

# Revisiting the controversy: The role of fungi in chronic rhinosinusitis

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## Abstract

In the last two decades, the development of culture-independent genomic techniques has facilitated an increased appreciation of the microbiota-immunity interactions and their role in a multitude of chronic inflammatory diseases such as chronic rhinosinusitis (CRS), asthma, inflammatory bowel disease and dermatitis. While the pathologic role of bacteria in chronic inflammatory diseases is generally accepted, the understanding of the role of fungi remains controversial. Chronic rhinosinusitis, specifically the phenotype linked to nasal polyps, represents a spectrum of chronic inflammatory diseases typically characterized by a type 2 immune response. Studies on the microbiota within sinus cavities from healthy and diseased patients have focused on the bacterial community, mainly highlighting the loss of diversity associated with sinus inflammation. Within the various CRS with nasal polyps (CRSwNP) phenotypes, allergic fungal rhinosinusitis presents an opportunity to investigate the role of fungi in chronic type 2 immune responses as well as the antifungal immune pathways designed to prevent invasive fungal diseases. In this review, we examine the spectrum of fungi-associated sinus diseases highlighting the interaction between fungal species and host immune status on disease presentation. With a focus on fungi and type 2 immune response, we highlight the current knowledge and its limitations of the sinus *mycobiota* along with cellular interactions and activated molecular pathways linked to fungi.

## KEY WORDS

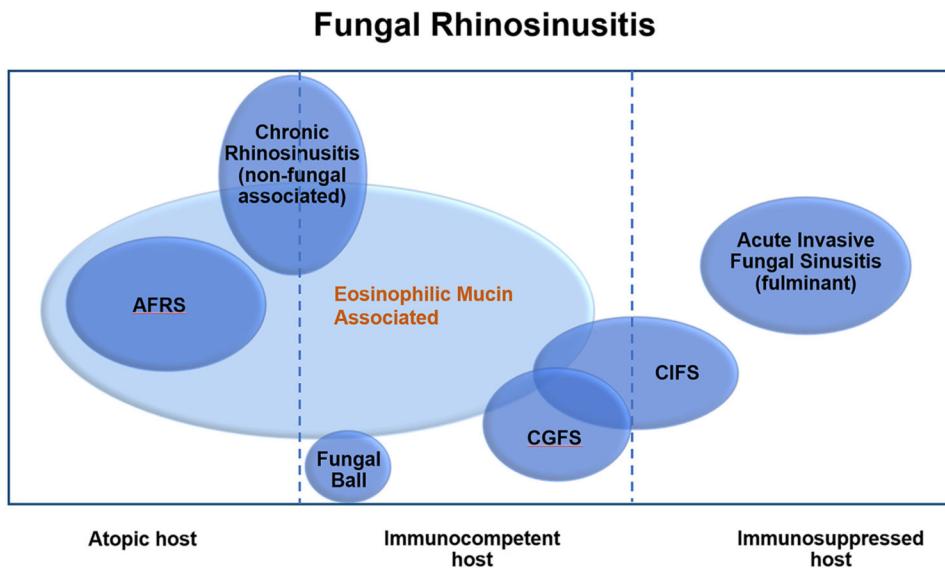
allergic fungal sinusitis, chronic rhinosinusitis, fungal sinusitis, superantigen, innate immunity and rhinosinusitis

## INTRODUCTION

Chronic rhinosinusitis (CRS) was initially considered an extension of acute bacterial rhinosinusitis. However, the classification of CRS correctly pivoted from infection to inflammation as molecular studies on diseased mucosa

highlighted inflammatory immune responses. Several possible etiologic agents of CRS have been studied including viruses, bacteria, environmental damage, allergens, and fungi. Unlike bacteria, which are typically cultured from purulent sinus drainage with standardized laboratory techniques, fungi were rarely cultured from sinus secretions.





**FIGURE 1** Fungal rhinosinusitis. Diagram representing the relationships among fungal associated rhinosinusitis subtypes relative to host immune status. AFRS is characterized by an exaggerated type 2 inflammatory response in patients with fungal sensitivity. Conversely, a fungus ball incites little underlying mucosal inflammation in the non-atopic, immunocompetent host. The immune compromised host can suffer frank tissue invasion with necrosis. Abbreviations: AFRS, allergic fungal rhinosinusitis; CGFS, chronic granulomatous fungal sinusitis; CIFS, chronic invasive fungal sinusitis

However, clinical presentations of various types of fungal sinusitis such as fungal balls or allergic fungal rhinosinusitis (AFRS) suggested an active role of fungi in driving local inflammatory immune responses.

In the late 1990s, Ponikau and colleagues showed that fungi could reliably be cultured from nasal secretions by treating it with a reducing agent prior to culture. He and others showed that peripheral blood mononuclear cells from some CRS patients with nasal polyps (CRSwNP) including AFRS but not healthy controls, exhibited T cell memory expressing type 2 cytokines such as interleukin 4 (IL-4) and IL-5 upon challenge with fungal extracts. Ponikau proposed that fungi were the drivers of all CRS inflammation. This bold hypothesis motivated numerous, although heterogenous, antifungal clinical trials in CRS including patients with and without polyps that ultimately showed no clear role of antifungals in the treatment of CRS. However, no randomized controlled trials with antifungals have specifically been reported in AFRS or fungal-sensitive CRSwNP. Nonetheless, these negative clinical trials called into question a significant etiologic role of fungi in the immunopathology of CRSwNP.

Over the last 20 years, new studies have identified molecular pathways activated by various fungal components leading to the type 2 immune response characteristic of chronic eosinophilic airway inflammation. These studies not only support the etiologic role of fungi in the pathophysiology of CRS, but also serve to highlight potential novel therapeutic targets. And more importantly, these

studies may identify molecular mechanisms by which to prevent invasive disease.

## SPECTRUM OF FUNGAL SINUSITIS

Fungus has been linked to a spectrum of sinus disease processes characterized by the presence of pathogenic fungus in the secretions or in the actual sinus mucosal tissues. From a clinical standpoint, fungal rhinosinusitis is classified both by the presence (or absence) of fungal elements within the soft tissue lining of the sinuses and the host's response to the presence of the fungus. More than 20 years ago, de Shazo et al.<sup>1,2</sup> codified the classification of fungal sinusitis as follows: noninvasive fungal sinusitis, including AFRS and sinus mycetoma (fungus ball), and invasive fungal sinusitis, including acute (fulminant) invasive fungal sinusitis and chronic invasive fungal sinusitis. More recently, Chakrabarti et al.<sup>3</sup> added the category of saprophytic fungal infestation to the noninvasive category, and provided distinguishing characteristics for chronic granulomatous fungal sinusitis as a distinct category of chronic invasive fungal sinusitis (Figure 1).

In the immunocompetent host, saprophytic fungal disease is characterized by fungal colonization of the paranasal sinuses (typically confined to a small area within a single sinus cavity) provoking minimal underlying inflammatory changes.<sup>3,4</sup> Similarly, a sinus fungus ball is an accumulation of nonviable fungal elements mixed

with a fibrinous, often necrotic, exudate of sinus secretions that typically involves one sinus cavity in an immunocompetent, nonatopic host. Although sinus fungus balls incite some inflammatory response in the sinus mucosa, usually in the form of sinus secretions that make up part of the fungus ball, de Shazo et al.<sup>1</sup> emphasized the presence of an intact sinus mucosal lining (absence of tissue invasion), the absence of granulomatous inflammation, and the absence of eosinophilic inflammation, as distinguishing features of this subtype of fungal disease. This is in contrast to AFRS, which affects atopic immunocompetent patients whose affected sinuses exhibit a robust local sinonasal inflammatory response characterized by polyps, elevated eosinophils, mast cells, and increased expression of interleukin 4 (IL-4), IL-5, and IL-13. Although affecting more than one sinus cavity, AFRS often involves a limited number of sinuses within an affected host. AFRS is often unilateral, although bilateral presentations are frequent as well. This unilateral presentation is unlike other subtypes of CRS, and supports the role of environmental fungi in initiating AFRS.

Initially difficult to culture from sinus secretions, Braun et al.,<sup>5</sup> by treating secretions with a reducing agent, demonstrated the ubiquitous nature of fungi by identifying the presence of fungi within nasal secretions in more than 90% of both CRS patients and healthy controls. Porter et al.<sup>6</sup> found that fungi were not necessarily as prevalent in sinus secretions. They found a higher presence of fungi from secretions obtained directly from sinus cavities when compared to nasal secretions from CRS patients, especially those with AFRS. In addition, 64% of patients with CRSwNP and over 98% of AFRS patients were found to have immunologic memory, and hence exposure of the immune system to the fungi, which was not in patients without CRS. Clearly, other factors in addition to fungal exposure, including host-specific factors, influence the development of phenotypic presentation of the various forms of fungal rhinosinusitis.

## AIRWAY MYCOSIS LINKED TO CHRONIC INFLAMMATORY RESPIRATORY DISEASES

Fungi represents a common trigger for chronic airway inflammation in asthma and CRS. Asthma severity and morbidity have been linked with fungal exposure.<sup>7,8</sup> Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) are two such severe asthma subtypes linked to fungal colonization.<sup>9</sup> Patients with SAFS were more likely to have early onset asthma, worse asthma control test (ACT) scores with multiple fungal sensitizations, increased rates of intensive

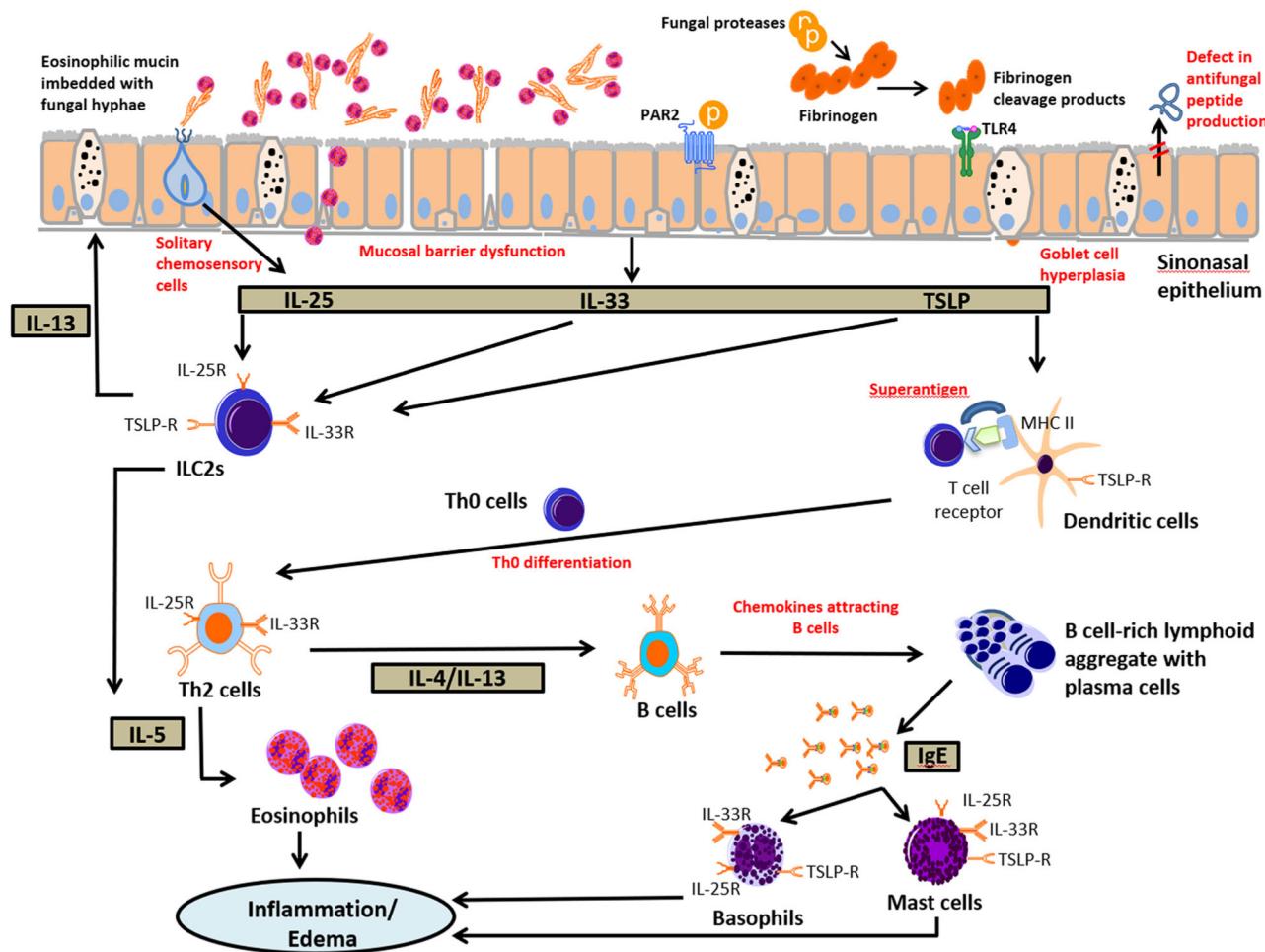
care unit admissions, and greater need for mechanical ventilation.<sup>8,10</sup> With this link between fungi and asthma severity, there has been increasing interest in the pulmonary fungal microbiome, or *mycobiome*.

Analysis of lung mycobiome has demonstrated higher fungal loads in patients with ABPA and SAFS when compared to healthy controls.<sup>9,11</sup> Although *Aspergillus fumigatus* has been linked with ABPA,<sup>9</sup> other fungi including *Candida*, *Penicillium*, and *Cladosporium* species have been identified in SAFS patients. Loss of diversity in the bacterial microbiome has also been associated with poorer respiratory health; and similarly, decreases in mycobiome diversity may contribute to chronic respiratory disease.<sup>11–14</sup> Complex interactions in the airway microbiome, including those among bacterial, fungal, and viral organisms, may be important for modulating microbiome diversity and host immune interactions.

Culture-independent techniques for microbial identification have been instrumental in elucidating a more complete understanding of the human microbiome. Polymerase chain reaction (PCR)-based technologies, including next generation sequencing (NGS) and whole metagenome shotgun (WMS) techniques, were introduced in the mid-1990s.<sup>15</sup> These methods have identified many more organisms in the bacterial microbiome of the paranasal sinuses than traditional microbiology culture techniques,<sup>15,16</sup> and are being applied to the sinus mycobiome.<sup>17,18</sup> Frequent targets for NGS in identifying fungal organisms include 18S ribosomal RNA (rRNA) and the internal transcribed spacer (ITS) region between 18S and 26S rRNA sequences.<sup>14,17</sup> DNA extraction from mucus and sputum samples is a critical step in performing culture-independent techniques. Dithiothreitol (DTT), applied to mucus samples as a fluidizing agent, has been shown to decrease the variation of extracted DNA.<sup>19</sup> To this extent, DTT may have a role in improving consistency of microbial sequencing from airway mucus samples.

Culture-independent techniques have been used to characterize the mycobiome in CRS, and *Aspergillus* species have been implicated in these fungal-related disease subtypes associated with nasal polyps. The mean relative abundance of *Aspergillus* species was demonstrated to be increased in all CRS patients where fungal organisms were detected with ITS sequencing.<sup>17</sup> *Aspergillus* species, specifically *A. fumigatus* and *A. flavus*, were found to be present in patients with AFRS.<sup>20</sup> The increased relative abundance of *Aspergillus* species in fungal CRS subtypes mirrors similar increases in pulmonary conditions such as ABPA and SAFS.

These findings of higher fungal presence within inflamed pulmonary and sinus cavities of patients with asthma and CRSwNP, respectively, support a role of fungi in the pathophysiology of these diseases. However, only



**FIGURE 2** Immune pathogenesis of fungal-induced chronic rhinosinusitis. Loss of antimicrobial peptides, increased epithelial permeability, fungal proteases, and other fungal elements promote release of innate inflammatory cytokines, including IL-25, IL-33, and TSLP. Several fungi-activated receptors including PAR2, TLR4, and solitary chemosensory cells can drive release of these epithelial-derived cytokines. Innate lymphoid cells, which are IL-25-responsive and IL-33-responsive, express IL-5, resulting in eosinophil recruitment and activation. Dendritic cells respond to innate mediators, such as TSLP, and antigenic stimulation by fungal (and other) microbiota, which then promotes  $T_h2$  cell development. T-cell activation can be activated further by superantigens.  $T_h2$  activation affects B-cell isotype switching and differentiation to IgE-producing and IgG-producing plasma cells, further promoting a “hyperallergic” inflammation in the airway. Abbreviations: IgE, immunoglobulin E; IgG, immunoglobulin G; IL-5, interleukin 5; IL-25, interleukin 25; IL-33, interleukin 33; ILC2, type 2 innate lymphoid cell; PAR2, protease-activated receptor 2;  $T_h2$ , T helper 2; TLR4, Toll-like receptor 4; TSLP, thymic stromal lymphopoietin; TSLP-R, TSLP receptor.

recently have some of the molecular pathways activated by fungi leading to the characteristic type 2 inflammation been described.

## FUNGAL-INDUCED TYPE 2 INFLAMMATION

Our understanding of the pathophysiology of fungi-mediated chronic airway inflammation is complex and rapidly evolving. Current research suggests that altered mucosal barrier physiology, fungi-activated molecular signaling, and an exaggerated type 2 immune response rep-

resent some of the primary drivers of airway inflammation in response to fungus, especially in certain subtypes of CRS (i.e., AFRS, CRSwNP) and allergic asthma.<sup>21,22</sup> The following sections will review salient basic science research supporting a role for fungi-mediated chronic airway inflammation, summarized in Figure 2.

## Mucosal barrier dysfunction

The respiratory mucosa of the sinuses and lower airways, its epithelium, and a diversity of proteins and peptides make up a constitutive line of defense in the prevention

of pathogen growth, infection, and immune stimulation in the airway. This innate barrier function seems to be altered in chronic airway inflammation.<sup>23–26</sup> Studies have found a higher recovery of positive fungal cultures from patients with type 2 airway disease, including CRSwNP and AFRS, suggesting there exists a molecular environment in the mucosa that is permissive for fungal overgrowth.<sup>27</sup> Patients with AFRS demonstrate reduced expression of antimicrobial peptides, including surfactant protein (SP)-D, lactoferrin, and a family of antifungal peptides, the histatins.<sup>28–30</sup> In vitro studies have shown that AFRS epithelial resistance is lowered when compared to controls, which may be the result of increased expression of type 2 inflammatory cytokines, such as IL-4 and IL-13.<sup>31,32</sup> Defective barrier function has also been explored as a mechanism of chronic airway inflammation in allergic asthma.<sup>23–25,33</sup> Altered expression of tight junctions has been proposed as a model for lung injury and allergic asthma.<sup>23,34–36</sup> As an example, claudin 18.1 is a lung-specific tight junction protein that is deficient in T helper 2 (Th2)-high patients with asthma, and is associated with significantly elevated immunoglobulin E (IgE) levels in claudin-18 null mice.<sup>37</sup> Additionally, biologically active fungal proteases, including the Pen c 13 allergen secreted by *Penicillium citrinum*, causes junctional structure alterations and increased epithelial permeability.<sup>38</sup> Determining if epithelial integrity compromise is the result of chronic type 2 inflammation, biologically active fungal molecules, or a combination of both, represents an important area of future research endeavors. Regardless of the initiating event, the loss of epithelial barrier integrity appears to be permissive for a “positive feedback loop” in fungal-mediated type 2 chronic airway inflammation.

## The role of respiratory epithelial signaling in fungi-mediated airway inflammation

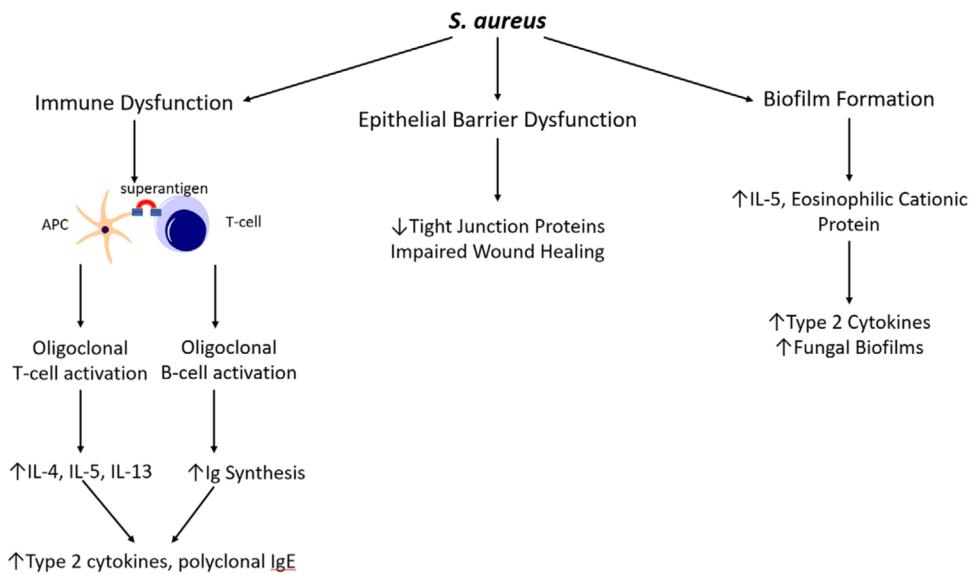
The respiratory epithelium represents an integral mediator of type 2 inflammation in response to fungi in CRS and allergic asthma. Several immune receptors that interact directly with fungal molecules are expressed on respiratory epithelium. These have been reviewed in detail elsewhere.<sup>39</sup> Briefly, these include pattern recognition receptors (PRR), including toll-like receptors (TLRs), c-type lectin receptors (CLRs), receptor for advanced glycation end products (RAGE), and protease activated receptors (PARs).<sup>39</sup> In response to fungi, the respiratory epithelium of the sinuses and lower airway release innate inflammatory cytokines (also referred to as epithelial-derived cytokines [EDCs]), including IL-33, thymic stromal lymphopoietin (TSLP), and IL-25, all of which potentiate type 2 inflammation.<sup>40–47</sup> For instance, *Alternaria* extract insti-

gates rapid airway release of IL-33 and an asthma exacerbation phenotype in a mouse model of reactive airway disease.<sup>45</sup> Using sinonasal epithelial cell cultures, both *Alternaria a* and *Aspergillus f* extracts stimulated solitary chemosensory cell expansion and IL-25 release.<sup>47</sup> Proteases from *Aspergillus* sp. can activate the innate immune response, inducing IL-4, IL-5, IL-13 production, eosinophilia, and airway goblet hyperplasia, mediated partially by cleavage of fibrinogen, which signals through TLR4.<sup>48</sup> Furthermore, *A. fumigatus* extract is capable of stimulating IL-33 production in epithelial cells derived from CRSwNP.<sup>42,43</sup> Dietz et al.<sup>42</sup> demonstrated that IL-33 expression could be triggered in CRSwNP-derived epithelium from *A. fumigatus* protease in a manner dependent on protease-activated receptor 2 (PAR-2) activity. Taken together, these studies highlight the importance of respiratory epithelium and associated EDCs in fungi-mediated type 2 airway inflammation.

## Group 2 innate lymphoid cells

T helper 2 cells have classically been recognized as the main effector cells of type 2 inflammation. More recently, increasing emphasis has been placed on the importance of innate lymphoid cells (ILCs), which are capable of orchestrating type 2 inflammation without antigen priming, and thus represent a more instantaneous source of type 2 inflammatory signaling in the airway.<sup>39,49,50</sup> There are three major subsets of innate lymphoid cells: group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s). ILCs are the innate cell counterparts of T lymphocytes, and as such, ILC2s represent the innate counterpart of Th2 cells. Expressing receptors to IL-25, IL-33, and TSLP, ILC2s can be activated by these cytokines to express type 2 inflammatory cytokines, including IL-13, IL-5, IL-4, and IL-9.<sup>50,51</sup>

ILC2s have emerged as an important cell type capable of eliciting type 2 inflammation in response to fungal stimulation both in CRS and asthma.<sup>39,52,53</sup> Mice challenged intranasally with *Alternaria* extract induce ILC2 expansion, eosinophilia, Th2 recruitment, and Th2 proliferation.<sup>54</sup> Furthermore, *Alternaria* can stimulate increased IL-33, followed by IL-5, IL-13, and airway eosinophilia in mice lacking T or B cells.<sup>53</sup> In a mouse model of allergic asthma, inhaled chitin stimulated production of IL-33, IL-25, and TSLP, which activated ILC2 expression of IL-13 and IL-4.<sup>55</sup> Shaw et al.<sup>43</sup> also demonstrated that IL-33-responsive ILC2s are present in CRSwNP, and that several different fungal antigens could promote IL-33 expression in epithelium derived from CRSwNP. In addition, ILC2s can promote expansion of Th2 memory and adaptive immune signaling, indicating



**FIGURE 3** Proposed mechanisms of *S. aureus* mediated amplification of type 2 inflammation. *S. aureus*, capable of forming biofilms, release numerous enzymes and exotoxins which can cause epithelial barrier dysfunction and enhance immune dysfunction activated by fungi. Abbreviations: APC, antigen presenting cell; Ig, immunoglobulin; IL, interleukin

a potential link between fungi-mediated ILC2 innate and adaptive immunity in chronic airway inflammation.<sup>56–58</sup> Taken together, fungi promote type 2 inflammation in the airway, in part, by stimulating the expression of important EDCs, including IL-33, IL-25, and TSLP, which in turn activates ILC2s expression of type 2 inflammatory cytokines.

## The role of adaptive immunity

Both innate and adaptive immune signaling together contribute to the type 2 inflammation characteristic of CRS and allergic asthma, especially eosinophilic subtypes.<sup>30,59,60</sup> Indeed, B cell and plasma cell expansion, local activation, class switch recombination, and antibody production occur within follicle-like structures in nasal polyps.<sup>61–63</sup> Whole genome analysis performed in AFRS identified nearly 3000 unique gene expression variations that differentiated it from CRSwNP, many of which were genes associated with adaptive immunity, including those involved in antigen-sensing pathways, co-stimulatory signaling, and T-cell receptor signaling.<sup>60</sup> Pant and Macardle<sup>64</sup> demonstrated AFRS tissue possesses deficient CD8+ T-cell response to fungi, suggesting that this alteration may permit local fungal accumulation, resulting in an inflammatory response characterized by a localized allergic fungal hypersensitivity. Porter et al.<sup>27</sup> also demonstrated that both CRSwNP and AFRS patients exhibit IgE-specific fungal reactivity and enhanced IL-4 memory recall from patients' peripheral blood mononuclear cells

(PBMCs) stimulated with fungal antigen, suggesting that the adaptive immune arm contributes to propagating fungal stimulated inflammation. Therefore, it is likely that in severe chronic airway inflammation, there is a complex and potentially synergistic interplay between respiratory epithelium, ILC2s, and adaptive immune B-cells and T-cells, which perpetuate type 2 inflammation in response to the presence of fungus.<sup>65</sup>

## Interaction between fungi and *Staphylococcus aureus* superantigen

*Staphylococcus aureus* has long been associated with pathophysiology of CRSwNP. Figure 3 highlights several of these mechanisms. Although commonly identified in nasal cavities, colonization of sinonasal mucosa with *S. aureus* has been reported higher in CRSwNP and AFRS patients.<sup>66–68</sup> In a meta-analysis, an odds ratio (OR) of 4.85 (95% confidence interval [CI], 1.80–13.05) of positive *S. aureus* culture in CRSwNP compared to control was reported.<sup>67</sup> An intriguing possible interaction between bacteria and fungi was proposed based upon histopathologic observations of sinonasal mucosa from eosinophilic CRS patients, which noted a bacterial presence in 17 of 22 specimens with fungal colonization versus in only five of 22 specimens without fungal colonization.<sup>69</sup> Similar bacterial-fungal co-occurrences were associated with asthma with characteristic bacteria-fungal microbiota profiling associated with asthma phenotypes.<sup>11</sup> Investigating the types of interactions between bacteria and fungi

important in CRS, Lee et al.<sup>18</sup> demonstrated synergistic interactions in growth and virulence between *Malassezia sympodialis* (a dominant fungal taxa of sinus microbiome) and *S. aureus*, and induction of type 2 and type 17 cytokines within sinonasal mucosa of mice co-infected intranasally.

Capable of producing multiple virulence factors including enzymes and exotoxins, *S. aureus* is effective in evading the host immune response while concurrently activating a nonspecific inflammatory response. *S. aureus* is capable of producing exotoxins, which exhibit the ability to act as superantigens. Rather than being processed and presented to T cell receptors (TCRs) on T cells by antigen-presenting cells via major histocompatibility complex (MHC) class II, superantigens can cause polyclonal activation of numerous T cells by binding the TCR and MHC II molecule directly. Through this mechanism, rather than activating the typical <0.01% of T cells as processed antigens, *S. aureus* superantigens can activate up to 30% of the T cell population.<sup>70,71</sup> In the presence of fungi that skews toward a type 2 immune response, *S. aureus* enterotoxin (SAE) further stimulates the type 2 immune response. T cells are skewed toward Th2 cells. *Staphylococcus* superantigens also activate B cells and in combination with type 2 cytokines drive polyclonal IgE expression.

## Antifungal activity of eosinophils in fungi-induced inflammatory airway diseases

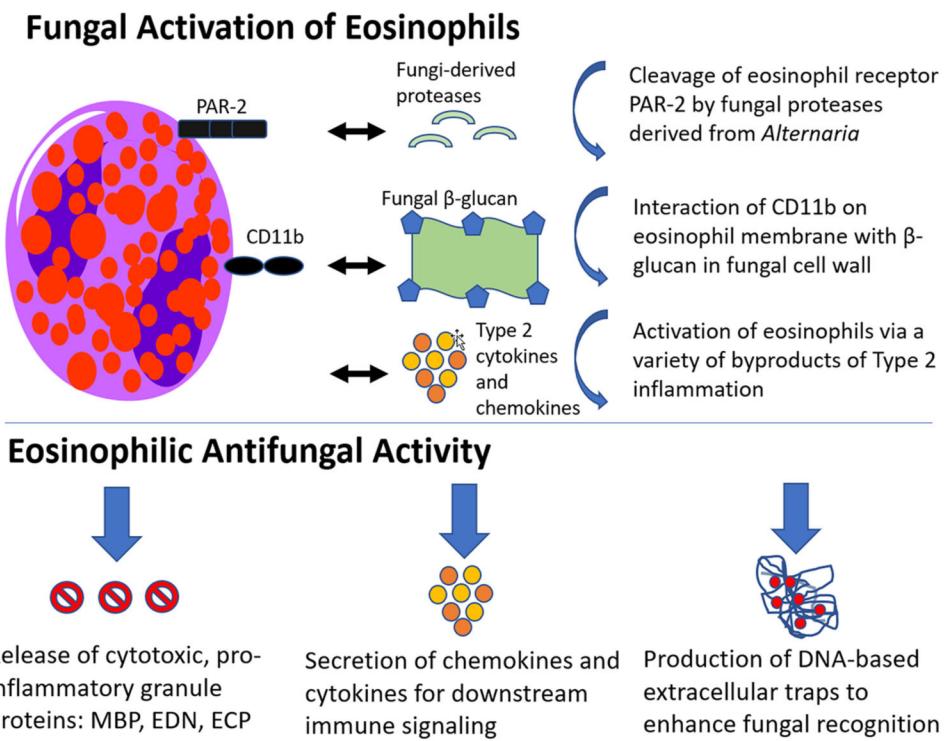
With activation of the innate and adaptive immunity, effector immune cells serve as downstream end products that simultaneously enhance host defenses against fungi to prevent tissue invasion but also contribute to the immunopathology of inflammation. Eosinophils are important effector immune cells that provide protection against pathogenic infections not only by fungi, but also by parasites, bacteria, and viruses.<sup>72</sup> Eosinophils, characterized by an abundance of intracellular crystalloid-bearing granules, provide a robust source of highly protective and pro-inflammatory proteins, such as major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and eosinophil peroxidase. Upon their release, these granule proteins are implicated in the eosinophils' cytotoxic activities toward infectious pathogens. Furthermore, an array of cytokines, chemokines, and growth factors are also secreted by eosinophils, which allow for cellular signaling and the coordination of other immune cells. The influx of eosinophils into inflamed tissue sites nonetheless is associated with the detrimental effects of tissue damage and remodeling, as evident in chronic inflammatory diseases of the airway. Elevated levels of local and systemic

eosinophils are thus common characteristic features of chronic inflammatory airway diseases related to fungal exposure.<sup>73</sup>

The immunologic mechanism by which eosinophils exert an innate immune response to fungal products is generally poorly understood, but the current evidence highlights the capacity of eosinophils to recognize common environmental fungi like *A. alternata* through various cellular surface receptors. In 2008, Yoon et al.<sup>74</sup> demonstrated in vitro that eosinophilic activity is induced when human eosinophils are incubated with *A. alternata* spores and hyphae. The group also underscored the critical role that CD11b, a β-2 integrin adhesion molecule expressed by eosinophils, in mediating contact with fungal β-glucans found in their cell walls, thereby initiating fungicidal activity of eosinophils. Other studies have additionally reported the importance of *Alternaria* proteases in the activation of eosinophilic inflammation by cleaving the PAR-2 on the eosinophil surface membrane.<sup>75</sup>

In addition to *Alternaria*, *A. fumigatus* has also been shown in murine models to stimulate the inherent antifungal activity of eosinophils. In a study by Lilly et al.,<sup>76</sup> eosinophil-deficient mice acutely challenged with *Aspergillus* were found to exhibit impaired fungal clearance and increased fungal germination in the lungs. This antifungal activity of eosinophils against *Aspergillus* occurred through pro-inflammatory cytokines and chemokines, as opposed to direct cell contact between fungal products and eosinophils.<sup>77</sup> Chitin, a component of the fungal cell wall, induces through an unknown molecular mechanism causing increased pulmonary eosinophilia and transcription of Th2-associated chemokines in mice.<sup>76</sup> Additional investigations are necessary to comprehensively elucidate the immunophysiologic mechanisms underlying eosinophilic antifungal activity.

Recent research on understanding eosinophil mediated immunity against fungi has focused on production and release of DNA-based extracellular traps (ETosis).<sup>72,78</sup> ETosis refers to an innate immune process in which various leukocytes, including neutrophils, mast cells, and macrophages extrude nuclear cellular components to create networks of filamentous and decondensed chromatin that bind, immobilize, and potentially eliminate microbial pathogens. The use of extracellular traps (ETs) is recognized as a method for immune cells to enhance microbial recognition and killing. Eosinophils are capable of forming eosinophil extracellular traps (EETs), which are released with granule proteins into the extracellular matrix in response to bacteria and other stimuli. In 2018, Muniz et al.<sup>78</sup> demonstrated that *A. fumigatus* induces the release of EETs in vitro into the bronchial secretions of patients with ABPA. Although the identified EETs in the ABPA mucus lacked fungicidal or fungistatic activities, the group



**FIGURE 4** Antifungal effects mediated by eosinophils in chronic rhinosinusitis. The stimulation of eosinophils in the immunologic response to fungal exposure is postulated to rely on several pathomechanisms, including the activation of the PAR-2, CD11b, and other cellular receptors by various fungal components and upstream immunologic signaling mediators. Once the eosinophils are activated, they contribute to antifungal response through the release of cytotoxic granule proteins, such as MBP, EDN, and ECP. Other effects from eosinophils include the formation of eosinophilic extracellular traps, which enhance microbial recognition and killing, and the secretion of cytokines and chemokines to further effect downstream immunologic signaling. Abbreviations: ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; MBP, major basic protein; PAR-2, protease-activated receptor 2

speculated that the excessive release of EETs in ABPA may contribute to the formation of the sticky and viscous mucus that characterizes this pulmonary disease (Figure 4).

### Future directions in understanding immune response to fungi and chronic inflammatory respiratory disease

Indeed, several important research topics in fungi-mediated airway inflammation deserve closer attention. A better understanding of the specific fungi-receptor interactions is warranted. What are the important epithelial cell surface receptors responsible for mediating epithelial cell-derived cytokine release in response to fungi? Furthermore, is this interaction species-specific? Additional important research studies will uncover the relative contributions of innate and adaptive immune arms in maintaining chronic and severe type 2 airway inflammation in response to fungal stimulation. Are ILC2s important for instantaneous airway inflammation and

Th2 cells important for maintaining chronicity? Is there redundancy in these pathways? Or, is there cross-talk between pathways that amplifies a type 2 inflammatory response? Of additional interest is gaining an understanding of the molecular environment in the sinuses and lower airway that is permissive for fungus progression through its life cycle, from spore to the generation of fungal hyphae. Furthermore, does the immune response differ at different stages of the fungi's life cycle? Given the antifungal activities of eosinophils, what are the effects, if any, on fungal load and/or mycobiome associated with therapeutics that have led to depletion of eosinophils, including anti-IL5 biologics? Further studies are indicated in appropriate mouse models of airway disease, which also represents another important area of future research. In comparison to allergic asthma, there is a relative paucity of animal models available to study chronic rhinosinusitis.<sup>79–81</sup> Thus, a mouse model of fungi-mediated sinusitis is greatly needed to better elucidate the role of fungi in the pathophysiology of CRS.

## SUMMARY

Certainly, the role of fungus in chronic respiratory inflammation and infection has been controversial in the past, but advances in microbial isolation techniques, coupled with a better understanding of the underlying inflammatory pathways that contribute to fungal-mediated disease burden, have provided a clearer picture of fungi's role in chronic airway disease. Still, more work is needed. Future research endeavors applied toward a more detailed understanding of the respiratory microbiome (and mycobiome), and the important associated inflammatory pathways, will result in more sophisticated, personalized therapies for patients suffering from the variety of airway conditions mediated by the presence of fungus.

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## CONFLICTS OF INTEREST

Martin J. Citardi serves as a consultant for Acclarent (Irvine, CA, USA), Intersect ENT (Palo Alto, CA, USA), Medical Metrics (Houston, TX, USA), and Stryker (Kalamazoo, MI, USA). Amber U. Luong serves as a consultant for Aerin Medical (Austin, TX, USA), Lyra Therapeutics (Watertown, MA, USA), Sanofi (Paris, France), GlaxoSmithKline (Brentford, UK) and Stryker (Kalamazoo, MI, USA); she also serves on the advisory board for ENTvantage Dx (Austin, TX, USA).

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