

IgE-Mediated Fungal Allergy in Allergic Fungal Sinusitis

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This review will address the current knowledge of the pathogenic mechanisms in allergic fungal sinusitis (AFS) and the basis for the current classification of a subgroup of chronic rhinosinusitis patients. Special attention is directed to the role of immunoglobulin E (IgE)-mediated fungal allergy in the pathogenesis of AFS. Concepts relating to the mucosal inflammatory response are introduced, as a knowledge of the reactions of the sinus mucosal cells can lead to a better understanding of the mechanisms perpetuating and maintaining the chronic inflammation.

Key Words: Allergic fungal sinusitis, allergy, chronic rhinosinusitis, mucosal inflammatory response.
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INTRODUCTION

Allergic fungal sinusitis (AFS) is a descriptive term for an important subset of chronic rhinosinusitis (CRS) patients. AFS is defined by a constellation of pathological features that include eosinophilic mucus (EM) in the sinuses, IgE-mediated allergy to fungi and fungi in the sinus mucus.¹ The pathogenesis of AFS is poorly understood. IgE-mediated allergy (type I hypersensitivity) is considered an important etiological factor in AFS. Type I hypersensitivity refers to an IgE-mediated allergic reaction, whereas allergy refers to an exaggerated or inappropriate immune-mediated reaction to an antigen at a dose tolerated by normal subjects.² In the AFS literature, allergy has become synonymous with type I hypersensitivity and will be used in this context in this paper. Although, fungal allergy is included in the diag-

nostic criteria of AFS, there is limited supporting evidence for its primary pathogenic role. A significant proportion of the population and CRS patients have fungal allergy but do not have the clinical condition of AFS. Also, several subgroups of CRS patients with clinical features similar to AFS, including polyps and EM, do not have fungus in the EM or fungal allergy.^{3–6} Thus, it is still unclear how these patients fit into the disease spectrum of AFS and CRS.

ALLERGIC FUNGAL SINUSITIS

Historical Background

The clinical features of nasal obstruction, hard and blood-tinged nasal casts, positive *Aspergillus fumigatus* cultures of sinus contents and nasal polyps were first described in a patient suspected to be suffering from allergic bronchopulmonary aspergillosis (ABPA).⁷ It was not until 1981 when the first analogy of the sinus contents to the mucus plugs found in the lungs of patients with ABPA was made.⁸ The authors described the classical histopathology appearance of the sinus inspissated debris from five ABPA patients where *Aspergillus fumigatus* was cultured in one patient and the authors proposed the term “allergic aspergillosis” of the paranasal sinuses. In 1983, a retrospective review of surgical specimens from the sinuses of CRS patients showed histological features indistinguishable from mucoid impaction found in the lungs of ABPA patients.⁶ This mucoid material was termed “allergic mucin”. Fungal hyphae resembling *Aspergillus* species were present in the mucus, and this was defined as a distinct pathological entity from ABPA and the term “allergic *Aspergillus* sinusitis” was introduced. Further studies established that fungal culture of the sinus mucus yielded fungi other than from the *Aspergillus* species and hence, the term “allergic fungal sinusitis”.⁹

Eosinophilic Mucus

Eosinophilic mucus is a diagnosis made following examination of paraffin-embedded mucus specimens stained with haematoxylin and eosin. EM is also known as allergic or fungal-like mucin. Mucin is a misnomer as

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it refers to "any group of protein containing glyco-conjugates with high sialic acid or sulfated polysaccharide content that compose the chief constituent of mucus"¹⁰ Eosinophils are the predominant and a consistent cellular component that defines EM. Eosinophil breakdown products known as Charcot-Leyden crystals, other leukocytes, respiratory epithelial cells; and debris are also present.¹¹ Macroscopically, EM is typically thick, viscous to almost solid, and colored, and it is often described as being of axle grease, peanut butter, or cottage cheese-like consistency.¹²

Diagnostic Criteria of Allergic Fungal Sinusitis

The generally accepted definition of AFS is that put forward by Bent and Kuhn and encompasses: a) EM, b) positive fungal stain of sinus contents, without fungal invasion into sinus tissue, c) nasal polyposis, d) characteristic radiological signs, and e) type I hypersensitivity to fungi.¹ However, there are several variations to this definition. In some, the macroscopic appearance of EM is sufficient for diagnosis, whereas others consider positive fungal cultures from nasal or sinus mucus in the absence of fungal elements adequate.^{4,13,14} Some dispute the need to demonstrate fungal allergy.^{13,14} Recently, an international panel redefined AFS as "histological confirmation of EM and presence of type I hypersensitivity to fungi in CRS patients."¹⁵ The various definitions have arisen because the pathogenic importance of fungi and fungal allergy in AFS is uncertain. The plethora of definitions of AFS has made it difficult to make direct comparisons between published studies and consequently hindered progress in understanding this disease.

Clinical Characteristics

Allergic fungal sinusitis, defined by the presence of allergic mucin with fungal forms noted upon histology or by allergic mucin with positive fungal cultures, affects young adults with a mean age at diagnosis of 20 to 35 years.^{3,5,16} Men and women are equally affected. As definitive diagnosis can only be made by examination of surgical specimens, the true prevalence of AFS is unknown. Also, there appears to be a geographical variation in the prevalence of AFS. However, based on the criteria of Bent and Kuhn, studies show that AFS affects 4–10% of patients undergoing sinus surgery.⁴

Typically, AFS affects immune-competent individuals. There is usually a long history of nasal obstruction, hyposmia or anosmia, and of blowing out nasal casts. Nasal polyps are present in almost all patients and extra-sinus complications in a proportion of patients. The mucosal inflammatory cell component is characterized mainly by eosinophils and lymphocytes.^{17,18} By definition, fungal allergy is present, although not necessarily to the same fungal species identified in the EM. In many cases, fungal allergy to more than one genera and species is present, a common feature for fungal allergies in humans.^{3,19,20} Allergy to nonfungal allergens is also common.^{3,21–23} Total serum IgE levels, peripheral blood eosinophil count, serum eosinophil cationic pro-

tein, erythrocyte sedimentation rate, and C reactive protein may be elevated,^{3,5} but none of these changes are consistent. Serum *Aspergillus* precipitins have been reported, but are often negative.¹² Asthma and aspirin sensitivity may be present in AFS patients, but coexisting AFS and ABPA is uncommon.^{24,25} However, many patients with AFS have significant lower respiratory tract symptoms but do not fulfill the diagnostic criteria for ABPA, including IgE levels greater than 1,000 kU/L and bronchiectasis.³ Whether the lower respiratory tract disease in AFS patients represents a different disease process from ABPA is speculative.

Fungi commonly identified in the EM are from the dematiaceous family and *Aspergillus* species.^{3,4,12} Dematiaceous fungi include the genera *Alternaria*, *Bipolaris*, *Cladosporium*, *Curvularia*, *Drechslera*, and *Helminthosporium*. These fungi are darkly pigmented due to dihydroxynaphthalene melanin in the cell walls of the hyphae or conidia.²⁶ Although fungal invasion of the sinus mucosa is not characteristic, fungi in the sinus mucosa has been documented.²⁷ Sinus computed tomography (CT) scans may show areas of heterogeneous signal intensity within the affected sinuses known as double densities.²⁸ These are due to accumulation of heavy metals including iron, manganese, and calcium, and are associated with fungi. Expansion of the sinus cavity with bone remodeling resulting in extension of sinus disease into adjacent sites may also be observed.²⁹ However, all these CT scan appearances are nonspecific for AFS.

Treatment and Prognosis

At present, there is no long-term successful treatment for AFS. Combinations of surgical and medical therapies are used to manage complications, provide symptomatic relief from extensive nasal polyps, and to delay recurrences.^{12,30,31} Corticosteroids are the most effective agent in preventing recurrences and in selected cases used as first line treatment. Topical antifungal treatment and immunotherapy are also used, although their clinical efficacy is variable.^{32,33} Despite treatment, the recurrence rate of nasal polyps and EM is extremely high.³⁴ Hence, there is a clear need for a better understanding of this disease and for the development of effective therapies.

CURRENT MODEL OF PATHOGENESIS IN ALLERGIC FUNGAL SINUSITIS

AFS and ABPA: Striking Similarities and Obvious Differences

The model for the pathogenesis of AFS was based on that for ABPA. In 1952, ABPA was first described as a condition of the airways characterized by prominent eosinophil and lymphocyte populations in the tissue, increased levels of serum IgE, and precipitating antibodies to *Aspergillus* antigens.³⁵ As for AFS, the prevalence of ABPA, typically seen in patients with severe asthma and cystic fibrosis, remains highly speculative and varies between 2% and 38% for asthmatic patients and between 35% and 81% for cystic fibrosis patients.

A hypersensitivity immune response to *Aspergillus fumigatus* antigens is believed to cause ABPA.³⁶ *Aspergillus* hyphae is often present in the lungs of ABPA patients, but it is unclear from the literature whether other fungal species are also cultured. Levels of total IgE, *Aspergillus*-specific IgE, IgG, and IgA, and *Aspergillus*-specific peripheral blood mononuclear cell proliferation are elevated in ABPA patients, and cytokine studies show either a type 1 or type 2 profile depending on the fungal antigen extract used.³⁷ Eosinophils and lymphocytes in the mucosa, EM, fungal elements and allergy are present in both ABPA and AFS patients. Similar to ABPA, fungi in the mucosa are also found in some AFS patients,^{27,38,39} and corticosteroids are the mainstay of medical therapy. However, in AFS, fungi other than *Aspergillus* appear to be common, *Aspergillus* precipitins are usually not present, and the total IgE level is often normal or only slightly elevated. Although fungal allergy is proposed as the major mechanism in ABPA and AFS, it remains to be explained why all patients with fungal allergy do not develop these severe clinical phenotypes. Similar to AFS, there is no direct evidence that fungal allergy is the central pathogenic factor in ABPA.

IgE-Mediated Allergy to Fungi in Allergic Fungal Sinusitis

Type I hypersensitivity depends on the permanent sensitization of mast cells in the target organ where the high affinity receptor for IgE, FcεRI, is critical in mediating allergen-specific IgE responses.⁴⁰ Type I hypersensitivity results from cross-linking by a specific allergen of two or more IgE molecules engaged on the surface of effector cells by FcεRI. Subsequent effector-cell activation causes release of preformed and newly formed mediators that cause local inflammation, recruitment of leucocytes, including basophils and eosinophils, leading to the clinical symptoms and signs. The diagnosis of type I hypersensitivity requires the detection of allergen-specific IgE in peripheral blood or a positive skin prick test to an allergen.⁴¹

Depending upon the definition of AFS used, up to 100% of patients have fungal allergy.^{1,16,42} This is not surprising given that fungal allergy is a prerequisite for the diagnosis of AFS in most studies. However, it is well established that fungal allergy is not present in every CRS patient with sinus EM, and fungal allergy may be present in individuals with no evidence of sinonasal disease.^{13,14,43} In addition, when present, fungal allergy may not be to the same fungal species identified in the sinus EM. Also, there is a poor correlation between the prevalence of allergy and nasal polyps, a common feature in patients with AFS.⁴⁴ Fungal allergy is estimated to affect 3% to 10% of adults and children worldwide.⁴⁵ Accordingly, fungal allergy in AFS may just represent the prevalence of fungal allergy in the population, as 4% to 10% of CRS patients with EM have fungal allergy.⁴ A comprehensive examination of the allergic parameters including systemic fungal-specific IgE, and total IgE levels showed no significant difference between AFS patients and controls (allergic rhinitis with fungal allergy).²⁰ How-

ever, when type I allergy is present, a worse clinical outcome may be observed. These findings indicate that fungal allergy may coexist in patients with AFS, and may exacerbate an underlying inflammatory disorder rather than be a major contributor to pathogenesis.

Role of Local IgE in Allergic Fungal Sinusitis

In the absence of systemic fungal allergy, local mucosal allergy to fungi has been suggested to mediate the inflammation in AFS.²³ There is evidence for local IgE synthesis in sinonasal and lung mucosa.⁴⁶ However, mucosal studies from CRS patients have generated inconsistent results. In one study, most nasal polyps did not contain IgE secreting plasma cells.⁴⁷ By contrast, others demonstrated IgE in nasal and polyp tissue. One study showed a good correlation between IgE levels in nasal tissue and sera from allergic patients,⁴⁸ whereas another found that specific IgE was not present in the mucosa of every fungal-allergic patient tested.²³ A comparison between allergic and nonallergic CRS patients showed no significant difference in total IgE or antigen-specific IgE in the mucosa.^{49,50} However, mucosal studies in allergic and nonallergic rhinitis patients showed that IgE-positive cells and mast cells are increased compared with controls.⁵¹ Interestingly, cell-bound IgE is also present in the upper airway and gut mucosa in asymptomatic individuals.^{52,53} These inconsistent findings may be due, in part, to the functional diversity of mast cells and antigen-specific IgE. Other than allergic responses, mucosal mast cells and IgE mediate nonallergic antigen-specific and nonspecific homeostatic and defense functions. Mast cells are also activated directly by pathogens via toll-like receptors.⁵⁴ Mast cells and IgE are implicated in responses to viral and bacterial infections⁵⁵ and in some autoimmune diseases.⁵⁶ Monomeric IgE may bind to FcεRI without causing degranulation and increase mast-cell survival. Also, several subsets of mast cells are described.⁵⁷ Thus, in the absence of systemically detectable allergen-specific IgE or a positive skin prick test, the pathogenic importance of local IgE in allergen-specific hypersensitivity responses is yet to be established.

EOSINOPHILIC MUCUS CHRONIC RHINOSINUSITIS

CRS patients with polyps and sinus EM but no detectable fungal elements in the sinus mucus or fungi allergy have been recognized since the first description of allergic *Aspergillus* sinusitis.¹¹ Although a review concluded that AFS patients were clinically and pathologically distinct from other patients with EM, termed eosinophilic mucin rhinosinusitis, this review was based on a retrospective analysis of studies in which the definitions of AFS were inconsistent.⁵ Hence, it remains unclear whether CRS patients with EM but no fungal elements or fungal allergy represent a different clinical and pathological entity from AFS patients.

A study in our department compared clinical, radiological, microbiological, and immunological parameters between AFS and CRS patients who had EM with and

without fungal allergy and fungi in sinuses. This study demonstrated no significant difference in the parameters tested between the study groups regardless of the presence of allergy or fungi in the sinuses.³ Importantly, the presence of EM in CRS patients characterized a more severe form of disease when compared to the CRS patients who did not have EM.^{3,58} Except for their detection in the sinuses, the effect of fungi on the sinus mucosa and fungal-specific immune responses have not been examined thoroughly in AFS patients. Thus, at present the role of fungi in this disease is speculative.

It appears then that EM is the main consistent feature correlating with severe sinus disease. Eosinophilic mucus is almost always seen in CRS patients with nasal polyps.^{3,5} However, fungal allergy and fungi may be demonstrated in patients with nonpolypoid sinus disease, patients with allergic rhinitis without sinusitis, and in seemingly healthy individuals. Based on these findings, we propose that all CRS patients with EM be included as one term, eosinophilic mucus chronic rhinosinusitis (EMCRS). The EMCRS patients fulfill the following clinical subclassifications documented in the literature:

1. AFS, where fungal allergy is present and fungi are detected in EM.^{1,5}
2. AFS-like, where fungal allergy is present but fungi are not detected in EM.⁴
3. Nonallergic, fungal eosinophilic sinusitis,⁴ nonallergic fungal eosinophilic rhinosinusitis,⁵⁹ or chronic fungal sinusitis,²³ where fungal allergy is absent and fungi are detected in EM.
4. Nonallergic, nonfungal, eosinophilic sinusitis (NANFES) or chronic eosinophilic sinusitis (CES),⁴ where fungal allergy is absent and fungi are not detected in EM.

Given the mounting evidence that fungal allergy is unlikely to have a major pathogenic role, and that the role of fungi is still being evaluated, further subdividing EMCRS patients as above may become redundant. However, it is important to identify coexisting medical problems, including atopy, asthma, and bacterial and fungal culture results as they may influence the clinical management in individual cases. Whatever the etiology of EM, there appears to be considerable evidence that EM has an important role in the prognosis of CRS patients and appears to signify a distinct form of CRS associated with an intense mucosal inflammatory response. EMCRS patients have a worse clinical course and a greater prevalence of lower respiratory tract disease including asthma and bronchiectasis compared with polypoid CRS patients without EM. However, it is yet to be determined whether EMCRS is different from other polypoid CRS in terms of its pathogenesis.

PATHOGENESIS OF MUCOSAL INFLAMMATION IN EMCRS PATIENTS

The mucosal inflammation in EMCRS patients (Pant et al., unpublished data), including AFS patients,⁵⁹ is characterized by populations of eosinophils and lympho-

cytes. These cells are also common in most forms of CRS, yet there are only few studies that have examined them. Factors implicated in the etiology of CRS include responses to microorganisms, altered function of the innate and adaptive immune systems, and IgE-mediated allergy. Mucosal studies in some CRS patients have shown microorganism biofilm,^{60,61} planktonic bacteria and fungi,¹³ and altered levels of proteins involved in innate immunity,⁶²⁻⁶⁹ yet their pathological significance is unclear as they may represent a secondary effect or a normal response to underlying inflammation. Allergic and nonallergic mechanisms contribute to eosinophilic inflammation. There is evidence of eosinophil recruitment in nonallergic CRS patients whose T cells are sensitized to fungal antigens.⁷⁰ Other than a mechanism for eosinophil accumulation in the absence of fungal allergy, the significance in CRS patients is uncertain as this may represent a normal antifungal response by eosinophils.⁷¹ Although fungi are implicated, it also remains to be explained why recalcitrant inflammation is present only in some individuals although exposure to fungal antigens is ubiquitous.

Superantigens have been proposed as a mechanism of inflammation in CRS patients. Superantigens are produced by viruses, bacteria, and fungi and are capable of stimulating a large proportion of T cells resulting in an inflammatory microenvironment. Persistence of bacteria and fungi in planktonic and biofilm form may be the source of superantigens contributing to the inflammation in some patients. The critical supporting link between these etiological factors and eosinophil and lymphocyte populations and their effector functions in the mucosa of CRS patients is yet to be established.

The major effector function of eosinophils in the CRS mucosa is believed to be tissue destruction occurring as collateral damage during antimicrobial and immunostimulatory activation. However, the role of eosinophils extend beyond phagocytosis to tissue remodeling and immune modulation via antigen presentation and cytokine release.^{72,73} Differential eosinophil phenotypes, and patterns of degranulation depending on the immune response are also proposed.^{74,75} Hence, defining the nature of an inflammatory response by an analysis of eosinophils alone is currently limited, in part, due to technical reasons, as cell function and cell surface antigen expression may alter depending on isolation and identification strategies.⁷⁶

Lymphocytes are abundant in the CRS mucosa and are integral in an immune response. In addition to generating antigen-specific responses, immune surveillance and regulation of adaptive and innate immunity is a vital function of lymphocytes. Regulation of an antigen-activated immune response is essential to prevent damage to normal and uninvolved cells in the vicinity.⁷⁷ Currently there are no published studies examining the lymphocyte populations and effector function in EMCRS patients. A study of lymphocyte phenotypes including clonality, antigen specificity, level of cellular differentiation, and effector functions can provide critical insight into the type of an immune response driving the chronic inflammation.

CLINICAL IMPLICATIONS AND THERAPEUTIC STRATEGIES

The mucosal immune system is a complex milieu in which the innate and adaptive immune systems work in concert to provide adequate immunity yet maintain a level of tolerance to commonly inhaled environmental antigens and mucosal flora. It is feasible that chronic inflammation may occur due to a dysregulated mucosal immune system in genetically susceptible individuals. This hypothesis would in part explain such intense inflammation in some patients in response to microorganisms that are normally tolerated by others. A systematic study of the fundamental inflammatory processes and of the key regulatory cells in the sinus mucosa is vital to define the type of immune response, the significance of etiological agents including microorganisms and genetic predisposition in CRS. Regardless of etiology, chronic inflammation can continue even when the inciting agent has long been eradicated. This form of inflammation is largely mediated by lymphocytes, where the persistence of the primary agent may not be required, such as in autoreactive states. Several specialized functional subsets of T cells, including regulatory T cells are described.⁷⁸ Recent findings show changes in the numbers and function of discriminating lymphocyte subsets, even when changes in the bulk cell populations are not evident; thus, analysis of cell subsets may provide powerful diagnostic and prognostic information and permit therapeutic modulation of various components of the innate and adaptive mucosal immune system.⁷⁹

CONCLUSION

The current evidence supports the notion that AFS is part of a spectrum of severe CRS with polyps that is characterized by EM in the sinuses. Fungal allergy in EMCRS patients is unlikely to be the primary pathogenic event. At best, in some patients allergy may be responsible for exacerbating the underlying inflammation. Understanding and defining the mucosal immune responses in these patients will enable a better understanding of the underlying pathological mechanisms. This includes examination of mucosal responses to common environmental antigens, including fungi and bacteria, that may help define the role of microorganisms in the pathogenesis of CRS and provide a basis for specific and effective antimicrobial and immunomodulatory therapies.

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