symptoms)
Kennedy *et al.* [16]	2005	United States	25	28	Terbinafine	Not applicable	625 mg/day	6 weeks	Oral	Randomized placebo-controlled double-blind single centre study	Negative
Helbling *et al.* [65]	2006	Switzerland	21	0	Amphotericin B	Sterile water	1% (0.1 ml) three times daily in each nostril	3 months	Nasal spray	Nonplacebo-controlled single centre study	Negative
Ebbens *et al.* [31]	2006	Netherlands, UK, Spain, Belgium	59	57	Amphotericin B	Glucose 2.5%	100 μg/ml (20 ml) twice daily in each nostril	13 weeks	Nasal lavage	Randomized placebo-controlled double-blind multi centre study	Negative
Liang *et al.* [23•]	2008	Taiwan	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 weeks	Nasal lavage	Randomized placebo-controlled study	Negative

antifungals in the treatment of patients with CRS is not substantiated by the majority of publications.

Immunotherapy: effective in patients with chronic rhinosinusitis with concurrent fungal allergy

If CRS stems from hypersensitivity to retained fungal elements (a conclusion that should be questioned as mentioned previously), the removal of fungal elements may minimize ongoing stimulation; however, when the underlying hypersensitivity remains untreated, the disease is expected to recur. From 1994 onwards, Mabry *et al.* [68] prospectively treated 23 patients with AFS with antifungal immunotherapy following thorough exenteration of the involved sinuses. A decreased need for both 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 1–3 years in 11 patients. Cessation of immunotherapy after 3 years did not result in recurrence of symptoms in the 7–17 months of follow-up [69]. When interpreting these data, one should note that no placebo group was included, that controls included those patients who dropped out from the immunotherapy group, that several patients were lost to follow-up, that most patients were treated with immunotherapy to both fungal and nonfungal antigens and that all patients were treated with nasal irrigations and topical steroids for a variable period of time postoperatively. But, even though many confounders are present, the results of this study are intriguing. Even if one assumes that fungal allergy is not causative of CRS, one may conclude that antifungal immunotherapy is effective in reducing signs and symptoms in patients with CRS with concurrent fungal allergy. Future placebo-controlled studies are necessary to reveal the true role of antifungal immunotherapy in the treatment of patients with CRS.

Conclusion

The role of fungi in CRS remains to be defined. Although different studies have agreed that fungi can be detected in the nose and paranasal sinuses of nearly all patients with CRS, they are present in healthy controls as well. Currently, there are more questions than answers concerning the cause of CRS and the role of fungi. Recent studies suggest that there are many mechanisms by which fungi can exert an effect on sinus mucosa in susceptible individuals. Future studies will have to clarify the role of fungi in CRS, which fungal organisms, if at all, may be pathogenic and what exactly characterizes the immunological response to fungi that may potentially result in the development of disease. Presently, in the absence of convincing immunological data and evidence of clinical improvement in CRS upon therapy with antifungal agents, the case against the fungus remains unproven.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 67).

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