Role of fungi in pathogenesis of chronic rhinosinusitis: the hypothesis rejected

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Purpose of review
Fungi have been suggested to play an important role in the pathogenesis of chronic rhinosinusitis (CRS). This review describes the recent knowledge concerning the role of fungi in the pathogenesis of CRS and allergic fungal rhinosinusitis (AFRS) and the clinical implications for treatment.

Recent findings
Recent studies show that, although there are several potential deficits in the innate and potentially also in the acquired immunity of CRS patients that might reduce or change their ability to react to fungi, there are not many arguments to suggest a causative role for fungi in CRS with or without nasal polyps. However, due to the intrinsic or induced change in immunity of CRS patients, fungi might have a disease-modifying role. The fact that AFRS is more prevalent in warm and humid areas may point to fungi as a factor in this disease.

Summary
Almost a decade after the launching of the hypothesis by Ponikau, the absence of convincing immunological data or evidence for clinical improvement of CRS upon therapy with antifungal agents now means that the hypothesis that fungi play a role in a majority of the cases of CRS has to be rejected and antifungal treatment should not be used.

Keywords
allergic fungal rhinosinusitis, fungus, paranasal sinus disease, rhinosinusitis, sinusitis, treatment

INTRODUCTION

Since the 1980s, it has been recognized that, in analogy to allergic bronchopulmonary aspergillosis, fungi may play a role in some forms of rhinosinusitis. Especially in warm and humid areas, like the south of the USA, the disease was recognized as a subgroup of chronic rhinosinusitis (CRS). In the 1990s, diagnostic criteria for the disease were outlined in which the presence of 'allergic mucin' containing fungal hyphae was an important aspect. The discovery of better culture techniques for fungi in mucus in the first years of the 21st century led to the conclusion that fungi can be found in mucus of all patients with CRS and even in mucus of healthy controls. This resulted in the hypothesis that fungi might play a role in all forms of CRS. The hypothesis started a flood of new research on the potential role of fungi in CRS. More than 500 articles have been written on the subject. In this review, we will give an overview of the data that led to this interesting hypothesis and to its rejection. Finally, we will discuss the recent literature on what the role of fungi in CRS might be, the certainties and the questions that remain to be answered.

WHAT IS 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 that 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]. The overall prevalence of CRS by criteria of the European Positions Paper on

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KEY POINTS

- The hypothesis that fungi cause most forms of chronic rhinosinusitis (CRS) can be rejected.
- Although there are not many arguments to suggest a causative role for fungi in CRS with or without nasal polyps, fungi might have a disease-modifying role.
- There is no significant benefit of topical or systemic antifungals over placebo in the treatment of CRS.

Rhinosinusitis and Nasal Polyps (EP3OS) in Europe is 11% (range 7–27%) [3▪]. CRS is considered to be a multifactorial disease in which a large number of factors may eventually lead to impaired ciliary function, more mucus with increased viscosity, and mucosal swelling, resulting in symptoms of CRS. CRS is also considered to be a common denominator for several diseases that cannot or can only with great difficulty be differentiated clinically. Patients with CRS with and without nasal polyps can be differentiated clinically, although even this differentiation is not always easy. In recent years, it has been suggested that CRS with and without polyps can also be discriminated based on inflammatory profile [4,5]. However, very different forms of inflammation seem to be able to result in the same phenotype [6,7].

THE CONCEPT OF ALLERGIC FUNGAL RHINOSINUSITIS

In 1995, deShazo *et al.* [8] suggested diagnostic criteria for different forms of fungal disease affecting the nose and paranasal sinuses. Acute, chronic, and granulomatous invasive forms of fungal rhinosinusitis are considered to be rare, fortunately, and generally only occur in immunocompromised hosts. Noninvasive forms of fungal rhinosinusitis include sinus mycetoma (fungal ball), in general affecting only one sinus, and noninvasive (allergic) fungal rhinosinusitis (AFRS), affecting multiple sinuses and generally occurring in immunocompetent individuals. AFRS is defined as a noninvasive localized hypersensitivity response to fungal colonization that arises in areas of compromised mucus drainage. Diagnostic criteria were suggested to include the presence of chronic rhinosinusitis (nearly always in association with nasal polyposis), the presence of 'allergic mucin' (also referred to as 'eosinophilic mucin') containing noninvasive fungal hyphae in one or more sinus cavities, immunocompetence, and fungal allergy. Sinus CT would show heterogeneity of opacification with the less intense density of mucosal thickening and the more intense density of the mucin plugs [9,10].

ALLERGIC FUNGAL RHINOSINUSITIS, EOSINOPHILIC FUNGAL RHINOSINUSITIS, OR EOSINOPHILIC MUCIN RHINOSINUSITIS?

On the basis of the finding that only three-fourths of patients diagnosed with AFRS were atopic, the role of type 1 hypersensitivity was disputed and the term 'eosinophilic fungal rhinosinusitis' (EFRS) was introduced [11]. After the introduction of the term EFRS, the necessity of the presence of fungal hyphae in eosinophilic mucin rhinosinusitis was disputed and the term 'eosinophilic mucin rhinosinusitis' (EMRS) was introduced [12]. Recently, the International Society for Human and Animal Mycology formed a working group on fungal rhinosinusitis [13▪▪]. They concluded that the role of fungi in AFRS, EFRS, and EMRS remains unclear. This consensus group proposed to use the term 'eosinophilic mucin' for thick mucus containing clusters of eosinophils and eosinophil-degraded products with or without Charcot–Leyden crystals either in the presence or in the absence of fungal hyphae. Furthermore, they propose that the terms AFRS, EFRS, and EMRS are imprecise and require better definition and propose a scheme for subclassifying these entities, including 'aspirin-exacerbated rhinosinusitis', allowing an overlap in histopathological features, and an overlap with granulomatous, chronic invasive, and other forms of rhinosinusitis. Whatever the optimal terminology, it seems clear that there is a subgroup of patients with CRS with thick mucus and polyps who react positively to surgical removal of all the mucus and aggressive treatment with local and systemic corticosteroids. The prevalence of this form of CRS seems to vary depending on the climate, being more prevalent in warm and humid areas, but usually does not constitute more than 20% of CRS patients [14▪]. The role of allergy and fungi in this phenotype remains utterly unclear.

FUNGI AS THE CAUSE OF MOST FORMS OF CHRONIC RHINOSINUSITIS: THE HYPOTHESIS

Contrary to the prevailing belief that fungi were responsible for CRS in only a selective group of patients with a distinct pathophysiology, Ponikau *et al.* [11], in 1999, demonstrated the presence of fungi in the nose and paranasal sinuses in nearly all CRS patients [202 of 210 (96%) consecutive CRS patients] and all healthy controls [14 of 14 (100%) healthy controls] by using novel collection and culturing methods. They further progressed their hypothesis by demonstrating high levels of toxic major basic protein (MBP) from eosinophils in the mucus of patients with CRS, and postulated that

MBP damages the nasal epithelium from the luminal side.

Ponikau *et al*. proposed that certain fungi could elicit eosinophilic inflammation in the absence of type I hypersensitivity reactions in patients with CRS and proposed the term EFRS. The authors supported this concept of nonatopic eosinophilia from fungi by studies that demonstrated that peripheral blood mononuclear cells (PBMCs) from patients with CRS show exaggerated immunological, both Th1 and Th2, responses after exposure to common airborne fungi, particularly of the *Alternaria* species, which were absent in PBMCs from healthy controls. The authors claimed that anomalous immune and inflammatory responses to ubiquitous fungi might explain the chronic eosinophilic inflammation of CRS. Later the authors claimed that, in uncontrolled trials, antifungal treatment improved signs and symptoms of CRS (for review and references, see [15]).

FUNGI AS THE CAUSE OF MOST FORMS OF CHRONIC RHINOSINUSITIS: THE HYPOTHESIS REJECTED

In recent years, a number of review articles have been published that question the role of fungi in the pathophysiology of CRS [15–20]. The arguments put forward are that fungal spores are everywhere and that, if the detection method is sensitive enough, fungi can be found in every patient and control. Also, there are no arguments to suggest that either a specific fungal species or the fungal load is relevant for disease development. However, just as with allergens, fungi may contain proteolytic activity, which may diminish epithelial integrity and thus expose the epithelium to fungal elements [21]. Mechanical barriers, effective mucociliary clearance, and optimal healing limit the degree of antigenic stimulation of immune cells residing in the mucosa. A number of studies have looked at potential dysfunction of mucus components but were unable to link a specific dysfunction to the pathophysiology of CRS [17]. Both bacteria and fungi possess a number of mechanisms for both the evasion and modulation of host immune responses, including the formation of biofilms and the production of superantigens. Recently, fungus has been shown to be a component of biofilms in a significant percentage of patients with CRS [22▪,23]. However, whether the fungus contributes to the disease or is just an irrelevant component of the biofilm is unclear. Of the receptors of the innate immunity on the epithelial cells for the innate immunity reaction to fungi, toll like receptor (TLR)2, TLR4, and TLR6 seem to be the most important. *Alternaria* and *Aspergillus* have been shown to enhance the production of interleukin (IL)-8 and granulocyte-macrophage colony-stimulating factor from nasal epithelial cells. When nasal epithelial cells were activated by the fungi, TLR2, TLR3, and TLR4 mRNAs were more strongly expressed than in the nonactivated cells. Protease inhibitors and antihuman TLR4 antibodies [24] inhibited cytokine production. Epithelial cells from CRS patients have also been shown to have a poor TLR2-induced release of neutrophil-attracting chemokines such as IL-8 [25]. The exact role of TLRs and whether they contribute to a reduced or a changed reaction to fungi in CRS patients is still not clear. Fungi have important protease activity. In this way, they can activate epithelial cells via their protease-activated receptors (PARs). Activation of nasal epithelial cells with fungi results in an upregulation of PAR2 and PAR3 mRNAs [26]. Proteases present in fungal extracts like *Alternaria* have been shown to interact with epithelial cells, most likely through a PAR2 receptor-driven mechanism, leading to morphologic changes, cell desquamation, and induction of proinflammatory cytokines like IL-6 and IL-8 [27].

However, through this mechanism, eosinophilic inflammation does not seem to be induced; in at least one study in CRS patients, PAR2 stimulation did not lead to release of eosinophil-attracting cytokines like eotaxin or RANTES [28]. So, although fungi interact with the epithelium and the innate immune system, until now no explanatory differences have been found between epithelial cells of CRS patients and normal controls.

Eosinophils are an important hallmark of CRS, especially with nasal polyps, in the western world. Eosinophils are present in the late phase of allergic rhinitis and are also prominent in the reaction to parasitic infections. The concurrent presence of fungi and eosinophils in nearly all CRS tissue specimens has led to the suggestion of a cause and effect relationship. In follow-up studies, a concentration-dependent increase in eosinophil migration toward both nasal mucin and nasal tissue extracts was shown, and PBMCs from CRS patients exposed to *Alternaria* fungal extracts generated a mixed Th1/Th2 cytokine profile, whereas cells from normal patients did not respond [29]. Also, a component of *Alternaria* was shown to degranulate eosinophils from CRS patients by acting on PARs, implying that fungi can trigger inflammatory cells to initiate a complex localized eosinophilic reaction [30].

The data produced by Ponikau *et al*. could be interpreted to be consistent with a T-cell-driven, non-IgE-mediated response that resulted in the attraction and specific targeting of eosinophils

against colonized fungi in the nasal lumen of CRS patients, with subsequent degranulation and mucosal damage implying an acquired immune response. However, the absence of specific T-cell responses and, maybe even more importantly, the recognition that activated eosinophils from patients with asthma (both allergic and nonallergic asthma) are also known to exhibit a primed phenotype resulting in increased eosinophil migration, adhesion, and degranulation capacities make this hypothesis unlikely [17]. Moreover, recently, Orlandi *et al.* [19] found that IL-5 was produced following *Alternaria* exposure not only by PBMCs of patients, but also by those of controls, and, furthermore, this response was heterogeneous and did not correlate with the presence of CRS [31▪▪]. In addition, *Alternaria*-induced levels of IL-13, the principal chemoattractant for eosinophils, did not differ between CRS patients and controls. Moreover, IL-5 levels correlated strongly with fungal-specific IgE but not with fungal-specific IgG, as was earlier suggested by the group Ponikau and coworkers [29].

One of the major arguments for an important role of fungi in CRS would be a positive reaction to antifungal treatment. A pooled meta-analysis of the five double-blinded and randomized studies investigating topical antifungals [32–36] and one double-blinded and randomized study investigating systemic antifungals [37] showed no statistically significant benefit of topical or systemic antifungals over placebo for any outcome [38▪▪]. Symptom scores in fact statistically favored the placebo group and adverse event reporting was statistically significantly higher in the antifungal group.

CONCLUSION

Although there are several potential deficits in the innate and potentially also in the acquired immunity of CRS patients that might reduce or change their ability to react to fungi, there are not many arguments to suggest a causative role for fungi in CRS with or without nasal polyps. However, due to the intrinsic or induced change in immunity of CRS patients, fungi might have a disease-modifying role. The fact that AFRS is more prevalent in warm and humid areas may point to fungi as an important factor. It is possible that in some cases of CRS fungal hyphae trapped in the stasis of mucus or even in the biofilm may indeed intensify and/or perpetuate the inflammatory reaction that was already present. Further research can be done to elucidate the role of fungus as disease modifier or potentially also as cause of some forms of CRS. These studies preferably have to be done in regions with a high prevalence of AFRS.

However, almost a decade after the launching of the hypothesis by Ponikau, the absence of convincing immunological data or evidence for clinical improvement of CRS upon therapy with antifungal agents now means that the hypothesis that fungi play a role in a majority of the cases of CRS has to be rejected. Even whether the combination of eosinophils and hyphae points to an allergic reaction to the fungi in allergic fungal rhinosinusitis is far from clear. Although anecdotal stories of a positive reaction of patients to (systemic) antifungal treatment have been reported, the significant side effects and the chance of placebo effects should be a strong reason not to prescribe this medication until a proper randomized placebo-controlled trial shows significant benefit [38▪▪].

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Conflicts of interest

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 73).

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