



Airway mycosis in allergic airway disease

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Abstract

The allergic airway diseases, including chronic rhinosinusitis (CRS), asthma, allergic bronchopulmonary mycosis (ABPM) and many others, comprise a heterogeneous collection of inflammatory disorders affecting the upper and lower airways and lung parenchyma that represent the most common chronic diseases of humanity. In addition to their shared tissue tropism, the allergic airway diseases are characterized by a distinct pattern of inflammation involving the accumulation of eosinophils, type 2 macrophages, innate lymphoid cells type 2 (ILC2), IgE-secreting B cells, and T helper type 2 (Th2) cells in airway tissues, and the prominent production of type 2 cytokines including interleukin (IL-) 33,

IL-4, IL-5, IL-13, and many others. These factors and related inflammatory molecules induce characteristic remodeling and other changes of the airways that include goblet cell metaplasia, enhanced mucus secretion, smooth muscle hypertrophy, tissue swelling and polyp formation that account for the major clinical manifestations of nasal obstruction, headache, hyposmia, cough, shortness of breath, chest pain, wheezing, and, in the most severe cases of lower airway disease, death due to respiratory failure or disseminated, systemic disease.

The syndromic nature of the allergic airway diseases that now include many physiological variants or endotypes suggests that distinct endogenous or environmental factors underlie their expression. However, findings from different perspectives now collectively link these disorders to a single infectious source, the fungi, and a molecular pathogenesis that involves the local production of airway proteinases by these organisms. In this review, we discuss the evidence linking fungi and their proteinases to the surprisingly wide variety of chronic airway and systemic disorders and the immune pathogenesis of these conditions as they relate to environmental fungi. We further discuss the important implications these new findings have for the diagnosis and future therapy of these common conditions.



1. Fungi in diseases of the upper and lower airways

Many distinct clinical syndromes have been linked, if inconsistently, to allergic inflammation as defined by the presence of circulating and tissue eosinophils and/or elevated serum IgE levels. Only more recently have these same syndromes been consistently linked to airway mycosis, a form of superficial infection of the airway by fungi. In this section, we review the association between diverse chronic diseases of the upper and lower airways and fungi and argue that these disorders share the same underlying fungal-based pathophysiology, whether the fungal involvement is primary or secondary. We further argue that in many instances of primary fungal-based airway disease, occult underlying immunodeficiency is a major contributor to disease expression (Fig. 1).

1.1 Fungal-related chronic rhinosinusitis

Chronic rhinosinusitis (CRS) describes a heterogeneous syndrome defined by inflammation of the sinonasal mucosa outlining the paranasal sinuses and nasal cavity. Phenotypically, CRS is categorized by the absence or presence of nasal polyps (Fig. 2). Clinically, CRS affects over 35 million people in America and represents a societal cost of over \$12.8 billion annually, which includes direct healthcare expenditures and indirect costs related to loss of productivity (DeConde & Soler, 2016). Symptoms attributable to CRS

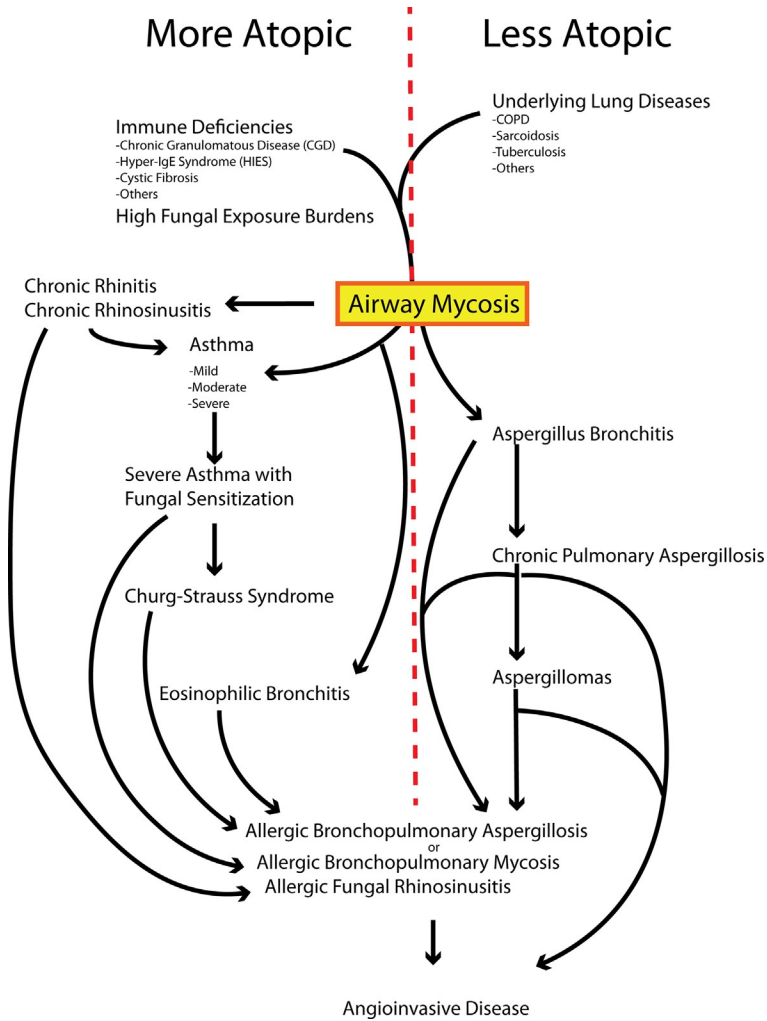


Fig. 1 Spectrum of airway mycosis-related airway diseases (AMRADs). All airway disorders involving primarily or secondarily airway mycosis begin with the inhalation of fungal conidia or yeast forms. Subsequent development of symptomatic disease, if any, depends importantly on modifying factors that may be intrinsic to the host or largely the result of environmental influences. A large number of AMRADs are primary in nature, involving innate or acquired immune deficiency involving the inability to efficiently eliminate incipient airway mycosis. This issue, potentially combined with excessive exposure to environmental conidia, leads to primarily atopic disorders such as allergic asthma, CRSwNP, Churg-Strauss syndrome, and ABPM. Conversely, airway mycosis can develop primarily as a result of pre-existing structural lung disease that results from prior tuberculosis, COPD, and other conditions. This secondary form of airway mycosis presents with atopic features either late in the disease process or not at all. Regardless of the origin of the airway mycosis, AMRADs tend to evolve similarly, developing into advanced ABPM and rarely terminal angioinvasive disease.

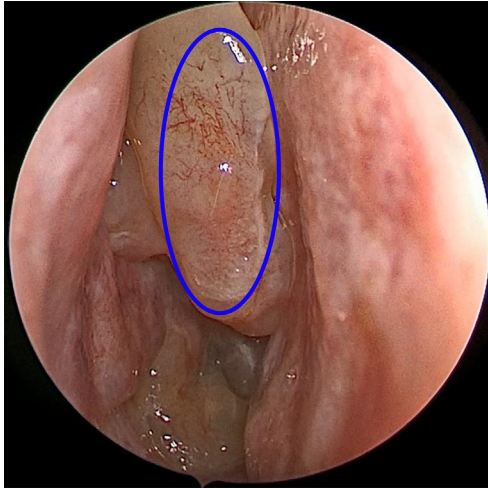


Fig. 2 Nasal polyp in CRS. Pictured is the nasal cavity of a patient with CRS complicated by nasal polyposis (blue circle) completely obstructing the lumen.

including headache, nasal congestion, loss of the sense of smell, lethargy, and cough markedly impair patients' quality of life and contribute to an overall decrease in general health. CRS patients therefore frequently seek medical treatment. However, with the heterogeneity of CRS combined with a poor understanding of its pathophysiology render the common treatment options of topical or oral corticosteroids and sinus saline irrigations minimally effective and no current treatment options are curative.

The chronic inflammation of the paranasal sinuses in CRS is analogous to asthma affecting the lungs. In addition to the physical link between the upper and lower respiratory organs via the pharynx and trachea, the sinuses and lungs share similar direct environmental exposures and tissue types, especially regarding the airway epithelium. Consequentially, there is significant co-morbidity between asthma and CRS with approximately 80% of asthmatic patients being diagnosed with a concurrent upper respiratory inflammatory diagnosis and 60–80% of CRS patients have asthma ([Promsopa et al., 2016](#); [Spector et al., 1998](#)). Histologically, ciliated pseudo-stratified respiratory epithelial cells are the initial barrier for both the sinuses and lungs to the environment. With similar exposures, these respiratory epithelial cells within both the upper and lower airway orchestrate like immune responses. Not surprisingly then, CRS, specifically CRS with nasal polyps (CRSwNP), and asthma have related epidemiology and immune

pathophysiology characterized by elevated local tissue levels of eosinophils, mast cells and T helper type 2 (Th2) cells and associated cytokines such as interleukin (IL)-4, IL-5, and IL-13 (Tomassen et al., 2016). Therefore, CRS serves as an excellent disease model for understanding the pathophysiology of asthma and vice versa.

The pathophysiology of CRS initially focused on environmental triggers such as allergens and microbes as etiologic agents. In 1999, Ponikau et al. suggested that fungal exposure was almost universal in CRS and subsequent studies further suggested that immune responses to fungi initiated the inflammation found in most CRS (Ponikau et al., 1999; Shin et al., 2004). Prior to these studies, fungi were rarely identified within sinus cavities. By treating mucus with a reducing agent prior to culture, these investigators cultured fungi from 96% of CRS patients and 100% of normal controls, raising questions as to the specificity of identifying fungi from airway samples even when using an optimized culture method. However, CRS patients demonstrated T cell memory recall responses to fungi most commonly identified from the sinuses, with induction of IFN- γ , IL-5 and IL-13 from peripheral blood mononuclear cells stimulated with fungal extracts, particularly that of *Alternaria alternata*. In contrast, fungus-specific IL-5 and IL-13 recall responses were absent in non-CRS normal control subjects and neither CRS nor control subjects exhibited IgE specific for any of the fungi tested. Based primarily on these studies that did not distinguish CRS by the absence or presence of nasal polyps, they concluded that fungus-specific cellular, and not humoral, immune responses were the primary drivers of inflammation found in CRS (Shin et al., 2004).

In light of the non-specific culture results of these studies, many other investigators began to challenge these findings. The most compelling arguments against the overarching role of fungi in CRS pathophysiology were based on the generally negative results of clinical trials that were designed to test the efficacy of oral and topical antifungals as treatment in CRS (Head, Sharp, Chong, Hopkins, & Philpott, 2018). Designed to test the hypothesis that fungi are the main drivers of inflammation found in all forms of CRS, these trials did not limit the inclusion criteria enrolling all CRS subtypes, with or without nasal polyps or fungal sensitivity. A majority of these clinical trials showed no symptomatic improvement with antifungals over placebo, providing the primary basis for the consensus view that antifungals should not be used in the standard treatment of CRS (Head et al., 2018; Orlandi et al., 2016). However, several smaller trials with criteria limiting enrollment

to CRSwNP patients that demonstrate fungal sensitivity showed positive effects to oral antifungals (Chan, Genoway, & Javer, 2008; Patro, Verma, Panda, Chakrabarti, & Singh, 2015; Rains & Mineck, 2003; Seiberling & Wormald, 2009). Rather than a ubiquitous trigger for all sinus mucosal inflammation, these studies suggested that the role of fungi in the pathophysiology of CRS is selective, pertinent specifically to patients with CRSwNP.

Allergic fungal rhinosinusitis (AFRS) is one such CRSwNP phenotype that highlights the role of fungi in the local Type 2 immunopathology associated with allergic chronic respiratory inflammation. AFRS is clinically defined by the presence of nasal polyps, fungal hypersensitivity as noted by elevated fungal specific IgE levels and the presence of thick mucus infiltrated with high levels of eosinophils and fungal debris within sinus cavities but without evidence of fungal tissue invasion (Bent & Kuhn, 1994). Peripheral blood mononuclear cells from AFRS patients challenged with fungal extracts demonstrated a predominant Th2 cell memory recall response with elevated IL-4 and IL-5 levels not elicited from non-CRS normal controls, without significant expression of Th1 cytokines to the fungal extract (Luong, Davis, & Marple, 2009).

The issue of non-specificity regarding isolation of fungi from upper airway specimens was finally resolved by the culturing, using again an optimized technique involving mucolysis, surgically-obtained maxillary sinus lavage fluid, avoiding the nasal passage, which essentially always contains culturable fungi (Ponikau et al., 1999). We demonstrated that filamentous fungi are significantly more commonly cultured from patients with CRSwNP or AFRS as compared to control patients with neither of these conditions (74% vs 16%, respectively) (Porter et al., 2014). This study was extraordinary because we were able to include a control group undergoing maxillary sinus surgery to address issues involving the central nervous system, but having normal maxillary sinuses. We further demonstrated that fungus-specific memory T helper cell type 2 (Th2) recall responses are highly specific and sensitive to having a diagnosis of CRSwNP, as opposed to IgE responses that lack both sensitivity and specificity.

Thus, the demonstration of unique CRS phenotypes that associate with airway fungi, fungus-specific Th2 memory recall responses in the same patients, and positive responses to antifungals involving CRSwNP patients together support a central role for fungi as drivers of the inflammation and clinical expression of CRSwNP.

1.2 Asthma

Asthma is a heterogeneous disease entity defined by the presence of persistent lung inflammation leading to symptoms of cough, shortness of breath, wheezing, chest tightness, with variable degrees of airway obstruction and hyperreactivity (Chung et al., 2014). Despite marked advances in our understanding of pathophysiology, asthma remains a poorly understood disease. Recent attempts to categorize asthma into distinct subsets have yet to lead to significant changes to clinical practice. There are currently four validated pathophysiologically distinct subsets, or endotypes, although a variety of other phenotypes have been proposed. These include pauci-granulocytic (<3% sputum eosinophils, <64% sputum neutrophils), eosinophilic (>3% sputum eosinophils, <64% sputum neutrophils), neutrophilic (<3% sputum eosinophils, >64% sputum neutrophils) and mixed granulocytic (>3% eosinophils, >64% sputum neutrophils) asthma (Svenningsen & Nair, 2017). Four validated phenotypes based upon these endotypes are (1) early onset, mild asthma, with good lung function and high Th2 inflammation; (2) moderate, persistent asthma with mild airway obstruction, sputum eosinophilia and high Th2 inflammation; (3) mixed asthma severity with fixed lung obstruction, sputum neutrophilia and low Th2 inflammation; and (4) severe uncontrolled, severe reversible obstruction, mixed granulocytic, with moderate Th2 inflammation (Loza et al., 2016). There is currently a strong body of evidence associating fungal sensitivity and eosinophilia to severe asthma with neutrophilia being more associated with bacterial infection or colonization (Caillaud, Leynaert, Keirsbulck, Nadif, & Awg, 2018; Fairs et al., 2010; Farrant, Brice, Fowler, & Niven, 2016; Goh et al., 2017; Medrek, Kao, Yang, Hanania, & Parulekar, 2017). Airway fungal load has also been shown to lead to more severe disease in non-atopic patients (Dannemiller, Gent, Leaderer, & Peccia, 2016). However, fungal mediated asthma is known to be steroid refractory and often severe, a characteristic shared with neutrophilic asthma. Sputum inflammatory profiles in asthma moreover are not stable and airway mycosis likely can cause both neutrophilia and eosinophilia at different times during the course of a person's disease (Fleming, Tsartsali, Wilson, Regamey, & Bush, 2012; Majewski, Ciebiada, Domagala, Kurmanowska, & Gorski, 2015).

We demonstrated this phenomenon in wild-type mice that were challenged intranasally with different doses of the common fungus *Aspergillus niger* (Porter, Fields, et al., 2011). Challenged over a very wide dose range of inhaled conidia, we showed that currently recognized asthma

immune endotypes, including eosinophil-predominant and neutrophil-predominant, are readily reproduced in mice with this single agent (Porter, Fields, et al., 2011). Thus, airway mycosis could be a major driver of even severe, neutrophilic asthma, mediated by immune mechanisms that do not manifest as eosinophilia or specific fungal IgE sensitization (Fei et al., 2011; Fukahori et al., 2014; Hadebe et al., 2015; Lathrop et al., 2014; Valladao, Frevert, Koch, Campbell, & Ziegler, 2016).

Since airway mycosis and fungal sensitivity are currently greatly underestimated by current methods of testing, it is likely that fungal-induced neutrophilia could continue undetected for years and potentially be exacerbated by inhaled and systemic corticosteroids (Fraczek et al., 2018; Li et al., 2018). In mice, predominantly neutrophilic allergic airway disease was only seen in the context of extremely high fungal airway burdens and marked a critical change in the cytokine phenotype from predominant type 2 to type1/17 cytokine secretion, a phenomenon we termed “immune phenotype switching.” Murine neutrophilic allergic airway disease was further a harbinger of invasive aspergillosis marked by dissemination to the brain (Porter, Fields, et al., 2011).

Not surprisingly then, severe asthma patients demonstrate the highest rate of sensitization to fungi as compared to other subsets (Masaki et al., 2017) and fungal sensitization is associated with overall higher IgE, the presence of multi-allergen sensitivity, frequent exacerbations, systemic corticosteroid dependence, increased intensive care utilization, and increased need for mechanical ventilation (Dannemiller et al., 2016; Medrek et al., 2017). Despite growing evidence of the causal effect of fungi in asthma pathogenesis, recognition of airway mycosis as a fundamental driver of asthma persistence and progression is low (Porter, Onger, Luong, Kheradmand, & Corry, 2011). Nonetheless, several retrospective and small, randomized prospective trials have shown that oral terbinafine, triazole antifungals, and even nebulized amphotericin are effective in the treatment of SAFS (Chishimba, Langridge, Powell, Niven, & Denning, 2015; Chishimba, Niven, Cooley, & Denning, 2012; Pasqualotto, Powell, Niven, & Denning, 2009; Rodriguez-Goncer, Niven, & Denning, 2017; Ward, Woodfolk, Hayden, Jackson, & Platts-Mills, 1999).

In contrast, two major randomized, prospective placebo controlled trials assessing the efficacy of systemic antifungals for the treatment of fungal sensitive asthma had markedly different outcomes, with the first trial (FAST study) showing itraconazole to be effective in improving scores on the Asthma Quality of Life Questionnaire (AQLQ) in patients with severe

asthma with fungal sensitization (SAFS) (Denning et al., 2009) and the second trial (EVITA-3) showing voriconazole to be no better than placebo (Agbetile et al., 2014).

The disparate results of these studies contribute to the existing controversy over whether or not antifungals should be utilized in the treatment of asthma. However, the study designs were not identical and key differences in patient enrollment and other factors likely directly led to their respective outcomes. The EVITA-3 study included significantly older patients, more of whom were dependent on systemic corticosteroids and had higher total serum IgE blood eosinophil levels. The EVITA-3 patients also received markedly less treatment as compared to FAST (3 months for EVITA-3 vs 8 months for FAST). These differences highlight the fact that the EVITA-3 trial enrolled older, sicker patients who received a shorter course of antifungal therapy than the subjects from the FAST trial, potentially explaining the apparent lack of efficacy seen in the EVITA-3 trial. A significant percentage of EVITA-3 enrollees further had significant smoking histories, likely precluding their ability to show improvement in airway obstruction, a key study objective, regardless of how well the antifungal therapy otherwise performed.

Evidence was also cited by the authors of EVITA-3 that itraconazole (used in the FAST study) inhibits corticosteroid metabolism, leading to its persistence in the body as the reason for the apparent positive results of the FAST trial (Lebrun-Vignes et al., 2001; Varis, Kivisto, Backman, & Neuvonen, 1999, 2000). However, while no in-depth studies of the impact of voriconazole on steroid metabolism have been conducted, case reports have indicated that voriconazole has effects on corticosteroid metabolism similar to itraconazole (Duman & Fulco, 2017; Jones, Chastain, & Wright, 2014). Furthermore, numerous other studies have shown that ketoconazole, fluconazole and posaconazole reduce corticosteroid metabolism when administered concurrently (Hoover, Britton, Gardner, Jackson, & Gutierrez, 2011; Pilmis et al., 2017; Seidegard, Nyberg, & Borga, 2008, 2012), strongly suggesting that this effect is likely common to all triazole antifungals, including voriconazole. Both studies also enrolled patients on daily systemic prednisolone, metabolism of which is uniquely unaffected by itraconazole (Lebrun-Vignes et al., 2001; Varis, Kivisto, & Neuvonen, 2000). The serum concentration of voriconazole has also been shown to decrease as a result of increased metabolism by an enzyme induced by concurrent corticosteroid use, an effect that has not been reported for itraconazole (Dolton et al., 2012; Hashemizadeh et al., 2017; Imataki,

Yamaguchi, Uemura, & Fukuoka, 2018). Thus, confounding by complex drug-drug interactions has the potential to significantly affect outcomes of antifungal trials in asthma and represents a key issue to be addressed as part of any future clinical trials of antifungals in asthma.

1.3 Other allergic airway disease syndromes

The airway eosinophilia syndromes are defined by BAL differentials containing at least 25% eosinophils and encompass diverse conditions in which airway mycosis may be a major driver (Philitt et al., 2002). The major pulmonary eosinophilia syndromes include lymphoid and myeloid hyper-eosinophilic syndromes (HES) that are usually pre-malignant or frankly malignant disorders, helminthic infections, allergic drug reactions, adverse reactions to inhaled toxins, eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome), and ABPA. Beyond these syndromes with well-defined criteria, all other cases are considered either acute or chronic idiopathic eosinophilic pneumonia (CIEP) (Sade, Mysels, Levo, & Kivity, 2007). The acute form of eosinophilic pneumonia is more fulminant, often presenting with respiratory failure, frequently resolving spontaneously. Reports of asthma preceding CIEP or CIEP patients eventually manifesting asthma have been described, implying that CIEP may simply be a more severe form of eosinophilic asthma (Marchand et al., 2003).

The approximate upper limits for bronchoalveolar lavage fluid (BALF) eosinophilia of 3% for eosinophilic asthma and 25% for CIEP are arbitrary and like asthma, ABPA and CIEP have been linked to airway mycosis and disseminated fungal disease (Dogra, Sinha, Saxena, & Talwar, 2016; Gupta et al., 2010; Kawayama, Fujiki, Honda, Rikimaru, & Aizawa, 2003; Maturu & Agarwal, 2015; Ogawa et al., 1997; Pacheco et al., 1998; Sade et al., 2007; Swartz & Stoller, 2009). Moreover, eosinophilic granulomatosis with polyangiitis overlaps strongly with regard to clinical features of both asthma and ABPA (Ishiguro, Takayanagi, Takaku, et al., 2016). Thus, asthma, ABPA, eosinophilic granulomatosis with polyangiitis, and CIEP exist as a continuum of the same disease entity linked by airway mycosis. We propose that ABPA, eosinophilic granulomatosis with polyangiitis, and CIEP should be viewed as extreme phenotypes of this airway mycosis continuum, potentially representing the final stage of severity before systemic dissemination of the fungi occurs.

In addition to these classical atopic airway diseases, airway mycosis can present with a less atopic, more indolent form of chronic disease

characterized by initial sputum production and cough that progresses to bronchitis, and in a minority of severely afflicted patients, bronchiectasis, an abnormal widening of the larger conducting airways that reduces the effectiveness of cough for mucus clearance. While pre-existing chronic lung conditions such as asthma and chronic obstructive pulmonary disease (COPD) predispose patients to the development of bronchiectasis, by far the most common subset of adult non-cystic fibrosis-related bronchiectasis is idiopathic (Altenburg, Wortel, van der Werf, & Boersma, 2015; Habesoglu, Ugurlu, & Eyuboglu, 2011; King, Holdsworth, Freezer, Villanueva, & Holmes, 2006). The microbiological correlates of bronchiectasis are frequently bacterial, with necrotizing pneumonias due to *Mycobacterium tuberculosis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and many others leading to chronic, irreversible bronchiectasis. Increasingly, however, airway mycosis is being recognized as a contributor or primary cause of bronchiectasis.

Chronic fungal bronchitis likely reflects a compromised pulmonary milieu where unresolved fungal growth and fungal-induced inflammation drives progressive structural airway damage, chronic sputum production and cough. The subsequent lung damage and bronchiectasis promotes more fungal colonization and impairs clearance, leading to chronic progression in a positive feedback loop manner that can become complicated by subsequent bacterial bronchitis (Niimi & Chung, 2004). It is likely in these cases that both chronic bacterial and fungal infection drive the progressive airway damage and loss of pulmonary function, but the fungal contribution to this disease process is often overlooked and poorly treated. This subset of patients, even if not overtly atopic or sensitized to fungi, with enough airway fungal burden, can develop ABPA or ABPA-like conditions with fungal sensitization (allergic bronchopulmonary mycosis) (Chowdhary et al., 2014; O'Driscoll, Hopkinson, & Denning, 2005; Seki et al., 2014).

In a large retrospective cross-sectional survey of 4232 consecutive sputum samples collected from patients with chronic bronchitis seen at the Firestone Institute for Respiratory Health from 2004 to 2008, 21% of the patients had chronic cough not associated with asthma, COPD or any other pulmonary disease, with 24% of their sputum samples being positive for eosinophils (D'Silva et al., 2011). One Japanese group recovered fungi from sputum of 62.5% of patients with chronic idiopathic cough compared to 17% in patients with cough from other reasons (Ogawa, Fujimura, Takeuchi, & Makimura, 2009). In a follow up study the group found 54% of patients with chronic idiopathic cough to have *Aspergillus fumigatus*

in their sputum (Ogawa, Fujimura, Ohkura, & Makimura, 2014). The same group then demonstrated improvement in the subjective sensation of mucus in the throat after one dose of nebulized amphotericin as well as 2 weeks of low dose oral itraconazole, suggesting fungi as a primary source of chronic cough with sputum even in the absence of clinical bronchitis (Ogawa, Fujimura, Ohkura, & Makimura, 2015; Ogawa, Fujimura, Takeuchi, & Makimura, 2013).

Bronchitis as a clinical entity has always been a loosely defined term characterizing patients with chronic cough and sputum production, but who do not fit neatly into more phenotypically discreet entities such as asthma. Directly connecting bronchitis to asthma is the presence of eosinophils in sputum. Studies have shown a relationship exists between eosinophilic bronchitis with atopic cough and cough variant asthma, with a large proportion of patients with eosinophilic bronchitis eventually developing cough variant asthma. Moreover, sputum eosinophils are found in a large proportion of patients with atopic cough and cough variant asthma (Ayik et al., 2003; Fujimura, Ogawa, Yasui, & Matsuda, 2000; Gibson, Fujimura, & Niimi, 2002; Niimi et al., 1998). The presence of sputum eosinophilia in cough variant asthma also appears to be an excellent predictor of eventual classical asthma (Kim, Kim, Kang, Yoo, & Koh, 2003).

There are nonetheless key differences among this set of clinically similar conditions that stratifies them into separate entities. Eosinophilic bronchitis is characterized by sputum production with eosinophilia without airway hyperreactivity (Ayik et al., 2003), while atopic cough and cough variant asthma are characterized by dry cough with some patients having eosinophilia in induced sputum (Fujimura, Ogawa, Nishizawa, & Nishi, 2003). Atopic cough is differentiated from cough variant asthma by lack of bronchial hyperresponsiveness. However, the effort to compartmentalize these very similar phenotypes based on specific, yet minor differences fails to reflect the likelihood that these conditions represent different stages of most likely the same entity that has a fungal etiology (Corrao, Braman, & Irwin, 1979; Fujimura et al., 2000; Krasnick, Patterson, & Roberts, 1995; Roth & Schatz, 2013).

Aspergillus bronchitis is a distinct entity manifesting as airway mycosis suggestive of ABPA, but not strictly satisfying ABPA diagnostic criteria (Baxter et al., 2013; Brandt et al., 2018; Chrdle et al., 2012; Shoseyov, Brownlee, Conway, & Kerem, 2006). Diagnostic criteria for aspergillus bronchitis include having aspergillus precipitins, fungal culture positivity, cough with tenacious sputum, aspergillus IgE positivity, elevated total serum

IgE, and responsiveness to antifungals, but precise diagnostic algorithms have yet to be defined. This entity perhaps best illustrates the airway mycosis continuum concept as patients with aspergillus bronchitis clinically are indistinguishable from ABPA aside from not meeting all serological requirements. Aspergillus bronchitis can occur in any person with chronic lung disease but has been most studied in patients with cystic fibrosis (CF), who frequently develop ABPA (Chrdle et al., 2012). ABPA in CF presents as a heterogeneous syndrome with patients having sputum with detectable fungal loads and having both, none or either IgE and IgG responses to aspergillus (Baxter et al., 2013). However, patients without detectable fungi in sputum can also present with identical patterns of antibody responses to aspergillus (Baxter et al., 2013). Although the clinical significance of finding fungi in the sputum and airways of CF patients is controversial, small retrospective and prospective studies have shown at least a 50% clinical improvement in CF patients with aspergillus bronchitis after treatment with antifungals (Brandt et al., 2018; Shoseyov et al., 2006). A case series involving patients in long-term care facilities with predominantly *Candida* spp. growing in sputum noted 100% clinical response to oral voriconazole or nebulized amphotericin, illustrating that the spectrum of “aspergillus” bronchitis extends beyond aspergillus and suggesting that this syndrome is better termed fungal bronchitis. Moreover, these results dispel the common assumption that *Candida* spp. and other non-aspergillus fungi detected in sputum are simply harmless bystanders (Johnson, 2012).

1.3.1 Allergic bronchopulmonary mycosis

Aspergillus and other fungal infections of the lung have been recognized and reported sporadically since the mid-1800s, but it was not until 1952 in a published series of eight cases was the term allergic bronchopulmonary aspergillosis (ABPA) proposed to describe three patients with asthma-like allergic respiratory tract disease who had evidence of concomitant fungal airway infections (Hinson, Moon, & Plummer, 1952). To distinguish these patients from many others with apparently distinct pulmonary syndromes that lacked allergic features, strict diagnostic criteria that were based on histological examination of lung sections, sputum cultures, clinical symptoms, radiographic studies, and blood and immunological testing were proposed and are largely still used today.

While strongly associated with asthma and *Aspergillus* spp., ABPA can develop in patients without significant history of atopy and, moreover, is linked to many different fungi, including the yeast *Candida albicans*

(Chowdhary et al., 2014; Oshikata et al., 2017; Saini, Boas, Jerath, Roberts, & Greenberger, 1998; Sandhu, Mehta, Khan, & Singh, 1979; Seki et al., 2014), and is therefore more appropriately termed allergic bronchopulmonary mycosis (ABPM). While genetic predisposition to atopy likely contributes to ABPM in certain asthmatics, some studies have also shown a negative correlation with other atopic diseases such as eczema and allergic rhinitis (Tay et al., 2016). Regardless, in both non-atopic and atopic ABPM patients, it is the enhanced exposure to airway fungal antigens due to overgrowth of airway fungi that eventually leads to fungus-specific hypersensitivity marked by often very high serum total and fungus-specific IgE levels (Bateman, 1994; Patterson, Rosenberg, & Roberts, 1977).

Co-morbid conditions such as tuberculosis, sarcoidosis, COPD, cystic fibrosis, AIDS with complicating airway cavitory lesions due to *Pneumocystis jirovecii* pneumonia can all produce cystic parenchymal pulmonary disease that favors intracavitary fungal growth that ultimately develops into ABPM (Adeyemo, Odelowo, & Makanjuola, 1984; Bafadhel et al., 2014; Galiatsatos, Melia, & Silhan, 2016; Wollschlaeger & Khan, 1984). Genetically-based immune deficiencies such as chronic granulomatous disease and HIES impose impairments in host defense against fungi, leading to the accumulation of fungi in even normal airways and extreme fungal hypersensitivity that result in APBM (Al Khatib et al., 2009; Eppinger, Greenberger, White, Brown, & Cunningham-Rundles, 1999; Ma et al., 2008; Netea, Kullberg, & van der Meer, 2005).

However, even in the absence of formally-defined ABPM, many patients with genetic and acquired immune deficiencies have some degree of airway mycosis that complicates their diseases. Within the narrow clinical spectrum of ABPM, remarkable variation exists in terms of disease presentation (i.e., with bronchiectasis vs no bronchiectasis) and co-existence of other fungal pulmonary disorders (e.g., chronic pulmonary aspergillosis, aspergillomas) (Agarwal, Garg, et al., 2012; Kumar, 2003; Li, Jiang, & Shao, 2018; Lowes, Chishimba, Greaves, & Denning, 2015). Despite the general consensus that ABPM represents only a heightened allergic response to airway fungi that are otherwise not pathogenic, evidence of limited tissue invasion in this condition has in fact been known for decades, strongly supporting the notion that ABPM is due to airway mycosis, with additional disease features that are due to systemic hypersensitivity (Henderson, 1968; Husebye, 1967; Riley, Mackenzie, Uhlman, & Edelman, 1975).

Steroids have long been established to be effective in ameliorating symptoms of ABPM and result in radiographic improvement and reductions

in serum IgE levels, although they do not reduced the incidence of disease flares (Imbeau, Nichols, Flaherty, Dickie, & Reed, 1978; Rosenberg, Patterson, Roberts, & Wang, 1978; Slavin, Million, & Cherry, 1970). Surprisingly, some of the earliest clinical studies of ABPM also suggested that aerosolized antifungals provided added benefits when added to corticosteroids and that this combined therapy was superior to corticosteroids alone in preventing disease recurrence (Slavin et al., 1970). Much more recently, oral antifungals have also been shown to be effective in ABPM when given as adjuncts to corticosteroids (Denning, Van Wye, Lewiston, & Stevens, 1991). Subsequent retrospective reports and case series continued to indicate that itraconazole was effective in improving pulmonary function and decreasing eosinophilia, aspergillus-specific IgG and IgE, and the need for systemic steroids (Pasqualotto et al., 2009; Rai, Panda, & Bhargava, 2004; Salez et al., 1999).

Current consensus guidelines recommend the use of antifungals in ABPM, but only weakly despite the growing evidence of their efficacy (Patterson et al., 2016). Comparison of oral prednisolone and itraconazole for treatment of ABPM in a prospective study demonstrated that while prednisolone yielded a slightly higher response rate, both agents were able to reduce the number of exacerbations and total IgE. This study further demonstrated that prednisolone had a much more detrimental side effect profile as compared to itraconazole (Agarwal et al., 2018). Nebulized amphotericin B has also been used in ABPM, most prominently in refractory or resistant cases, yielding positive clinical effects in all reported trials, all of which are small (Chishimba et al., 2015; Godet et al., 2015, 2017; Ram et al., 2016). Despite the lack of universal use of antifungals in any form, the many successful clinical studies of antifungals in ABPM confirm the fungal infectious nature of this disorder.

1.3.2 Cystic fibrosis

Although typically viewed as a bacterial infectious disease due to airway infection with mucoid strains of *Pseudomonas aeruginosa* and related pathogens, the autosomal recessive pulmonary inflammatory disease cystic fibrosis is also strongly linked to airway mycosis. Abnormally thick and tenacious mucus is secreted into the cystic fibrosis airway due to diminished or absent function of the cystic fibrosis transmembrane conductance regulator (CFTR) gene that is expressed on airway ionocyte cells, which has a primary function to regulate chloride ion transport (Montoro et al., 2018). Widespread blockade of airways due to thick mucus is likely a key factor that

promotes the overgrowth of bacteria behind the areas of blockade (Zhou-Suckow, Duerr, Hagner, Agrawal, & Mall, 2017). However, when optimal sputum culture methods are employed, sputum cultures from cystic fibrosis patients usually yield fungi, thousands of species of which have been found in this disorder, and the incidence of fungal bronchitis and ABPA complicating cystic fibrosis can be very high as well (Alyasin, Moghtaderi, Farjadian, Babaei, & Teshnizi, 2018; Brandt et al., 2018; Coron et al., 2018; Engel et al., 2018; Shoseyov et al., 2006). In this regard, cystic fibrosis differs little from all other allergic airway diseases that are marked by very high rates of airway mycosis. Because most of these disorders are characterized by immunodeficiency, cystic fibrosis should also be viewed as another form of immunodeficiency in which the abnormal airway promotes the overgrowth of diverse pathogens (Cohen & Prince, 2012). Given the very high rates of airway mycosis in cystic fibrosis, it is possible that the primary initiating factor in disease is airway mycosis, with bacterial processes representing a superinfection that subsequently complicates the clinical course.

The perennial concern with respect to airway mycosis in CF is when to treat, especially when the patient does not meet absolute criteria for ABPM, i.e., the patient is limited to fungal bronchitis or even asymptomatic colonization (Tracy & Moss, 2018). Airway mycosis complicating cystic fibrosis is associated with worsening of pulmonary function regardless of the species involved (AbdulWahab, Salah, Chandra, & Taj-Aldeen, 2017; Gileles-Hillel et al., 2015; Reece et al., 2017). Furthermore, cystic fibrosis patients as a whole frequently manifest elevated chitinase activity, a marker of both asthma and diverse fungal infections (Alonso, Pisa, Fernandez-Fernandez, Rabano, & Carrasco, 2017; Van Dyken et al., 2011; Zhu et al., 2004), in bronchoalveolar lavage fluid and serum as well as β -(1,3)-D-glucan (β -glucan) antigenemia (Hector et al., 2016; Rautemaa, Green, Jones, & Rautemaa-Richardson, 2017). The latter observation is of particular concern as elevated β -glucan levels typically signify invasive disease (Mori & Matsumura, 1999; Odabasi et al., 2004), although such invasion is not always clinically apparent (Rautemaa et al., 2017). Thus, airway mycosis is very common if not universal in cystic fibrosis and is linked to both worse prognosis and poorly controlled, invasive disease. Although no consensus exists as to how to manage airway mycosis in cystic fibrosis (Elphick & Southern, 2016), these findings argue in favor of adopting far more aggressive antifungal approaches in this context.

1.3.3 Chronic pulmonary aspergillosis (CPA): Aspergilloma, chronic cavitary aspergillosis, chronic necrotizing aspergillosis, fibrosing aspergillosis

CPA represents a group of primary fungal-associated airway disorders that are characterized by lung inflammation and damage that is presumed to be caused by progressive cavitary aspergillus infections in patients having symptoms for at least 1–3 months (Ando et al., 2016; Denning et al., 2016; Maghrabi & Denning, 2017; Smith & Denning, 2011). CPA reflects a more severe disease process as compared to non-invasive airway mycosis, involving semi-invasive necrotic infection of the airways and lung parenchyma, but without demonstrating overt fungal angioinvasive disease that is seen in highly fatal cases of invasive aspergillosis.

CPA almost always presents in patients with a chronic underlying disease and in whom damaged lung parenchyma creates an environment amenable for fungal proliferation (Kosmidis & Denning, 2015; Soubani & Chandrasekar, 2002). Permanent lung damage caused by tuberculosis is the major underlying predisposing factor for CPA, but other conditions leading to lung destruction and distortion such as sarcoidosis, CF, COPD, non-tuberculous mycobacterial disease, interstitial lung disease, and immune deficiencies are all linked to CPA (Kosmidis & Denning, 2015). CPA strongly overlaps with ABPA and may evolve from this entity (Harada et al., 2019; Sehgal et al., 2019). A high index of suspicion is required for diagnosis, which relies on a combination of airway cultures; serum precipitins for IgG; serum IgE and galactomannan; and lung imaging (Denning et al., 2016).

Several subsets of CPA have been recognized based mostly on radiographical and histological differences, with immunological differences being less clear (Agarwal, Aggarwal, et al., 2012; Izumikawa et al., 2014; Naito & Kurahara, 2017). A study comparing CPA with ABPA showed almost a quarter of patients with CPA satisfied criteria for ABPA, supporting the notion that CPA and ABPA are on the same spectrum of airway mycosis-related disease (Sehgal et al., 2019). The non-invasive forms of CPA are simple aspergillomas, in which fungal balls of up to several centimeters form in pre-existing lung cavities, and chronic cavitary pulmonary aspergillosis (CCPA) in which a focal area of fungal pneumonia undergoes necrosis, creating a central cavity marked by a rim of ongoing pneumonia. Whereas aspergillomas typically do not involve lung invasion, CCPA usually involves local parenchymal invasion. Surgical resection may be curative for

aspergillomas, but prolonged oral antifungals are the preferred treatment modality for CCPA. Relapse is common, with the potential for emergence of antifungal resistance and progression of disease to the semi-invasive chronic necrotizing pulmonary aspergillosis (CNAP) (Agarwal et al., 2013; Cucchetto et al., 2015; Fujita et al., 2012; Kohno et al., 2013; Newton, Harris, Morris, & Denning, 2016; Yoshida et al., 2012). ABPA commonly complicates CPA, and the combination of disorders can produce fibrosing aspergillosis, an end-stage process that can result in complete lung destruction (Kosmidis, Newton, Muldoon, & Denning, 2017). Treatment guidelines for CPA universally include the recommendation to treat for at least several months with systemic antifungals (Maghrabi & Denning, 2017).

1.4 Non-allergic airway mycosis-related lung diseases

In addition to frankly allergic airway diseases marked by the production of IgE and blood and tissue eosinophilia, multiple additional airway diseases begin as non-allergic disorders that then acquire airway mycosis that usually worsens the disease syndrome. Allergic features do not always ensue after the development of airway mycosis in these situations, but the onset of eosinophilia or atopy in the context of pre-existing chronic lung disease should raise the possibility of complicating airway mycosis.

1.4.1 Tuberculosis

Prior tuberculosis that results in chronic lung damage accounts for at least one third of all secondary cases of CPA (Smith & Denning, 2011). A Ugandan study of HIV-infected tuberculosis patients showed a prevalence of IgE sensitization to *Aspergillus* spp. of 10% with roughly 7% developing overt CPA (Kwizera et al., 2017). This study indicated that fungi can complicate active pulmonary tuberculosis, but conversely patients with airway mycosis and ABPA can develop pulmonary disease due to non-tuberculous mycobacteria, with one study showing a 17% incidence of non-tuberculous mycobacterial disease after ABPA diagnosis (Ishiguro, Takayanagi, Takaku, et al., 2016). In addition, non-tuberculous mycobacterial infections, most prominently *Mycobacterium avium* complex (MAC) infected patients are at equal risk for development of CPA (Ishiguro, Takayanagi, Baba, et al., 2016). Risk factors include underlying chronic lung disease, prior to TB infection, current smoking and low BMI (Ishiguro, Takayanagi, Baba, et al., 2016). The most common presentation of CPA in TB infected individuals is aspergillomas, which account for roughly a third of the cases with

risk of death being significantly higher compared to patients with TB without concurrent CPA (Koyama et al., 2015). Infection is not strictly relegated to *Aspergillus* spp. as *Candida albicans* and other non-albicans yeasts have also been reported as co-infections with tuberculosis (Kali et al., 2013). Treatment for isolated aspergilloma often includes surgical resection where possible or bronchial artery embolization in the context of severe hemoptysis where surgery is not feasible. However, antifungals are sometimes also used and are mandatory for all other subsets of CPA (Adeyemo et al., 1984; Agarwal et al., 2013; Cucchetto et al., 2015; Fujita et al., 2012; Gupta, Jain, & Kewlani, 2015; Kohno et al., 2013; Newton et al., 2016; Yoshida et al., 2012).

1.4.2 COPD

COPD is the second most common underlying lung condition in CPA after tuberculosis (Smith & Denning, 2011). Although the finding of fungi from airway secretions in COPD typically creates diagnostic uncertainty (Pashley, 2014), a study of 128 patients with COPD detected filamentous hyphae in 49% of patients while only present in 16% of control patients (Bafadhel et al., 2014). This study associated higher doses of inhaled corticosteroids taken by COPD patients to the recovery of filamentous hyphae, a finding that has been replicated in subsequent studies (Bafadhel et al., 2014; Fraczek et al., 2018; Tong et al., 2018). Immediate type hypersensitivity to *Aspergillus* spp. was further detected in 13% of patients with COPD (Bafadhel et al., 2014), which is similar to the 17% immediate hypersensitivity response against a panel of multiple fungal allergens that we observed in an asthma cohort (Li, Tsai, et al., 2018).

Subsequent studies have shown that sensitization to fungi among patients with COPD is associated with overall poor lung function and the development of bronchiectasis (Bafadhel et al., 2014; Everaerts et al., 2018; Jin, Liu, & Sun, 2014). In contrast to immediate hypersensitivity testing that lack sensitivity, up to 40% of COPD patients express aspergillus galactomannan antigen, positivity to which was more strongly associated with older age, presence of bronchiectasis, higher incidence of exacerbations and elevated mortality (Yoshimura et al., 2018). ABPA is further seen with greater frequency in COPD (2.9%) than in the general population (Jin et al., 2014). Regardless of the concomitant fungal-related disorder, COPD patients who develop airway mycosis carry an especially poor prognosis as compared to patients without such complications (Koyama et al., 2015).

Asthma–COPD overlap syndrome (ACOS) is an emerging, incompletely defined clinical syndrome in which multiple key clinical features of asthma and COPD are observed in the same patient (Postma & Rabe, 2015). Although only limited links between ACOS and airway fungal disease have yet been made (Chiba et al., 2017; Hirano et al., 2017), it is likely that many cases of ACOS reflect the development of airway mycosis in patients with preceding lung and airway damage related to COPD.

1.4.3 Sarcoidosis

Sarcoidosis is a systemic, idiopathic inflammatory disorder that most commonly affects the lungs, producing often extreme hilar lymph node enlargement, interstitial lung disease, and pulmonary fibrosis that can be fatal. The key diagnostic feature of sarcoidosis is the presence of non-caseating granulomas in affected organs that cannot be ascribed to any known agent. Although non-tuberculous mycobacteria have been linked etiologically to sarcoidosis (el-Zaatari et al., 1996), fungi have also (Tercelj, Rott, & Rylander, 2007; Tercelj, Salobir, Zupancic, & Rylander, 2011, 2014). Nonetheless, a consensus as to the underlying cause of sarcoidosis is not universally accepted.

Despite this ambiguity, fungi can clearly produce a comorbid infection that complicates pulmonary sarcoidosis. Initially considered to be an innocuous bystander in cavitary lesions in pulmonary sarcoidosis, aspergillus co-infection was eventually realized to be an important contributory factor in sarcoid-related morbidity (Lofgren & Lindgren, 1959). Approximately 3–12% of sarcoidosis patients demonstrate aspergillus precipitins and over 90% of these subjects will demonstrate radiographic aspergillomas (Denning, Pleuvry, & Cole, 2013; Uzunhan et al., 2017; Wollschlager & Khan, 1984) that may become symptomatic due to hemoptysis and lung parenchymal invasion with systemic spread of disease (Israel, Lenchner, & Atkinson, 1982). Overall, these studies demonstrate that airway mycosis strongly and adversely influences the course of sarcoidosis and may be a precursor condition to it.

In summary, the clinical burden of airway mycosis is much more widespread than currently appreciated and its consideration must be broadened to include not only patients with SAFS or ABPA, but also any individual with unexplained chronic cough, dyspnea and sputum production (Fig. 1). Clinicians must be especially wary of patients with any chronic non-fungal lung disease that becomes complicated by airway mycosis. Airway mycosis complicating these conditions does not usually present as overt atopic or

allergic disease, but with progression, serological and hematological markers of this complication will usually develop. However, by the time allergic disease becomes obvious, irreversible lung damage including bronchiectasis and advanced cavitory lung disease may have occurred, with attendant high morbidity and mortality. Given the high potential for morbidity related to airway mycosis, clinicians must implement antifungal therapy as early as possible.



2. Immune pathogenesis

Immunity against fungi remains relatively poorly understood, particularly in the context of allergic airway disease. However, a critical revelation over past decade is the realization that rather than representing aberrant responses to harmless environmental antigens, atopic (IgE) reactions, which underlie allergies, and eosinophilia are just two of many components of the complex allergic inflammatory program that have antimicrobial, including antifungal, activity. The allergic immune response involves both innate and adaptive components and is intricately connected to the coagulation system (Fig. 3).

2.1 Asthma and related allergic airway diseases

The pathogenesis of asthma has long been linked to the interaction of multiple environmental factors with genetically susceptible individuals. Sensitivity to foods and aeroallergens, pollutants, viral and bacterial infections are all implicated in asthma development and exacerbations (Chung et al., 2014), but in most cases it remains unclear what factors are etiologic and which are exacerbating. Complicating this picture are the proposed variety of different observable phenotypes (exercise-induced, adult onset, steroid resistant, etc.) and pathophysiologic endotypes (eosinophilic, neutrophilic, IgE-mediated, etc.) that suggest a complex and multifactorial etiology (Lotvall et al., 2011). Despite the syndromic nature of asthma as observed clinically, evidence increasingly suggests that asthma is etiologically linked to a single biochemical perturbation-enhanced proteolytic activity-occurring within the airway.

Among the first evidence that proteinases were directly linked to asthma were a series of reports beginning 50 years ago that described asthma occurring among workers in laundry detergent factories who developed a bronchial illness indistinguishable from asthma following exposure to the proteinase prepared from *Bacillus subtilis* (subtilisin) which was then newly

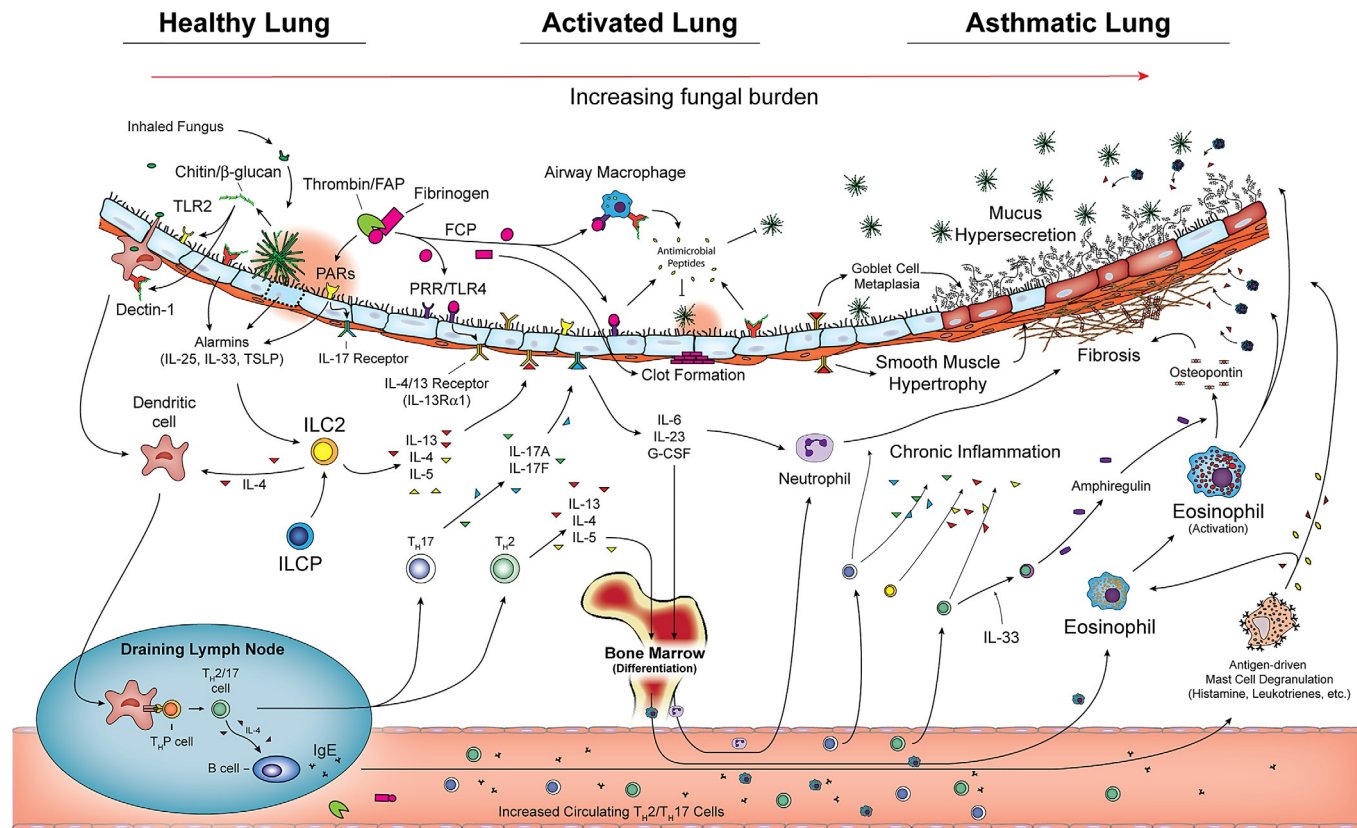


Fig. 3 See figure legend on next page.

added to the soap to improve its performance (Pepys, 1973; Pepys, Longbottom, Hargreave, & Faux, 1969). Sensitivity to the proteinase was shown by both skin prick and inhalational challenge, confirming the allergic nature of the reaction. Subsequently, asthma has been seen in other industrial contexts where active proteinases are used (Baur & Fruhmman, 1979; Gailhofer, Wilders-Truschnig, Smolle, & Ludvan, 1988) and asthma due to subtilisin use remains an industrial concern despite the introduction of safeguards against aerosolization (Cullinan et al., 2000).

Proteinases from dust mites (Kelada et al., 2011), cats (Pei et al., 2013), and the papaya fruit (papain (Sokol, Barton, Farr, & Medzhitov, 2008)) among other allergens were also shown to induce allergic airway disease in rodents. However, careful analysis of active proteinases from the house dust of the homes of asthmatic children disclosed that the dominant active proteinases were derived from fungi, especially *Aspergillus niger* (Porter et al., 2009). Subsequent studies demonstrated that *A. niger* (Porter et al., 2009) and many

Fig. 3 Immune pathogenesis of asthma due to airway mycosis. All initially healthy individuals are exposed to inhaled fungi that can produce at least transient airway mycosis. Depending on host and environmental factors, the airway mycosis can lead to airway inflammation that characterizes the activated, but not necessarily diseased, lung, and eventually progress to clinically significant asthma with attendant immune and structural sequelae. The initial wave of airway mycosis triggers innate allergic responses by activating pattern recognition receptors (PRR) including Toll like receptor 2 (TLR2) and Dectin-1 expressed on airway epithelial and immune cells that detect fungal sugars such as chitin and β -glucan. Epithelial cells then secrete cytokines such as interleukin 25 (IL-25), IL-33 and thymic stromal lymphopoietin (TSLP) into the subepithelial space to promote the development of innate lymphoid cells type 2 (ILC2) and dendritic cells that migrate to draining lymph nodes and promote development of T helper type 2 (Th2) and Th17 cells and IgE-secreting B cells. At the same time, endogenous and potentially fungal proteinases promote the breakdown of fibrinogen into fibrinogen cleavage products (FCP) that signal through TLR4 to initiate airway hyperreactivity, eosinophilia, and mucus hypersecretion. Proteinases, fungal and endogenous, may also interact with protease-activated receptor 2 (PAR2) to either promote or attenuate asthma-like disease. With greater degrees of airway mycosis, the low-grade, innate allergic inflammation that characterizes healthy and activated lungs converts to a chronic lung (asthma) phenotype characterized by more persistent and intense allergic inflammation that includes prominent Th2 cells and Th17 cells, eosinophils (under the direction of IL-5), and mast cells (under the direction of IL-4 and IgE), and neutrophils (under the direction of IL-17A and related cytokines) that promote severe allergic inflammation, airway hyperreactivity, and mucus hypersecretion that characterize various asthma endotypes. Most of these immune elements, including highly activated neutrophils, eosinophils, and antimicrobial peptide-secreting epithelial cells, have an antifungal role. Nonetheless, failure to resolve airway mycosis can result in permanent airway damage including airway fibrosis and bronchiectasis.

other common fungi (Porter, Fields, et al., 2011) could produce airway mycosis and induce asthma-like allergic airway disease in mice. Importantly, the ability of *A. niger* to induce allergic airway disease requires the secretion of proteinases (Porter et al., 2009) and without the ability to secrete proteinases, this organism grows better in the mouse airway as compared to its wild-type counterpart (Porter et al., 2009). These observations suggest that allergic inflammation, rather than being an aberrant, inappropriate immune response as often envisaged (Balzar, Strand, Nakano, & Wenzel, 2006; Clarke et al., 2014; Hongjia et al., 2014; Wiesner & Klein, 2017; Wills-Karp, 1999), is actually protective against airway mycosis. Subsequent studies have confirmed that many components of the allergic immune response including eosinophils, epithelial cells, macrophages, T cells, IL-5, and IL-13, play significant antifungal roles in this context (Millien et al., 2013; Porter, Fields, et al., 2011).

Despite the compelling link between fungal (and other) proteinases demonstrated in the pathogenesis of allergic airway disease and asthma, proteinases and their potentially infective sources have not been widely accepted as essential drivers of allergic airway diseases except for the most severe cases of asthma with fungal sensitization, with many clinicians continuing to underutilize antifungal therapies for severe asthma and ABPA. The lack of acceptance of antifungals in the treatment of asthma probably stems from the view that the airway, far from being a sterile environment, has many microbiological communities, with fungi being a part of the natural flora, an impression likely imposed by the uniquely ubiquitous nature of fungal aerosol exposure, and as such are perceived to be innocuous bystanders.

2.2 Asthma, airway mycosis, and immunodeficiency

Clinicians also often fail to recognize that allergic airway disease syndromes and fungal infections are frequently found as part of the clinical spectrum seen in both primary and acquired immunodeficiency syndromes (Agondi, Barros, Rizzo, Kalil, & Giavina-Bianchi, 2010; Antachopoulos, Walsh, & Roilides, 2007; Balzar et al., 2006; Chan & Gelfand, 2015; Chu et al., 2012; Kim et al., 2010; Mark et al., 2012; Ozcan, Metin, Erkocoglu, & Kocabas, 2014; Urm et al., 2013). Eosinophilia is also very frequently found in subjects with diverse immunodeficiency syndromes (Navabi & Upton, 2016). The hyper-IgE syndromes (HIES) due to mutations in signal transducer and activator of transcription 3 (STAT3-HIES; Job's syndrome), dedicator of cytokinesis 8 (DOCK8-HIES) and the

transforming growth factor receptors (TGFB β -HIES; Loeys-Dietz syndrome) are characterized to varying degrees by recurrent severe fungal and bacterial infections, congenital malformations, and disorders of connective tissue in addition to manifesting very high serum IgE levels. Asthma is especially frequently seen in DOCK8 HIES and Loeys-Dietz syndrome (Boos et al., 2014; Chu et al., 2012; Frischmeyer-Guerrero et al., 2013; Loeys & Dietz, 1993).

Elevated IgE levels and fungal lung disease are also seen in chronic granulomatous disease (CGD) that is due to defects in the NADPH oxidase system and can present with clinical features that strongly overlap with ABPM (Eppinger et al., 1999; Patioglu, Gungor, Lazaroski, & Unal, 2013). Strikingly, detailed analysis of IgE specificity in HIES has shown that most of the IgE is directed at the fungi that inevitably infect these patients and is therefore not a non-specific consequence of the gene defect (Eppinger et al., 1999). Thus, although many different immunodeficiency disorders have been identified that associate with asthma and elevated IgE levels, they most likely also share the inability to normally clear the airways and other sites of fungi. The inevitable airway mycosis that unites these syndromes is then a major factor that drives the elevated IgE and probably also the eosinophilia that is commonly found in these diverse disorders.

However, there is also likely a second mechanism driving IgE production that unites immunodeficiency-related airway mycosis, allergic airway disease, and atopy. Beyond sensitization to fungi, many genetically-based immunodeficiency syndromes are also linked to general atopy and atopy-related allergic disorders such as atopic dermatitis and food allergy (Boos et al., 2014; Chu et al., 2012; Mark et al., 2012; Ramirez-Vargas et al., 2014; Zhang et al., 2014). Human immunodeficiency virus (HIV)-related acquired immunodeficiency is also linked to elevated IgE levels (Wright et al., 1990) and asthma (Foster et al., 2008; Mirani et al., 2015). To understand how immunodeficiency might be linked to general atopy, we simulated immunodeficiency-related airway mycosis by repeatedly challenging wild-type mice intranasally with the spores of the mold *Aspergillus niger* in doses that were insufficient to alone provoke canonical features of asthma such as airway hyperresponsiveness (AHR) or fungus-specific IgG1, a mouse IgG isotype that is driven by IL-4 and is closely linked to IgE production (Coffman, Shrader, Carty, Mosmann, & Bond, 1987; Erazo et al., 2007; Finkelman et al., 1988; Snapper, Finkelman, & Paul, 1988).

Mice challenged in parallel with ovalbumin, a bland “bystander” antigen, also failed to develop AHR or ovalbumin-specific IgG1 as expected

given the tolerogenic nature of ovalbumin when administered intranasally (Seymour, Gershwin, & Coffin, 1998). However, *A. niger* spores and ovalbumin administered concomitantly were sufficient to provoke both AHR and IgG1 specific for ovalbumin, but surprisingly not to *A. niger* lysates (Porter et al., 2009). Thus, similar to what has previously been demonstrated for other allergenic pathogens such as helminths (Hurst, Seymour, Muchamuel, Kurup, & Coffin, 2001), airway mycosis promotes atopic reactions to bystander antigens, potentially including food-derived antigens, but not always to the fungi themselves. Through this mechanism, airway mycosis may drive non-airway atopic syndromes as diverse as food allergy, seasonal pollen allergy, atopic dermatitis, and possibly urticaria.

We propose then a reformulation of the concept of the atopic march, the predilection of allergic disease syndromes to begin in infancy, often as atopic dermatitis, and then progress to allergic rhinitis and more serious conditions such as asthma, chronic rhinosinusitis, urticaria, and food allergy (Ito & Mikawa, 1990; Spergel & Paller, 2003) (Fig. 4). Central to this new concept is the role that airway mycosis plays both in directing type 4 hypersensitivity (i.e., Th2 cell and eosinophil) responses that drive some airway disease syndromes (e.g., CRS, asthma) and type 1 immediate hypersensitivity (atopic) responses that underlie allergic rhinitis, anaphylaxis, food allergies and other IgE-dependent reactions. Although environmental factors, especially mold exposure, are critically involved, this synthesis emphasizes the concept that airway mycosis also involves diverse genetic factors that conspire to inhibit clearance of airway fungi.

In response to the unresolvable airway mycosis that develops and slowly worsens in these patients, subjects may mount compensatory atopic reactions, comprised of diverse antifungal programs, to control the mycosis (Porter et al., 2009; Porter, Fields, et al., 2011). In this regard, even subjects with mild allergic airway disease, if genetically susceptible, may make the gradual transition toward more advanced disease that eventually results in severe and potentially irreversible structural changes such as bronchiectasis and advanced airway fibrosis that lead to fixed airway obstruction (Figs. 3 and 4). A major concern, therefore, with current allergic airway disease management strategies that emphasize the use of immunosuppressive corticosteroids and, increasingly, biologics that interrupt key antifungal programs (e.g., mepolizumab (Flood-Page, Menzies-Gow, Kay, & Robinson, 2003; Ortega et al., 2014) that reduces airway eosinophilia) is that such therapy may promote worsening of airway mycosis over periods of years to decades. It is possible that asthmatics who without any treatment would not progress

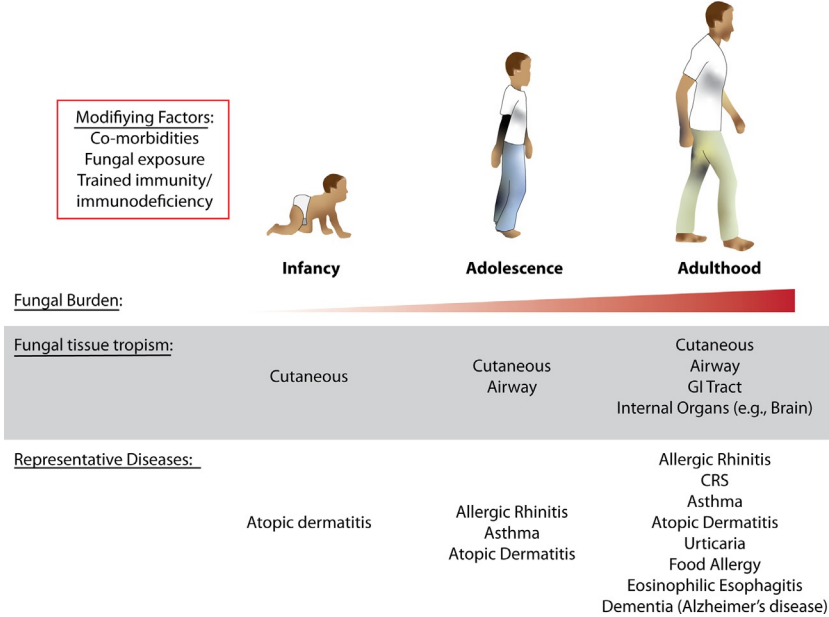


Fig. 4 The fungal atopic disease march. The overall burden of mycosis provides a potential explanation of the tendency of allergic or atopic diseases to worsen, with respect to both diversity and severity, with age. According to this concept, the total body burden of fungi (assessed as fungal mass with respect to weight), including airway, cutaneous, and GI tract mycoses, may grow over time in susceptible individuals. Beginning with cutaneous inoculation at birth, mycosis soon spreads to other epithelial surfaces, including the respiratory and GI tracts. As the fungal biomass increases at each site, the diversity of fungal-related disorders increases concomitantly, potentially leading to distant spread of both mycosis and mycosis-related diseases, potentially including the central nervous system. Critical factors modifying the rate of progression and severity of these disorders, if they ever appear, include the precise nature of genetic and acquired immune deficiencies, the intensity of exposure to environmental fungi, and other co-morbidities.

beyond mild to moderate disease become destined to develop through modern therapy severe, very severe, and ultimately life-threatening, end stage disease.

Although a full discussion is beyond this review, it is worth noting here that there are several end-stage outcomes of untreated or improperly treated airway mycosis. Experimental low-grade dissemination of yeast from mucosal sites leads to a brain infection having Alzheimer’s disease-like features (Wu et al., 2019). With higher degrees of immune compromise, more virulent disseminated disease might be seen involving acute illness and massive

organ dysfunction that carries a high mortality rate even with optimal antifungal therapy (Azie et al., 2012). Finally, angioinvasive disease of the lungs that produces hemoptysis can be rapidly fatal and is difficult to manage medically and surgically (Denning et al., 2016). These poor outcomes strongly argue for the early and aggressive use of antifungals at the earliest sign of airway mycosis in any context.

2.3 Airway innate immune activation by fungi

The earliest immune events initiated by fungi are triggered by the key danger signals secreted by or expressed on yeasts and molds (Fig. 3). Beta glucan, a sugar found in the cell walls of most fungi (yeasts such as *Candida albicans* express only low levels of beta glucan due to regulated expression (Galán-Díez et al., 2010)) is detected by the pathogen associated molecular pattern (PAMP) receptor Dectin 1 (*Clec7a*) that is expressed on highly phagocytic cells such as macrophages, dendritic cells, and neutrophils. Activation of these cells through Dectin 1 promotes phagocytosis of fungi and the release of many cytokines including IL-17A, IL-22, TNF, and GM-CSF that promote Th17 responses as well as secretion of antimicrobial peptides from airway epithelial cells (Lilly et al., 2012; Sun et al., 2012; Werner et al., 2009, 2011). Dectin-1 further recognizes a ligand expressed on CD4+ T cells and therefore serves a co-stimulatory role that promotes T cell survival and differentiation (Drummond et al., 2016).

A second polysaccharide derived from the cell walls of all fungi, chitin (poly-*N*-acetylglucosamine), is recognized by airway cells and triggers acute inflammation involving the recruitment of eosinophils, basophils, and alternatively activated macrophages (Reese et al., 2007; Van Dyken et al., 2011). Chitin further stimulates airway epithelial cells to secrete IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), cytokines that collectively promote type 2 immune responses (Vannella et al., 2016) and the recruitment to lung of innate lymphoid cells type 2 (ILC2) (Van Dyken et al., 2014). The mammalian receptors for chitin are not known with certainty, but TLR2 has been implicated in mediating cytokine production and cell proliferation in response to airway delivery of chitin (Da Silva, Pochard, Lee, & Elias, 2010).

The most important factor identified thus far that drives the airway type 2 inflammatory phenotype during airway mycosis is fungus-associated proteinase (FAP; Fig. 3). Active proteinases secreted by molds, especially *Aspergillus* spp., are especially potent drivers of the type 2 immune response,

able to provoke the same degree of inflammation and lung physiological change (e.g., airway hyperresponsiveness) as the whole organism during active airway infection (Kheradmand et al., 2002; Porter et al., 2009). Partial or complete genetic ablation of fungal proteinases further markedly impairs or obliterates the ability of fungi to mediate allergic airway disease through the induction of both Th2 and Th17 responses (Kheradmand et al., 2002; Porter et al., 2009). Many different molds are capable of producing allergic airway disease in mice, and proteinases derived from non-*aspergillus* fungi (Boitano et al., 2011), but also bacteria (Florsheim et al., 2015) and plants (Kheradmand et al., 2002; Sokol et al., 2008), are equally capable of inducing robust phenotypes. Careful analysis of the human asthmatic airway suggests that fungal proteinases are capable of penetrating the airway epithelium and embedding in the submucosa, potentially to act directly on airway smooth muscle cells to drive airway hyperresponsiveness (Balenga et al., 2015).

Neutralization of fungal proteinase activity abolishes their allergenic potential (Kheradmand et al., 2002), suggesting that allergenicity requires the hydrolytic cleavage of endogenous substrates that in turn must be detected by specific immune receptors. In part, this concept was confirmed by the discovery that when the clotting factor fibrinogen is cleaved through a series of proteolytic steps involving thrombin, the resulting fibrinogen cleavage products (FCPs) signal through the innate immune receptor Toll like receptor 4 (TLR4). This signaling cascade results in two relevant outcomes: modest recruitment to the airway of eosinophils and innate lymphoid cells type 2 (ILC2) and mucin gene induction when FCPs are delivered to the airway, and the induction of potent antifungal activity when FCPs are incubated with macrophages and airway epithelial cells (Millien et al., 2013). Additional studies are pending to determine if FAP can also cleave fibrinogen into cleavage products having similar activity.

Thus, proteinase-cleaved fibrinogen signals innately through TLR4 to drive antifungal immunity and modest degrees of allergic airway inflammation. Fungal proteinases can also activate protease-activated receptor-2 (PAR-2) to in part drive eosinophilic airway inflammation (Boitano et al., 2011; Hiraishi et al., 2018). However, additional studies are required to understand in molecular detail how fungal proteinases drive the Th2 and Th17 responses that are essential for the expression of robust allergic airway disease. Although coagulation factors have also been implicated in CRS (Kim, Cho, Takabayashi, & Schleimer, 2015), additional work is needed to delineate the direct effect of this pathway in the pathophysiology of upper airway allergic disease.

2.4 Airway epithelium in fungal CRS

The respiratory epithelium serves as the most important physical and immunologic barrier against potential environmental threats such as microbes and allergens and deserves special consideration. The integrity of the epithelial barrier is largely dependent on tight junction proteins and compromise of these barrier proteins is thought to promote antigen transfer to the submucosal space, thereby promoting allergic inflammation (Sweerus et al., 2016; Wan et al., 1999). Using primary differentiated respiratory epithelial cells from CRSwNP patients, exposure to the cytokines IL-4 and IL-13 caused a significant decrease in transepithelial resistance (TER) reflecting an increase in epithelial permeability (Wise et al., 2014). Similar air liquid interface (ALI) cultures of primary differentiated sinonasal epithelial cells from AFRS patients demonstrated a significantly reduced TER and decreased expression of tight junction proteins at baseline without additional cytokine exposure as compared to non-CRS cells (Den Beste, Hoddeson, Parkos, Nusrat, & Wise, 2012). As ALI cultures take at least 4 weeks to differentiate, a decreased TER in AFRS cells relative to non-CRS controls suggests that the reduced expression of tight junction proteins could reflect either the effect of sustained exposure to type 2 cytokines in vivo or a pre-existing, potentially genetically-based difference in epithelial cells derived from AFRS patients. The increased epithelial permeability in AFRS potentially allows for enhanced exposure to fungal antigens, thus driving the very high levels of IgE and other immunological parameters that are typical of this disorder.

In addition to the barrier function they provide, respiratory epithelial cells also serve critical immune functions. Three epithelial cell derived cytokines (IL-33, IL-25 and thymic stromal lymphopoietin) orchestrate the innate and adaptive type 2 immune response that is characteristic of most airway mycosis-related airway diseases (AMRADs). In CRS, IL-33 is of particular importance as its receptors are found on numerous allergic effector cells including innate lymphoid cells type 2 (ILC2), eosinophils, mast cells, and basophils (Molofsky, Savage, & Locksley, 2015). We found that ILC2s are the primary source of secreted IL-13 in response to IL-33 stimulation (Shaw et al., 2013). IL-13 influences the expression of CRS and asthma through its ability to initiate goblet cell metaplasia within the airway epithelium and enhance both airway mucus secretion and airway hyperresponsiveness (Grunig et al., 1998; Wills-Karp et al., 1998).

Critically, extracts of fungi and fungal associated proteinases (Hiraishi et al., 2018; Kiss et al., 2007) promote the secretion of IL-33 from sinonasal

epithelial cells derived from CRSwNP patients (Shaw et al., 2013). IL-33 signals through its receptor subunit ST2 to drive production from nasal polyp Th2 cells of IL-5 and the epidermal growth factor receptor ligand amphiregulin, which respectively promote eosinopoiesis and activation of airway-recruited eosinophils to secrete osteopontin, a complex, cytokine-like molecule that in the context of allergic airway disease promotes sub-epithelial fibrosis that in turn may promote fixed obstruction of the upper and lower airways (Fig. 3) (Cantor & Shinohara, 2009; Morimoto et al., 2018).

As noted previously, the interaction between fungal proteinase and coagulation proteins has recently been implicated in allergic airway inflammation (Millien et al., 2013). In addition to promoting lower airway hyperreactivity in mice, fungal proteinase cleavage of fibrinogen can activate human SNECs to upregulate IL-13R α 1 and MUC5AC as well as antifungal activity, presumably involving release of antimicrobial peptides, confirming that such a pathway is present in humans (Fig. 3).

In addition to the fungal proteinase activity, sinonasal mucosa express proteinase inhibitors and lower levels of cystatin A and SPINK5 (cysteine and serine proteinase inhibitors, respectively) were noted in SNECs from eosinophilic CRSwNP patients (Kouzaki et al., 2016). Thus, an imbalance between endogenous proteinase inhibitors and exposure to exogenous proteinase activity contribute to compromises in the epithelial barrier as well as activation of respiratory epithelial cells to orchestrate a type 2 immune response (Kouzaki et al., 2016).



3. Summary and clinical applicability

The relatively new concept of airway mycosis, long known to be the basis of ABPM and selected other disorders, extends well beyond these conditions to involve a large number of chronic upper and lower airway disorders that frequently, but not always, present with allergic features of eosinophilia or elevated IgE levels. Although it remains largely unclear why these disorders present with distinct clinical features, a unifying concept aside from their fungal origin is immunodeficiency. While unique genetic mutations (e.g., chronic granulomatous disease, HIES syndromes) linking to allergic airway disease are known, it is likely that numerous additional polymorphisms will be described in the future. Understanding the function of the involved genes will further undoubtedly reveal additional layers of complexity of the mammalian antifungal immune response, about which

we today only have a rudimentary understanding. In addition to this essential fundamental work, it is also essential for clinicians to develop more effective methods to diagnose and treat airway mycosis at its earliest stages to prevent long term complications.

3.1 New diagnostic approaches

As with any infectious disease, having accurate, highly sensitive methods that are readily available to clinicians for diagnosis is essential. However, such methods have never been available for diagnosing airway mycosis, although more recently the situation has begun to improve. Lacking such methods, or worse, having only diagnostic methods that are insensitive, has greatly impaired the general acceptance of airway mycosis as a genuine diagnostic category and an entity requiring therapy.

Due to the uncommonness with which fungi are isolated from routine respiratory tract cultures, often combined with a lack of evidence of acute illness when fungi are recovered from the respiratory tract, positive fungal cultures are usually interpreted as evidence only of contamination or innocuous colonization rather than true infection. This issue is further supported by the frequency with which fungi are isolated from healthy control subjects. Older studies of the prevalence of yeasts from sputum found very high rates of fungal colonization in healthy control subjects even when compared to diseased cohorts, reaching the conclusion that *Candida albicans* is likely an innocuous finding from this specimen (Baum, 1960). Positive fungal cultures from the lower respiratory tract yielded a dismal 0% positive predictive value for serious fungal disease in patients with solid tumors who were ostensibly at high risk for invasive fungal disease, although isolation of some fungi, (e.g., *Scedosporium* spp.) was more likely to be found to clinically significant (Lionakis & Kontoyiannis, 2004). Ultimately, too few high quality studies have been conducted to rigorously understand the importance of fungi isolated from the airway in any clinical context (Krause et al., 2017).

A further concern with most prior studies is that significant fungal disease is typically defined as invasive disease, without consideration of the possibility that non-invasive airway fungal growth can also be a cause of significant disease. The widely accepted clinical model is that pathogen cultures are interpretable most easily in the context of flagrant disease that usually includes fever, major organ dysfunction, and other major signs of illness as seen in a hyperacute context. In contrast, genuine fungal diseases due to airway mycosis almost never follow this paradigm, and are instead

lengthy, afebrile illnesses playing out over years and decades, often without accompanying dramatic clinical signs and symptoms aside from the paroxysms of shortness of breath that are seen in asthma and ABPM.

The predominant perception of the “innocuous” nature of airway fungi has begun to change with the introduction of DNA-based analytical methods. The mycobiome of patients with non-specific bronchiectasis and cystic fibrosis trend toward a reduced microbial diversity with *Candida* and *Aspergillus* species being predominant (Kim, Clark, et al., 2015). Conventional antibiotic use does not seem to alter the lung mycobiota (Kim, Clark, et al., 2015), but corticosteroid use increases airway fungal burdens (Fraczek et al., 2018). This growing body of evidence of the fungal burden in human airways suggests that while some airway fungi may be innocuous (defined as such by the absence of serious apparent respiratory tract disease), the known pathogenic species such as *Candida* spp. and *Aspergillus* spp. are invariably present in the diseased lung.

The fact that respiratory tract sputum fungal cultures often fail to yield fungi is usually interpreted as lack of fungi, and therefore lack of airway mycosis and significant fungal disease. However, it is now clear that this is a false impression that is due in many instances to the difficulty in isolating fungi from such specimens using standard methods. In fact, sputum is notoriously inefficient at yielding pathogens potentially underlying any serious lung condition. Thus, even after three separate specimen cultures from the same individual, *Mycobacterium tuberculosis* is identified from sputum from at most 62% of children eventually diagnosed with intrathoracic tuberculosis (Hesseling et al., 2006). Isolation of the etiologic agent of community acquired bacterial pneumonia from even frankly purulent sputum has a much lower success rate such that when a putative pathogen is actually identified by culture, this rarely influences clinical decision making (Ewig, Schlochtermeyer, Goike, & Niederman, 2002). Similarly, fungi could only be cultured from 27% of sputa from cystic fibrosis (CF) patients, while sequencing was positive in 90% (Pashley et al., 2012). Fungi possess the added disadvantage, in distinction from pathogenic bacteria, that their airborne spores are always present, again contributing to the unfortunate interpretation that such culture results are not clinically meaningful.

We discovered that cultures of sputa using conventional methods typically do not yield fungi even in the context of obvious fungal disease in which microscopic examination of sputa reveals abundant fungal elements (Mak, Porter, Bandi, Kheradmand, & Corry, 2013). Such sputa, which are typically encountered in asthma and CRS, usually fail to yield fungi during

culture because the mucus is highly fungistatic, containing multiple elements including fibrinogen-activated macrophages (Millien et al., 2013) and eosinophils (Porter et al., 2009; Yoon, Ponikau, Lawrence, & Kita, 2008) that are potentially fungistatic (fungal growth inhibiting) and, at least in the case of macrophages, secrete soluble substances that inhibit fungal growth (Millien et al., 2013). We and others (Pashley et al., 2012) have shown that performing a mucolytic step that permits separation of fungal elements in sputum from the mucus that contains the bulk of the antifungal moieties greatly enhances the culture yield of fungi (Mak et al., 2013; Porter et al., 2014). These improved culture techniques increase fungal culture yields in diseased patients from 11–16% to 84–87%, leading to the identification of a subset of asthma patients with airway mycosis that strongly benefit from antifungal therapy (Li, Tsai, et al., 2018). Thus, in contrast to current practice where occasional conventional cultures of respiratory tract specimens that unexpectedly yield these organisms are considered “contaminants” or otherwise spurious, we believe that such results, especially if obtained following a mucolytic procedure, should be viewed as evidence of active fungal infection, especially of airway mycosis, until proven otherwise. These findings further support the widespread adoption of improved respiratory specimen culture techniques to enhance the yield of potentially pathogenic fungi. Nonetheless, improved culture techniques will never fully solve the technological problem of diagnosing airway mycosis as not all individuals with clinically significant airway mycosis are capable of producing sputum. As such, non-culture based diagnostic methods are clearly needed (Denning et al., 2017).

Third, underlying the failure to recognize the vast impact of fungi on allergic airway diseases is the false belief that entities such as mild/moderate asthma, severe asthma with fungal sensitization (SAFS), asthma with airway mycosis, ABPA and other conditions exist as distinct clinical entities having separate etiologies rather than representing a single disease entity that is expressed on a continuum. For example, although allergic bronchopulmonary mycosis (ABPM) and Churg–Strauss syndrome are usually considered distinct from asthma, in fact the vast majority (>95%) of patients with these conditions have asthma as a preceding condition (Kauffman, Tomee, van der Werf, de Monchy, & Koeter, 1995; Loughlin, Cole, Rothman, & Johnson, 2002). The allergic disease spectrum of the upper airway, including CRSwNP and AFRS, follows a similar continuum and perhaps should be considered part of the lower airway allergic disease continuum given the many similarities between asthma and CRS

and their frequent concurrence (Pakdaman, Corry, & Luong, 2011; Promsopa et al., 2016; Ragab, Clement, & Vincken, 2004; Rodrigues et al., 2016; Tsao, Chen, Yeh, & Huang, 2003).

A final impediment to recognizing the importance of airway mycosis in allergic airway disease is the insensitivity of current immunodiagnostic testing to confirm or refute a potential diagnosis of airway mycosis. When positive, standard testing such as immediate hypersensitivity testing (which measures fungus-specific IgE) can be used as a complementary assay to establish the relevance of airway fungal culture results. Alternatively, such testing can be used to tentatively assign a diagnosis of airway mycosis in the absence of airway culture results, although in reality most clinicians only view such results as evidence of fungal hypersensitivity and not necessarily fungal infection. Our retrospective analysis of asthmatic subjects' responses to antifungal treatment showed that only 14% of subjects showed any specific IgE sensitization to fungi while sputum culture (using our modified method of mucolysis prior to culture) revealed airway mycosis in 86% of asthmatics in the series (Li, Tsai, et al., 2018).

We further demonstrated in a prospective analysis of CRS patients that only 50% of CRS patients had IgE detectable by ELISA against a panel of several common fungi, but over 80% of these patients manifested fungus-specific IL-4 (Th2) responses detectable in peripheral blood by enzyme linked immunocell spot (ELISpot) assay as compared to 0% in controls (Porter et al., 2014). This new T cell-based immunodiagnostic assay was based on the use of a panel of antigens derived from 9 common fungi isolated from the airways of allergic patients. However, when the fungal antigen used in the ELISpot assay was matched to a fungus actually isolated from the same patient, the positive rate improved further to 95% (Porter et al., 2014). Essentially identical results were obtained when the primary immunological outcome was fungus-specific cytokine secretion as compared to serum IgE measurements (Shin et al., 2004). Thus, traditional immediate hypersensitivity (IgE) testing performed via skin prick testing, radioallergosorbent (RAST) testing, or ELISA lack adequate sensitivity to serve as screening assays for detecting fungal sensitization. In contrast, multiple groups have independently demonstrated that T cell based immunodiagnostic approaches offer far greater sensitivity and might be suitable for further development as diagnostic adjuncts for allergic airway diseases.

Although evidence exists to suggest that cross reactivity often confounds serologic-based diagnosis of fungal hypersensitivity (Soeria-Atmadja, Onell, & Borga, 2010), the magnitude of this issue is not known.

Nonetheless, where systematic attempts to culture respiratory tract samples of subjects with allergic airway disease have been made, the results generally indicate that many fungal species can be isolated (Agbetile et al., 2012; Porter et al., 2014), although in some contexts *Alternaria alternata* could be a dominant, single pathogen (Shin et al., 2004). Thus, human allergic airway diseases are overwhelmingly linked to airway mycosis immunodiagnostically and through the physical isolation of fungi from relevant specimens.

Metagenomic approaches that involve the amplification of fungal DNA from airway samples offer the opportunity of far greater sensitivity for diagnosing airway mycosis. However, while the DNA of pathogenic fungi are readily identified from the induced sputum of asthma patients, fungi can also be identified from healthy control subjects, although significant differences in the composition of the airway mycobiota exist between individuals with and without chronic lung disease (van Woerden et al., 2013). Moreover, it remains unclear if individuals with normal lung function and no respiratory tract disease have *viable* fungi growing in their airways as has been repeatedly demonstrated for asthma, CRS, and many other allergic airway diseases (Li, Tsai, et al., 2018; Pashley, 2014; Pashley et al., 2012; Porter et al., 2014). Unfortunately, normal individuals do not produce sputum that represents the most appropriate single sample for globally assessing the airways for any pathogen by culture. Thus, the value of metagenomic analysis of sputum for diagnosing airway mycosis remains unresolved.

Additional lines of study suggest that development of a biomarker assay, i.e., an assay having high throughput and sensitivity that can be run on readily available biological samples such as serum and exhaled breath, are within reach. One component of the overall immune response against fungi includes the production of chitinases (also termed chitotriosidase) that degrade fungal cell walls in a manner analogous to how penicillins degrade bacterial cell walls (Elias, Homer, Hamid, & Lee, 2005). The extended human chitinase gene family includes 8 members, only two of which possess chitinase activity, chitotriosidase (CHIT1) and acidic mammalian chitinase (AMCase); the others are of largely unknown function. These molecules are strongly induced in allergic airway disease contexts, including asthma and cystic fibrosis, and genetic mutations of which are linked to asthma in diverse contexts (Bierbaum et al., 2005; Bierbaum, Superti-Furga, & Heinzmann, 2006; Hector et al., 2016; Vicencio et al., 2010).

Although the chitinase-like protein YKL-40 shows some promise in distinguishing some forms of asthma and COPD (Wang et al., 2018), it has limited utility in distinguishing different levels of asthma severity, at least

in the pediatric context (Santos, Davidson, Covar, & Spahn, 2014). Additional studies of true chitinases in asthma, CRS, and cystic fibrosis biomarkers are warranted, especially in the context of documented airway mycosis.

Although analytical methods remain to be perfected, exhaled breath is an attractive medium to search for biomarkers in allergic airway disease, potentially including volatile fungal products (Smith & Spanel, 2015). Unlike sputum and serum, exhaled breath condensate (EBC) is both universally available and safe to collect. Remarkably, fungal DNA can be recovered from EBC of asthmatic, but not healthy control subjects, suggesting that metagenomics applied to EBC could be useful in the diagnosis of airway mycosis in diverse clinical contexts (Carpagnano et al., 2016).

3.2 Therapy of airway mycosis-related airway diseases

As discussed in the preceding sections, numerous studies now overwhelmingly support the concept of airway mycosis as being the central cause of diverse upper and lower airway allergic inflammatory disorders, including CRSwNP, asthma, and many others. In part, this evidence is based on the results of controlled clinical trials and retrospective studies involving the use of antifungal agents in the treatment of these disorders. However, negative studies also exist, complicating interpretation of the overall role of antifungals to treat airway mycosis, a situation no better illustrated than with asthma (Agbetile et al., 2012; Denning et al., 2009). The most sophisticated such trials furthermore were Phase 2; Phase 3 randomized controlled trials of antifungals in asthma and other airway mycosis syndromes are notably lacking, further impeding acceptance of the importance of antifungal therapy in these conditions for those who insist on achieving this gold standard before accepting any new medical therapy.

Due to their cost, phase 3 medical trials are conducted almost exclusively by the pharmaceutical industry, which has no interest in underwriting these hugely expensive studies because most antifungal agents are now off patent, precluding the ability to generate profits even when granted a new use indication. This situation could change with the advent of new antifungals still on patent and the development of new antifungal formulations that “recharge” the original patent. Inhaled antifungals such as liposomal amphotericin B are particularly attractive for use in airway mycosis given the convenience (one or twice weekly aerosol treatments) and potentially cost effectiveness. Moreover, aerosol delivery of antifungal agents promises

to drastically reduce or eliminate side effects and enhance efficacy due to the ability to overcome antifungal resistance given the ability to deliver far greater concentrations of drug directly to the airway. Far more detailed study of this modality is required in order to realize this promise (Casciaro et al., 2015; Casey, Garrett, & Eaton, 2002; Hayes, Murphy, Lynch, & Feola, 2010; Johnson, 2012; Ram et al., 2016).

Anti-inflammatory therapy, currently a mainstay for conditions such as CRSwNP, asthma, ABPM and others, will continue to be an essential adjunctive therapy regardless of the how successful antifungal therapies ultimately become. There are two principal reasons for this. First, antifungals are slow to act, requiring a minimum of 12 weeks to experience significant efficacy in asthma (Li, Tsai, et al., 2018), but up to 32 weeks or longer to achieve full benefits depending on the extent of the disease and endpoints examined (Denning et al., 2009; Pasqualotto et al., 2009). Therapy for semi-invasive cases of chronic pulmonary aspergillosis and aspergillomas may require even longer therapy. Thus, the periodic disease exacerbations that are characteristic of asthma will continue to occur before the burden of airway mycosis, and therefore the risk of exacerbation, is significantly reduced through the use of antifungals. Anti-inflammatory therapy will therefore be required well into the course of any antifungal treatment regime for most patients with severe asthma, ABPM, and related afflictions.

Second, treatment-related, pro-inflammatory side effects of antifungal therapy are likely to be seen in many patients beginning antifungal therapy. These side effects include the emergence of transient urticaria and severe asthma exacerbations occurring within 1 month of initiating antifungal therapy. We estimate that these reactions, which may be similar to the Jarisch-Herxheimer reaction seen after initial antibiotic therapy for many bacterial infections (Butler, 2017), occur in 10–20% of our antifungal treated asthma and CRS patients (D. Corry, unpublished observations). Although these reactions are most likely a complication of antifungal therapy involving the release of antigens from deteriorating fungi, they should be managed with anti-inflammatory agents as with any exacerbation of allergic airway disease.

For many decades, the choice of anti-inflammatory agents for use in allergic airway disease was inhaled, oral, or intravenous corticosteroids. The advent of high potency anti-inflammatory biologics such as monoclonal antibodies directed against IL-5 (e.g., mepolizumab (Ortega et al., 2014)), the IL-4/IL-13 receptor (dupilumab (Castro et al., 2018)), and others herald a new era of choice in this regard, a full discussion of which is beyond this review. However, regardless of the anti-inflammatory agent used, a prime

objective of antifungal therapy should be the reduction and ultimately elimination of the need for adjunctive anti-inflammatory therapy administered through any route.

Because airway mycosis sufficient to provoke significant allergic disease is likely in many individuals to be the result of an underlying immune deficiency (Fig. 4), combined with the ubiquity of fungal aerosols that guarantee continuous reinfection, airway mycosis recurrence with the re-emergence of allergic airway disease is likely to occur even after prolonged antifungal therapy that initially produces complete remissions. This important issue is yet poorly studied, but it is clear that recurrent sinus and lower airway allergic disease is very common, occurring in up to 75% of treated subjects regardless of the therapeutic protocol (Campbell et al., 2006; Chishimba et al., 2012; Khalil et al., 2011; Koyama et al., 2014; Rains & Mineck, 2003). However, initial use of antifungals may reduce the severity of recurrent sinus disease and reduce the need for repeat surgery (Khalil et al., 2011; Rains & Mineck, 2003). Ultimately, many if not most patients with serious allergic airway disease will require life-long antifungal and perhaps anti-inflammatory therapy. Whether such therapy most appropriately takes the form of intermittent, intensive antifungal regimens or continuous, less intensive treatment is currently entirely unresolved. Chronic antifungal therapy in any form raises important concerns regarding the emergence of antifungal resistance and serious side effects, some of which that are uniquely seen in the context of chronic antifungal therapy (Epaulard et al., 2013; Ezra, Jourabchi, & Mousdicas, 2016; Miller, Cowen, Nguyen, McCalmont, & Fox, 2010). In summary, the enormous advances in our understanding of the role of fungi in AMRADs clearly warrant properly designed clinical trials to resolve the many questions surrounding the use of antifungals for AMRADs and the role of adjunctive anti-inflammatory therapy.

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References

- AbdulWahab, A., Salah, H., Chandra, P., & Taj-Aldeen, S. J. (2017). Persistence of *Candida dubliniensis* and lung function in patients with cystic fibrosis. *BMC Research Notes*, 10(1), 326.
- Adeyemo, A. O., Odelowo, E. O., & Makanjuola, D. I. (1984). Management of pulmonary aspergilloma in the presence of active tuberculosis. *Thorax*, 39(11), 862–867.

- Agarwal, R., Aggarwal, A. N., Garg, M., Saikia, B., Gupta, D., & Chakrabarti, A. (2012). Allergic bronchopulmonary aspergillosis with aspergilloma: An immunologically severe disease with poor outcome. *Mycopathologia*, 174(3), 193–201.
- Agarwal, R., Dhooria, S., Singh Sehgal, I., Aggarwal, A. N., Garg, M., Saikia, B., et al. (2018). A randomized trial of itraconazole vs prednisolone in acute-stage allergic bronchopulmonary aspergillosis complicating asthma. *Chest*, 153(3), 656–664.
- Agarwal, R., Garg, M., Aggarwal, A. N., Saikia, B., Gupta, D., & Chakrabarti, A. (2012). Serologic allergic bronchopulmonary aspergillosis (ABPA-S): Long-term outcomes. *Respiratory Medicine*, 106(7), 942–947.
- Agarwal, R., Vishwanath, G., Aggarwal, A. N., Garg, M., Gupta, D., & Chakrabarti, A. (2013). Itraconazole in chronic cavity pulmonary aspergillosis: A randomised controlled trial and systematic review of literature. *Mycoses*, 56(5), 559–570.
- Agbetile, J., Bourne, M., Fairs, A., Hargadon, B., Desai, D., Broad, C., et al. (2014). Effectiveness of voriconazole in the treatment of *Aspergillus fumigatus*-associated asthma (EVITA3 study). *The Journal of Allergy and Clinical Immunology*, 134(1), 33–39.
- Agbetile, J., Fairs, A., Desai, D., Hargadon, B., Bourne, M., Mutalithas, K., et al. (2012). Isolation of filamentous fungi from sputum in asthma is associated with reduced post-bronchodilator FEV1. *Clinical and Experimental Allergy*, 42(5), 782–791.
- Agondi, R. C., Barros, M. T., Rizzo, L. V., Kalil, J., & Giavina-Bianchi, P. (2010). Allergic asthma in patients with common variable immunodeficiency. *Allergy*, 65(4), 510–515.
- Al Khatib, S., Keles, S., Garcia-Lloret, M., Karakoc-Aydiner, E., Reisli, I., Artac, H., et al. (2009). Defects along the T(H)17 differentiation pathway underlie genetically distinct forms of the hyper IgE syndrome. *The Journal of Allergy and Clinical Immunology*, 124(2), 342–348.
- Alonso, R., Pisa, D., Fernandez-Fernandez, A. M., Rabano, A., & Carrasco, L. (2017). Fungal infection in neural tissue of patients with amyotrophic lateral sclerosis. *Neurobiology of Disease*, 108, 249–260.
- Altenburg, J., Wortel, K., van der Werf, T. S., & Boersma, W. G. (2015). Non-cystic fibrosis bronchiectasis: Clinical presentation, diagnosis and treatment, illustrated by data from a Dutch Teaching Hospital. *The Netherlands Journal of Medicine*, 73(4), 147–154.
- Alyasin, S., Moghtaderi, M., Farjadian, S., Babaei, M., & Teshnizi, S. H. (2018). Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis and non-cystic fibrosis bronchiectasis. *Electronic Physician*, 10(1), 6273–6278.
- Ando, T., Tochigi, N., Gocho, K., Moriya, A., Ikushima, S., Kumasaka, T., et al. (2016). Pathophysiological implication of computed tomography images of chronic pulmonary aspergillosis. *Japanese Journal of Infectious Diseases*, 69(2), 118–126.
- Antachopoulos, C., Walsh, T. J., & Roilides, E. (2007). Fungal infections in primary immunodeficiencies. *European Journal of Pediatrics*, 166(11), 1099–1117.
- Ayik, S. O., Basoglu, O. K., Erdinc, M., Bor, S., Veral, A., & Bilgen, C. (2003). Eosinophilic bronchitis as a cause of chronic cough. *Respiratory Medicine*, 97(6), 695–701.
- Azie, N., Neofytos, D., Pfaller, M., Meier-Kriesche, H. U., Quan, S. P., & Horn, D. (2012). The PATH (prospective antifungal therapy) alliance registry and invasive fungal infections: Update 2012. *Diagnostic Microbiology and Infectious Disease*, 73(4), 293–300.
- Bafadhel, M., McKenna, S., Agbetile, J., Fairs, A., Desai, D., Mistry, V., et al. (2014). *Aspergillus fumigatus* during stable state and exacerbations of COPD. *The European Respiratory Journal*, 43(1), 64–71.
- Balenga, N. A., Klichinsky, M., Xie, Z., Chan, E. C., Zhao, M., Jude, J., et al. (2015). A fungal protease allergen provokes airway hyper-responsiveness in asthma. *Nature Communications*, 6, 6763.
- Balzar, S., Strand, M., Nakano, T., & Wenzel, S. E. (2006). Subtle immunodeficiency in severe asthma: IgA and IgG2 correlate with lung function and symptoms. *International Archives of Allergy and Immunology*, 140(2), 96–102.

- Bateman, E. D. (1994). A new look at the natural history of *Aspergillus* hypersensitivity in asthmatics. *Respiratory Medicine*, 88(5), 325–327.
- Baum, G. L. (1960). The significance of *Candida albicans* in human sputum. *The New England Journal of Medicine*, 263, 70–73.
- Baur, X., & Fruhmman, G. (1979). Allergic reactions, including asthma, to the pineapple protease bromelain following occupational exposure. *Clinical Allergy*, 9(5), 443–450.
- Baxter, C. G., Dunn, G., Jones, A. M., Webb, K., Gore, R., Richardson, M. D., et al. (2013). Novel immunologic classification of aspergillosis in adult cystic fibrosis. *The Journal of Allergy and Clinical Immunology*, 132(3), 560–566.e10.
- Bent, J. P., 3rd, & Kuhn, F. A. (1994). Diagnosis of allergic fungal sinusitis. *Otolaryngology and Head and Neck Surgery*, 111(5), 580–588.
- Bierbaum, S., Nickel, R., Koch, A., Lau, S., Deichmann, K. A., Wahn, U., et al. (2005). Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. *American Journal of Respiratory and Critical Care Medicine*, 172(12), 1505–1509.
- Bierbaum, S., Superti-Furga, A., & Heinzmann, A. (2006). Genetic polymorphisms of chitotriosidase in Caucasian children with bronchial asthma. *International Journal of Immunogenetics*, 33(3), 201–204.
- Boitano, S., Flynn, A. N., Sherwood, C. L., Schulz, S. M., Hoffman, J., Gruzina, I., et al. (2011). *Alternaria alternata* serine proteases induce lung inflammation and airway epithelial cell activation via PAR2. *American Journal of Physiology—Lung Cellular and Molecular Physiology*, 300(4), L605–L614.
- Boos, A. C., Hagl, B., Schlesinger, A., Halm, B. E., Ballenberger, N., Pinarci, M., et al. (2014). Atopic dermatitis, STAT3- and DOCK8-hyper-IgE syndromes differ in IgE-based sensitization pattern. *Allergy*, 69(7), 943–953.
- Brandt, C., Roehmel, J., Rickerts, V., Melichar, V., Niemann, N., & Schwarz, C. (2018). *Aspergillus* bronchitis in patients with cystic fibrosis. *Mycopathologia*, 183(1), 61–69.
- Butler, T. (2017). The Jarisch-Herxheimer reaction after antibiotic treatment of spirochetal infections: A review of recent cases and our understanding of pathogenesis. *The American Journal of Tropical Medicine and Hygiene*, 96(1), 46–52.
- Caillaud, D., Leynaert, B., Keirsbulck, M., Nadif, R., & Awg, m. (2018). Indoor mould exposure, asthma and rhinitis: Findings from systematic reviews and recent longitudinal studies. *European Respiratory Review*, 27(148), 30.
- Campbell, J. M., Graham, M., Gray, H. C., Bower, C., Blaiss, M. S., & Jones, S. M. (2006). Allergic fungal sinusitis in children. *Annals of Allergy, Asthma & Immunology*, 96(2), 286–290.
- Cantor, H., & Shinohara, M. L. (2009). Regulation of T-helper-cell lineage development by osteopontin: The inside story. *Nature Reviews. Immunology*, 9(2), 137–141.
- Carpagnano, G. E., Malerba, M., Lacedonia, D., Susca, A., Logrieco, A., Carone, M., et al. (2016). Analysis of the fungal microbiome in exhaled breath condensate of patients with asthma. *Allergy and Asthma Proceedings*, 37(3), 41–46.
- Casciaro, R., Naselli, A., Cresta, F., Ros, M., Castagnola, E., & Minicucci, L. (2015). Role of nebulized amphotericin B in the management of allergic bronchopulmonary aspergillosis in cystic fibrosis: Case report and review of literature. *Journal of Chemotherapy*, 27(5), 307–311.
- Casey, P., Garrett, J., & Eaton, T. (2002). Allergic bronchopulmonary aspergillosis in a lung transplant patient successfully treated with nebulized amphotericin. *The Journal of Heart and Lung Transplantation*, 21(11), 1237–1241.
- Castro, M., Corren, J., Pavord, I. D., Maspero, J., Wenzel, S., Rabe, K. F., et al. (2018). Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *The New England Journal of Medicine*, 378, 2486–2496.
- Chan, S. K., & Gelfand, E. W. (2015). Primary immunodeficiency masquerading as allergic disease. *Immunology and Allergy Clinics of North America*, 35(4), 767–778.

- Chan, K. O., Genoway, K. A., & Javer, A. R. (2008). Effectiveness of itraconazole in the management of refractory allergic fungal rhinosinusitis. *Journal of Otolaryngology—Head & Neck Surgery*, 37(6), 870–874.
- Chiba, S., Tsuchiya, K., Nukui, Y., Sema, M., Tamaoka, M., Sumi, Y., et al. (2017). Interstitial changes in asthma–COPD overlap syndrome. *The Clinical Respiratory Journal*, 11(6), 1024–1031.
- Chishimba, L., Langridge, P., Powell, G., Niven, R. M., & Denning, D. W. (2015). Efficacy and safety of nebulised amphotericin B (NAB) in severe asthma with fungal sensitisation (SAFS) and allergic bronchopulmonary aspergillosis (ABPA). *The Journal of Asthma*, 52(3), 289–295.
- Chishimba, L., Niven, R. M., Cooley, J., & Denning, D. W. (2012). Voriconazole and posaconazole improve asthma severity in allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization. *The Journal of Asthma*, 49(4), 423–433.
- Chowdhary, A., Agarwal, K., Kathuria, S., Gaur, S. N., Randhawa, H. S., & Meis, J. F. (2014). Allergic bronchopulmonary mycosis due to fungi other than *Aspergillus*: A global overview. *Critical Reviews in Microbiology*, 40(1), 30–48.
- Chrldle, A., Mustakim, S., Bright-Thomas, R. J., Baxter, C. G., Felton, T., & Denning, D. W. (2012). *Aspergillus* bronchitis without significant immunocompromise. *Annals of the New York Academy of Sciences*, 1272, 73–85.
- Chu, E. Y., Freeman, A. F., Jing, H., Cowen, E. W., Davis, J., Su, H. C., et al. (2012). Cutaneous manifestations of DOCK8 deficiency syndrome. *Archives of Dermatology*, 148(1), 79–84.
- Chung, K. F., Wenzel, S. E., Brozek, J. L., Bush, A., Castro, M., Sterk, P. J., et al. (2014). International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *The European Respiratory Journal*, 43(2), 343–373.
- Clarke, D. L., Davis, N. H., Champion, C. L., Foster, M. L., Heasman, S. C., Lewis, A. R., et al. (2014). Dectin-2 sensing of house dust mite is critical for the initiation of airway inflammation. *Mucosal Immunology*, 7(3), 558–567.
- Coffman, R. L., Shrader, B., Carty, J., Mosmann, T. R., & Bond, M. W. (1987). A mouse T cell product that preferentially enhances IgA production. I. Biologic characterization. *Journal of Immunology*, 139(11), 3685–3690.
- Cohen, T. S., & Prince, A. (2012). Cystic fibrosis: A mucosal immunodeficiency syndrome. *Nature Medicine*, 18(4), 509–519.
- Coron, N., Pihet, M., Frealle, E., Lemeille, Y., Pinel, C., Pelloux, H., et al. (2018). Toward the standardization of mycological examination of sputum samples in cystic fibrosis: Results from a French multicenter prospective study. *Mycopathologia*, 183(1), 101–117.
- Corrao, W. M., Braman, S. S., & Irwin, R. S. (1979). Chronic cough as the sole presenting manifestation of bronchial asthma. *The New England Journal of Medicine*, 300(12), 633–637.
- Cucchetto, G., Cazzadori, A., Conti, M., Cascio, G. L., Braggio, P., & Concia, E. (2015). Treatment of chronic pulmonary aspergillosis with voriconazole: Review of a case series. *Infection*, 43(3), 277–286.
- Cullinan, P., Harris, J. M., Newman Taylor, A. J., Hole, A. M., Jones, M., Barnes, F., et al. (2000). An outbreak of asthma in a modern detergent factory. *Lancet*, 356(9245), 1899–1900.
- Da Silva, C. A., Pochard, P., Lee, C. G., & Elias, J. A. (2010). Chitin particles are multifaceted immune adjuvants. *American Journal of Respiratory and Critical Care Medicine*, 182(12), 1482–1491.
- Dannemiller, K. C., Gent, J. F., Leaderer, B. P., & Peccia, J. (2016). Indoor microbial communities: Influence on asthma severity in atopic and nonatopic children. *The Journal of Allergy and Clinical Immunology*, 138(1), 76–83.e1.

- DeConde, A. S., & Soler, Z. M. (2016). Chronic rhinosinusitis: Epidemiology and burden of disease. *American Journal of Rhinology & Allergy*, 30(2), 134–139.
- Den Beste, K. A., Hoddeson, E. K., Parkos, C. A., Nusrat, A., & Wise, S. K. (2012). Epithelial permeability alterations in an in vitro air-liquid interface model of allergic fungal rhinosinusitis. *International Forum of Allergy and Rhinology*, 3(1), 19–25.
- Denning, D. W., Cadranel, J., Beigelman-Aubry, C., Ader, F., Chakrabarti, A., Blot, S., et al. (2016). Chronic pulmonary aspergillosis: Rationale and clinical guidelines for diagnosis and management. *The European Respiratory Journal*, 47(1), 45–68.
- Denning, D. W., O'Driscoll, B. R., Powell, G., Chew, F., Atherton, G. T., Vyas, A., et al. (2009). Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitization: The fungal asthma sensitization trial (FAST) study. *American Journal of Respiratory and Critical Care Medicine*, 179(1), 11–18.
- Denning, D. W., Perlin, D. S., Muldoon, E. G., Colombo, A. L., Chakrabarti, A., Richardson, M. D., et al. (2017). Delivering on antimicrobial resistance agenda not possible without improving fungal diagnostic capabilities. *Emerging Infectious Diseases*, 23(2), 177–183.
- Denning, D. W., Pleuvry, A., & Cole, D. C. (2013). Global burden of chronic pulmonary aspergillosis complicating sarcoidosis. *The European Respiratory Journal*, 41(3), 621–626.
- Denning, D. W., Van Wye, J. E., Lewiston, N. J., & Stevens, D. A. (1991). Adjunctive therapy of allergic bronchopulmonary aspergillosis with itraconazole. *Chest*, 100(3), 813–819.
- Dogra, V., Sinha, A. K., Saxena, R., & Talwar, D. (2016). Aspergillus march: From ABPA to aspergilloma to subacute invasive aspergillosis. *Allergy, Asthma and Clinical Immunology*, 12, 64.
- Dolton, M. J., Ray, J. E., Chen, S. C., Ng, K., Pont, L. G., & McLachlan, A. J. (2012). Multicenter study of voriconazole pharmacokinetics and therapeutic drug monitoring. *Antimicrobial Agents and Chemotherapy*, 56(9), 4793–4799.
- Drummond, R. A., Dambuza, I. M., Vautier, S., Taylor, J. A., Reid, D. M., Bain, C. C., et al. (2016). CD4(+) T-cell survival in the GI tract requires dectin-1 during fungal infection. *Mucosal Immunology*, 9(2), 492–502.
- D'Silva, L., Hassan, N., Wang, H. Y., Kjarsgaard, M., Efthimiadis, A., Hargreave, F. E., et al. (2011). Heterogeneity of bronchitis in airway diseases in tertiary care clinical practice. *Canadian Respiratory Journal*, 18(3), 144–148.
- Duman, A. K., & Fulco, P. P. (2017). Adrenal insufficiency with voriconazole and inhaled/intranasal corticosteroids: Case report and systematic review. *Journal of Pharmacy Practice*, 30(4), 459–463.
- Elias, J. A., Homer, R. J., Hamid, Q., & Lee, C. G. (2005). Chitinases and chitinase-like proteins in T(H)2 inflammation and asthma. *The Journal of Allergy and Clinical Immunology*, 116(3), 497–500.
- Elphick, H. E., & Southern, K. W. (2016). Antifungal therapies for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database of Systematic Reviews*, 11, CD002204.
- el-Zaatari, F. A., Naser, S. A., Markesich, D. C., Kalter, D. C., Engstand, L., & Graham, D. Y. (1996). Identification of *Mycobacterium avium* complex in sarcoidosis. *Journal of Clinical Microbiology*, 34(9), 2240–2245.
- Engel, T. G. P., Slabbers, L., de Jong, C., Melchers, W. J. G., Hagen, F., Verweij, P. E., et al. (2018). Prevalence and diversity of filamentous fungi in the airways of cystic fibrosis patients—A Dutch, multicentre study. *Journal of Cystic Fibrosis*, 52. <https://doi.org/10.1016/j.jcf.2018.11.012>.
- Epaulard, O., Villier, C., Ravaud, P., Chosidow, O., Blanche, S., Mamzer-Bruneel, M. F., et al. (2013). A multistep voriconazole-related phototoxic pathway may lead to skin carcinoma: Results from a French nationwide study. *Clinical Infectious Diseases*, 57(12), e182–e188.

- Eppinger, T. M., Greenberger, P. A., White, D. A., Brown, A. E., & Cunningham-Rundles, C. (1999). Sensitization to *Aspergillus* species in the congenital neutrophil disorders chronic granulomatous disease and hyper-IgE syndrome. *The Journal of Allergy and Clinical Immunology*, 104(6), 1265–1272.
- Erazo, A., Kutchukhidze, N., Leung, M., Christ, A. P. G., Urban, J. F., Jr., Curotto de Lafaille, M. A., et al. (2007). Unique maturation program of the IgE response in vivo. *Immunity*, 26(2), 191–203.
- Everaerts, S., Lagrou, K., Vermeersch, K., Dupont, L. J., Vanaudenaerde, B. M., & Janssens, W. (2018). *Aspergillus fumigatus* detection and risk factors in patients with COPD-bronchiectasis overlap. *International Journal of Molecular Sciences*, 19(2), 1–11.
- Ewig, S., Schlochtermeier, M., Goïke, N., & Niederman, M. S. (2002). Applying sputum as a diagnostic tool in pneumonia: Limited yield, minimal impact on treatment decisions. *Chest*, 121(5), 1486–1492.
- Ezra, N., Jourabchi, N., & Mousdicas, N. (2016). Voriconazole-induced subacute cutaneous lupus erythematosus in an adult with Aspergillosis. *Skinmed*, 14(6), 461–463.
- Fairs, A., Agbetile, J., Hargadon, B., Bourne, M., Monteiro, W. R., Brightling, C. E., et al. (2010). IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *American Journal of Respiratory and Critical Care Medicine*, 182(11), 1362–1368.
- Farrant, J., Brice, H., Fowler, S., & Niven, R. (2016). Fungal sensitisation in severe asthma is associated with the identification of *Aspergillus fumigatus* in sputum. *The Journal of Asthma*, 53(7), 732–735.
- Fei, M., Bhatia, S., Oriss, T. B., Yarlagadda, M., Khare, A., Akira, S., et al. (2011). TNF-alpha from inflammatory dendritic cells (DCs) regulates lung IL-17A/IL-5 levels and neutrophilia versus eosinophilia during persistent fungal infection. *Proceedings of the National Academy of Sciences of the United States of America*, 108(13), 5360–5365.
- Finkelman, F. D., Katona, I. M., Urban, J. F., Jr., Holmes, J., Ohara, J., Tung, A. S., et al. (1988). IL-4 is required to generate and sustain in vivo IgE responses. *Journal of Immunology*, 141(7), 2335–2341.
- Fleming, L., Tsartsali, L., Wilson, N., Regamey, N., & Bush, A. (2012). Sputum inflammatory phenotypes are not stable in children with asthma. *Thorax*, 67(8), 675–681.
- Flood-Page, P. T., Menzies-Gow, A. N., Kay, A. B., & Robinson, D. S. (2003). Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *American Journal of Respiratory and Critical Care Medicine*, 167(2), 199–204.
- Florsheim, E., Yu, S., Bragatto, I., Faustino, L., Gomes, E., Ramos, R. N., et al. (2015). Integrated innate mechanisms involved in airway allergic inflammation to the serine protease subtilisin. *Journal of Immunology*, 194(10), 4621–4630.
- Foster, S. B., McIntosh, K., Thompson, B., Lu, M., Yin, W., Rich, K. C., et al. (2008). Increased incidence of asthma in HIV-infected children treated with highly active antiretroviral therapy in the National Institutes of health women and infants transmission study. *The Journal of Allergy and Clinical Immunology*, 122(1), 159–165.
- Fraczek, M. G., Chishimba, L., Niven, R. M., Bromley, M., Simpson, A., Smyth, L., et al. (2018). Corticosteroid treatment is associated with increased filamentous fungal burden in allergic fungal disease. *The Journal of Allergy and Clinical Immunology*, 142(2), 407–414.
- Frischmeyer-Guerrero, P. A., Guerrero, A. L., Oswald, G., Chichester, K., Myers, L., Halushka, M. K., et al. (2013). TGFbeta receptor mutations impose a strong predisposition for human allergic disease. *Science Translational Medicine*, 5(195), 195ra94.
- Fujimura, M., Ogawa, H., Nishizawa, Y., & Nishi, K. (2003). Comparison of atopic cough with cough variant asthma: Is atopic cough a precursor of asthma? *Thorax*, 58(1), 14–18.
- Fujimura, M., Ogawa, H., Yasui, M., & Matsuda, T. (2000). Eosinophilic tracheobronchitis and airway cough hypersensitivity in chronic non-productive cough. *Clinical and Experimental Allergy*, 30(1), 41–47.

- Fujita, M., Tao, Y., Kajiki, A., Ouchi, H., Harada, E., Ikegame, S., et al. (2012). The clinical efficacy and safety of micafungin-itraconazole combination therapy in patients with pulmonary aspergilloma. *Journal of Infection and Chemotherapy*, 18(5), 668–674.
- Fukahori, S., Matsuse, H., Tsuchida, T., Kawano, T., Nishino, T., Fukushima, C., et al. (2014). Clearance of *Aspergillus fumigatus* is impaired in the airway in allergic inflammation. *Annals of Allergy, Asthma & Immunology*, 113(2), 180–186.
- Gailhofer, G., Wilders-Truschnig, M., Smolle, J., & Ludvan, M. (1988). Asthma caused by bromelain: An occupational allergy. *Clinical Allergy*, 18(5), 445–450.
- Galán-Díez, M., Arana, D. M., Serrano-Gómez, D., Kremer, L., Casasnovas, J. M., Ortega, M., et al. (2010). *Candida albicans* beta-glucan exposure is controlled by the fungal CEK1-mediated mitogen-activated protein kinase pathway that modulates immune responses triggered through dectin-1. *Infection and Immunity*, 78(4), 1426–1436.
- Galiatsatos, P., Melia, M. T., & Silhan, L. L. (2016). Human immunodeficiency virus and allergic bronchopulmonary aspergillosis: Case report and review of literature. *Open Forum Infectious Diseases*, 3(2), ofw116.
- Gibson, P. G., Fujimura, M., & Niimi, A. (2002). Eosinophilic bronchitis: Clinical manifestations and implications for treatment. *Thorax*, 57(2), 178–182.
- Gileles-Hillel, A., Shoseyov, D., Polacheck, I., Korem, M., Kerem, E., & Cohen-Cymberknob, M. (2015). Association of chronic *Candida albicans* respiratory infection with a more severe lung disease in patients with cystic fibrosis. *Pediatric Pulmonology*, 50(11), 1082–1089.
- Godet, C., Cateau, E., Rammaert, B., Grosset, M., Le Moal, G., Beraud, G., et al. (2017). Nebulized liposomal amphotericin B for treatment of pulmonary infection caused by *hormographiella aspergillata*: Case report and literature review. *Mycopathologia*, 182(7–8), 709–713.
- Godet, C., Goudet, V., Laurent, F., Le Moal, G., Gounant, V., Frat, J. P., et al. (2015). Nebulised liposomal amphotericin B for *Aspergillus* lung diseases: Case series and literature review. *Mycoses*, 58(3), 173–180.
- Goh, K. J., Yui, A. C. A., Lapperre, T. S., Chan, A. K., Chew, F. T., Chotirmall, S. H., et al. (2017). Sensitization to *Aspergillus* species is associated with frequent exacerbations in severe asthma. *Journal of Asthma and Allergy*, 10, 131–140.
- Grunig, G., Warnock, M., Wakil, A. E., Venkayya, R., Brombacher, F., Rennick, D. M., et al. (1998). Requirement for IL-13 independently of IL-4 in experimental asthma. *Science*, 282(5397), 2261–2263.
- Gupta, K., Das, A., Joshi, K., Singh, N., Aggarwal, R., & Prakash, M. (2010). *Aspergillus* endocarditis in a known case of allergic bronchopulmonary aspergillosis: An autopsy report. *Cardiovascular Pathology*, 19(4), e137–e139.
- Gupta, P. R., Jain, S., & Kewlani, J. P. (2015). A comparative study of itraconazole in various dose schedules in the treatment of pulmonary aspergilloma in treated patients of pulmonary tuberculosis. *Lung India*, 32(4), 342–346.
- Habesoglu, M. A., Ugurlu, A. O., & Eyuboglu, F. O. (2011). Clinical, radiologic, and functional evaluation of 304 patients with bronchiectasis. *Annals of Thoracic Medicine*, 6(3), 131–136.
- Hadebe, S., Kirstein, F., Fierens, K., Chen, K., Drummond, R. A., Vautier, S., et al. (2015). Microbial ligand costimulation drives neutrophilic steroid-refractory asthma. *PLoS One*, 10(8), e0134219.
- Harada, M., Imokawa, S., Miwa, S., Nishihashi, F., Aono, Y., Amano, Y., et al. (2019). Chronic pulmonary aspergillosis may cause eosinophilic granulomatosis with polyangiitis via allergic bronchopulmonary aspergillosis. *Oxford Medical Case Reports*, 2019(2), omy126.
- Hashemizadeh, Z., Badiie, P., Malekhoseini, S. A., Raeisi Shahraki, H., Geramizadeh, B., & Montaseri, H. (2017). Observational study of associations between voriconazole therapeutic drug monitoring, toxicity, and outcome in liver transplant patients. *Antimicrobial Agents and Chemotherapy*, 61(12), e01211–17.

- Hayes, D., Jr., Murphy, B. S., Lynch, J. E., & Feola, D. J. (2010). Aerosolized amphotericin for the treatment of allergic bronchopulmonary aspergillosis. *Pediatric Pulmonology*, 45(11), 1145–1148.
- Head, K., Sharp, S., Chong, L. Y., Hopkins, C., & Philpott, C. (2018). Topical and systemic antifungal therapy for chronic rhinosinusitis. *Cochrane Database of Systematic Reviews*, (9), CD012453.
- Hector, A., Chotirmall, S. H., Lavelle, G. M., Mirkovic, B., Horan, D., Eichler, L., et al. (2016). Chitinase activation in patients with fungus-associated cystic fibrosis lung disease. *The Journal of Allergy and Clinical Immunology*, 138(4), 1183–1189.e4.
- Henderson, A. H. (1968). Allergic aspergillosis: Review of 32 cases. *Thorax*, 23(5), 501–512.
- Hesseling, A. C., Schaaf, H. S., Beyers, N., Gie, R. P., Marais, B. J., & Enarson, D. A. (2006). The bacteriologic yield in children with intrathoracic tuberculosis. *Clinical Infectious Diseases*, 42(8), e69–e71.
- Hinson, K. F., Moon, A. J., & Plummer, N. S. (1952). Broncho-pulmonary aspergillosis; A review and a report of eight new cases. *Thorax*, 7(4), 317–333.
- Hiraishi, Y., Yamaguchi, S., Yoshizaki, T., Nambu, A., Shimura, E., Takamori, A., et al. (2018). IL-33, IL-25 and TSLP contribute to development of fungal-associated protease-induced innate-type airway inflammation. *Scientific Reports*, 8(1), 18052.
- Hirano, T., Yamada, M., Sato, K., Murakami, K., Tamai, T., Mitsushashi, Y., et al. (2017). Invasive pulmonary mucormycosis: Rare presentation with pulmonary eosinophilia. *BMC Pulmonary Medicine*, 17(1), 76.
- Hongjia, L., Caiqing, Z., Degan, L., Fen, L., Chao, W., Jinxiang, W., et al. (2014). IL-25 promotes Th2 immunity responses in airway inflammation of asthmatic mice via activation of dendritic cells. *Inflammation*, 37(4), 1070–1077.
- Hoover, W. C., Britton, L. J., Gardner, J., Jackson, T., & Gutierrez, H. (2011). Rapid onset of iatrogenic adrenal insufficiency in a patient with cystic fibrosis-related liver disease treated with inhaled corticosteroids and a moderate CYP3A4 inhibitor. *The Annals of Pharmacotherapy*, 45(7–8), e38.
- Hurst, S. D., Seymour, B. W., Muchamuel, T., Kurup, V. P., & Coffman, R. L. (2001). Modulation of inhaled antigen-induced IgE tolerance by ongoing Th2 responses in the lung. *Journal of Immunology*, 166(8), 4922–4930.
- Husebye, K. O. (1967). Serial studies in a case of pulmonary aspergillosis. *Diseases of the Chest*, 51(3), 327–332.
- Imataki, O., Yamaguchi, K., Uemura, M., & Fukuoka, N. (2018). Voriconazole concentration is inversely correlated with corticosteroid usage in immunocompromised patients. *Transplant Infectious Disease*, 20(4), e12886.
- Imbeau, S. A., Nichols, D., Flaherty, D., Dickie, H., & Reed, C. (1978). Relationships between prednisone therapy, disease activity, and the total serum IgE level in allergic bronchopulmonary aspergillosis. *The Journal of Allergy and Clinical Immunology*, 62(2), 91–95.
- Ishiguro, T., Takayanagi, N., Baba, Y., Takaku, Y., Kagiya, N., & Sugita, Y. (2016). Pulmonary nontuberculous mycobacteriosis and chronic lower respiratory tract infections in patients with allergic bronchopulmonary mycosis without cystic fibrosis. *Internal Medicine*, 55(9), 1067–1070.
- Ishiguro, T., Takayanagi, N., Takaku, Y., Kagiya, N., Kurashima, K., & Sugita, Y. (2016). Combined allergic bronchopulmonary aspergillosis and eosinophilic granulomatosis with polyangiitis: Three cases and a review of the literature. *Internal Medicine*, 55(7), 793–797.
- Israel, H. L., Lenchner, G. S., & Atkinson, G. W. (1982). Sarcoidosis and aspergilloma. The role of surgery. *Chest*, 82(4), 430–432.
- Ito, S., & Mikawa, H. (1990). Immunological aspects of asthma (prophylaxis). *Acta Paediatrica Japonica*, 32(2), 192–196.

- Izumikawa, K., Tashiro, T., Tashiro, M., Takazono, T., Kosai, K., Morinaga, Y., et al. (2014). Pathogenesis and clinical features of chronic pulmonary aspergillosis—Is it possible to distinguish CNPA and CCPA clinically? *Journal of Infection and Chemotherapy*, 20(3), 208–212.
- Jin, J., Liu, X., & Sun, Y. (2014). The prevalence of increased serum IgE and *Aspergillus* sensitization in patients with COPD and their association with symptoms and lung function. *Respiratory Research*, 15, 130.
- Johnson, D. C. (2012). Chronic candidal bronchitis: A consecutive series. *Open Respiratory Medicine Journal*, 6, 145–149.
- Jones, W., Chastain, C. A., & Wright, P. W. (2014). Iatrogenic cushing syndrome secondary to a probable interaction between voriconazole and budesonide. *Pharmacotherapy*, 34(7), e116–e119.
- Kali, A., Charles, M. P., Noyal, M. J., Sivaraman, U., Kumar, S., & Easow, J. M. (2013). Prevalence of candida co-infection in patients with pulmonary tuberculosis. *The Australasian Medical Journal*, 6(8), 387–391.
- Kauffman, H. F., Tomee, J. F., van der Werf, T. S., de Monchy, J. G., & Koeter, G. K. (1995). Review of fungus-induced asthmatic reactions. *American Journal of Respiratory and Critical Care Medicine*, 151(6), 2109–2115.
- Kawayama, T., Fujiki, R., Honda, J., Rikimaru, T., & Aizawa, H. (2003). High concentration of (1→3)-beta-D-glucan in BAL fluid in patients with acute eosinophilic pneumonia. *Chest*, 123(4), 1302–1307.
- Kelada, S. N., Wilson, M. S., Tavarez, U., Kubalanza, K., Borate, B., Whitehead, G. S., et al. (2011). Strain-dependent genomic factors affect allergen-induced airway hyper-responsiveness in mice. *American Journal of Respiratory Cell and Molecular Biology*, 45(4), 817–824.
- Khalil, Y., Tharwat, A., Abdou, A. G., Essa, E., Elsayy, A. H., Elnakib, O., et al. (2011). The role of antifungal therapy in the prevention of recurrent allergic fungal rhinosinusitis after functional endoscopic sinus surgery: A randomized, controlled study. *Ear, Nose, & Throat Journal*, 90(8), E1–E7.
- Kheradmand, F., Kiss, A., Xu, J., Lee, S. H., Kolattukudy, P. E., & Corry, D. B. (2002). A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *Journal of Immunology*, 169(10), 5904–5911.
- Kim, D. Y., Cho, S. H., Takabayashi, T., & Schleimer, R. P. (2015). Chronic rhinosinusitis and the coagulation system. *Allergy, Asthma & Immunology Research*, 7(5), 421–430.
- Kim, S. H., Clark, S. T., Surendra, A., Copeland, J. K., Wang, P. W., Ammar, R., et al. (2015). Global analysis of the fungal microbiome in cystic fibrosis patients reveals loss of function of the transcriptional repressor Nrg1 as a mechanism of pathogen adaptation. *PLoS Pathogens*, 11(11), e1005308.
- Kim, C. K., Kim, J. T., Kang, H., Yoo, Y., & Koh, Y. Y. (2003). Sputum eosinophilia in cough-variant asthma as a predictor of the subsequent development of classic asthma. *Clinical and Experimental Allergy*, 33(10), 1409–1414.
- Kim, J. H., Park, H. J., Choi, G. S., Kim, J. E., Ye, Y. M., Nahm, D. H., et al. (2010). Immunoglobulin G subclass deficiency is the major phenotype of primary immunodeficiency in a Korean adult cohort. *Journal of Korean Medical Science*, 25(6), 824–828.
- King, P. T., Holdsworth, S. R., Freezer, N. J., Villanueva, E., & Holmes, P. W. (2006). Characterisation of the onset and presenting clinical features of adult bronchiectasis. *Respiratory Medicine*, 100(12), 2183–2189.
- Kiss, A., Montes, M., Susarla, S., Jaensson, E. A., Drouin, S. M., Wetsel, R. A., et al. (2007). A new mechanism regulating the initiation of allergic airway inflammation. *The Journal of Allergy and Clinical Immunology*, 120(2), 334–342.
- Kohno, S., Izumikawa, K., Yoshida, M., Takesue, Y., Oka, S., Kamei, K., et al. (2013). A double-blind comparative study of the safety and efficacy of caspofungin versus

- micafungin in the treatment of candidiasis and aspergillosis. *European Journal of Clinical Microbiology & Infectious Diseases*, 32(3), 387–397.
- Kosmidis, C., & Denning, D. W. (2015). The clinical spectrum of pulmonary aspergillosis. *Thorax*, 70(3), 270–277.
- Kosmidis, C., Newton, P., Muldoon, E. G., & Denning, D. W. (2017). Chronic fibrosing pulmonary aspergillosis: A cause of 'destroyed lung' syndrome. *Infectious Diseases*, 49, 296–301.
- Kouzaki, H., Matsumoto, K., Kikuoka, H., Kato, T., Tojima, I., Shimizu, S., et al. (2016). Endogenous protease inhibitors in airway epithelial cells contribute to eosinophilic chronic rhinosinusitis. *American Journal of Respiratory and Critical Care Medicine*, 195(6), 737–747.
- Koyama, K., Ohshima, N., Suzuki, J., Kawashima, M., Okuda, K., Sato, R., et al. (2015). Evaluation of clinical characteristics and prognosis of chronic pulmonary aspergillosis depending on the underlying lung diseases: Emphysema vs prior tuberculosis. *Journal of Infection and Chemotherapy*, 21(11), 795–801.
- Koyama, K., Ohshima, N., Suzuki, J., Kawashima, M., Takeda, K., Ando, T., et al. (2014). Recurrence of chronic pulmonary aspergillosis after discontinuation of maintenance treatment by antifungal triazoles. *Journal of Infection and Chemotherapy*, 20(6), 375–379.
- Krasnick, J., Patterson, R., & Roberts, M. (1995). Allergic bronchopulmonary aspergillosis presenting with cough variant asthma and identifiable source of *Aspergillus fumigatus*. *Annals of Allergy, Asthma & Immunology*, 75(4), 344–346.
- Krause, R., Moissl-Eichinger, C., Halwachs, B., Gorkiewicz, G., Berg, G., Valentin, T., et al. (2017). Mycobiome in the lower respiratory tract—A clinical perspective. *Frontiers in Microbiology*, 7, 2169.
- Kumar, R. (2003). Mild, moderate, and severe forms of allergic bronchopulmonary aspergillosis: A clinical and serologic evaluation. *Chest*, 124(3), 890–892.
- Kwizera, R., Parkes-Ratanshi, R., Page, I. D., Sekaggya-Wiltshire, C., Musaaazi, J., Fehr, J., et al. (2017). Elevated aspergillus-specific antibody levels among HIV infected ugandans with pulmonary tuberculosis. *BMC Pulmonary Medicine*, 17(1), 149.
- Lathrop, M. J., Brooks, E. M., Bonenfant, N. R., Sokocevic, D., Borg, Z. D., Goodwin, M., et al. (2014). Mesenchymal stromal cells mediate *Aspergillus* hyphal extract-induced allergic airway inflammation by inhibition of the Th17 signaling pathway. *Stem Cells Translational Medicine*, 3(2), 194–205.
- Lebrun-Vignes, B., Archer, V. C., Diquet, B., Levron, J. C., Chosidow, O., Puech, A. J., et al. (2001). Effect of itraconazole on the pharmacokinetics of prednisolone and methylprednisolone and cortisol secretion in healthy subjects. *British Journal of Clinical Pharmacology*, 51(5), 443–450.
- Li, L., Jiang, Z., & Shao, C. (2018). Pulmonary aspergillus overlap syndromes. *Mycopathologia*, 183(2), 431–438.
- Li, E., Tsai, C.-L., Maskatia, Z. K., Kakkar, E., Porter, P., Rossen, R. D., et al. (2018). Benefits of antifungal therapy in asthma patients with airway mycosis: A retrospective cohort analysis. *Immunity, Inflammation and Disease*, 6, 264–275. <https://doi.org/10.1002/iid3.215>.
- Lilly, L. M., Gessner, M. A., Dunaway, C. W., Metz, A. E., Schwiebert, L., Weaver, C. T., et al. (2012). The beta-glucan receptor dectin-1 promotes lung immunopathology during fungal allergy via IL-22. *Journal of Immunology*, 189(7), 3653–3660.
- Lionakis, M. S., & Kontoyiannis, D. P. (2004). The significance of isolation of saprophytic molds from the lower respiratory tract in patients with cancer. *Cancer*, 100(1), 165–172.
- Loeys, B. L., & Dietz, H. C. (1993). *Loeys-Dietz syndrome*. Seattle: University of Washington.
- Lofgren, S., & Lindgren, A. G. (1959). Cavern formation in pulmonary sarcoidosis. *Acta Chirurgica Scandinavica. Supplementum*, (Suppl. 245), 113–118.

- Lotvall, J., Akdis, C. A., Bacharier, L. B., Bjermer, L., Casale, T. B., Custovic, A., et al. (2011). Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. *The Journal of Allergy and Clinical Immunology*, 127(2), 355–360.
- Loughlin, J. E., Cole, J. A., Rothman, K. J., & Johnson, E. S. (2002). Prevalence of serious eosinophilia and incidence of Churg-Strauss syndrome in a cohort of asthma patients. *Annals of Allergy, Asthma & Immunology*, 88(3), 319–325.
- Lowes, D., Chishimba, L., Greaves, M., & Denning, D. W. (2015). Development of chronic pulmonary aspergillosis in adult asthmatics with ABPA. *Respiratory Medicine*, 109(12), 1509–1515.
- Loza, M. J., Djukanovic, R., Chung, K. F., Horowitz, D., Ma, K., Branigan, P., et al. (2016). Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. *Respiratory Research*, 17(1), 165.
- Luong, A., Davis, L. S., & Marple, B. F. (2009). Peripheral blood mononuclear cells from allergic fungal rhinosinusitis adults express a Th2 cytokine response to fungal antigens. *American Journal of Rhinology & Allergy*, 23(3), 281–287.
- Ma, C. S., Chew, G. Y., Simpson, N., Priyadarshi, A., Wong, M., Grimbacher, B., et al. (2008). Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *The Journal of Experimental Medicine*, 205(7), 1551–1557.
- Maghrabi, F., & Denning, D. W. (2017). The management of chronic pulmonary aspergillosis: The UK National Aspergillosis Centre approach. *Current Fungal Infection Reports*, 11(4), 242–251.
- Majewski, S., Ciebiada, M., Domagala, M., Kurmanowska, Z., & Gorski, P. (2015). Short-term reproducibility of the inflammatory phenotype in different subgroups of adult asthma cohort. *Mediators of Inflammation*, 2015, 419039.
- Mak, G., Porter, P. C., Bandi, V., Kheradmand, F., & Corry, D. B. (2013). Tracheobronchial mycosis in a retrospective case-series study of five status asthmaticus patients. *Clinical Immunology*, 146(2), 77–83.
- Marchand, E., Etienne-Mastroianni, B., Chanez, P., Lauque, D., Leclerc, P., & Cordier, J. F. (2003). Idiopathic chronic eosinophilic pneumonia and asthma: How do they influence each other? *The European Respiratory Journal*, 22(1), 8–13.
- Mark, B. J., Becker, B. A., Halloran, D. R., Bree, A. F., Sindwani, R., Fete, M. D., et al. (2012). Prevalence of atopic disorders and immunodeficiency in patients with ectodermal dysplasia syndromes. *Annals of Allergy, Asthma & Immunology*, 108(6), 435–438.
- Masaki, K., Fukunaga, K., Matsusaka, M., Kabata, H., Tanosaki, T., Mochimaru, T., et al. (2017). Characteristics of severe asthma with fungal sensitization. *Annals of Allergy, Asthma & Immunology*, 119(3), 253–257.
- Maturu, V. N., & Agarwal, R. (2015). Acute invasive pulmonary aspergillosis complicating allergic bronchopulmonary aspergillosis: Case report and systematic review. *Mycopathologia*, 180(3–4), 209–215.
- Medrek, S. K., Kao, C. C., Yang, D. H., Hanania, N. A., & Parulekar, A. D. (2017). Fungal sensitization is associated with increased risk of life-threatening asthma. *The Journal of Allergy and Clinical Immunology. In Practice*, 5(4), 1025–1031.e2.
- Miller, D. D., Cowen, E. W., Nguyen, J. C., McCalmont, T. H., & Fox, L. P. (2010). Melanoma associated with long-term voriconazole therapy: A new manifestation of chronic photosensitivity. *Archives of Dermatology*, 146(3), 300–304.
- Millien, V. O., Lu, W., Shaw, J., Yuan, X., Mak, G., Roberts, L., et al. (2013). Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science*, 341(6147), 792–796.
- Mirani, G., Williams, P. L., Chernoff, M., Abzug, M. J., Levin, M. J., Seage, G. R., 3rd, et al. (2015). Changing trends in complications and mortality rates among US youth and young adults with HIV infection in the era of combination antiretroviral therapy. *Clinical Infectious Diseases*, 61(12), 1850–1861.

- Molofsky, A. B., Savage, A. K., & Locksley, R. M. (2015). Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity*, 42(6), 1005–1019.
- Montoro, D. T., Haber, A. L., Biton, M., Vinarsky, V., Lin, B., Birket, S. E., et al. (2018). A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature*, 1(7718), 319–324.
- Mori, T., & Matsumura, M. (1999). Clinical evaluation of diagnostic methods using plasma and/or serum for three mycoses: Aspergillosis, candidosis, and pneumocystosis. *Nihon Ishinkin Gakkai Zasshi*, 40(4), 223–230.
- Morimoto, Y., Hirahara, K., Kiuchi, M., Wada, T., Ichikawa, T., Kanno, T., et al. (2018). Amphiregulin-producing pathogenic memory T helper 2 cells instruct eosinophils to secrete osteopontin and facilitate airway fibrosis. *Immunity*, 49(1), 134–150.e6.
- Naito, M., & Kurahara, Y. (2017). Coexistence of pulmonary aspergilloma and allergic bronchopulmonary aspergillosis. *Internal Medicine*, 56(15), 2077–2078.
- Navabi, B., & Upton, J. E. (2016). Primary immunodeficiencies associated with eosinophilia. *Allergy, Asthma and Clinical Immunology*, 12, 27.
- Netea, M. G., Kullberg, B. J., & van der Meer, J. W. (2005). Severely impaired IL-12/IL-18/IFN γ axis in patients with hyper IgE syndrome. *European Journal of Clinical Investigation*, 35(11), 718–721.
- Newton, P. J., Harris, C., Morris, J., & Denning, D. W. (2016). Impact of liposomal amphotericin B therapy on chronic pulmonary aspergillosis. *The Journal of Infection*, 73(5), 485–495.
- Niimi, A., Amitani, R., Suzuki, K., Tanaka, E., Murayama, T., & Kuze, F. (1998). Eosinophilic inflammation in cough variant asthma. *The European Respiratory Journal*, 11(5), 1064–1069.
- Niimi, A., & Chung, K. F. (2004). Airway inflammation and remodelling changes in patients with chronic cough: Do they tell us about the cause of cough? *Pulmonary Pharmacology & Therapeutics*, 17(6), 441–446.
- Odabasi, Z., Mattiuzzi, G., Estey, E., Kantarjian, H., Saeki, F., Ridge, R. J., et al. (2004). Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: Validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clinical Infectious Diseases*, 39(2), 199–205.
- O'Driscoll, B. R., Hopkinson, L. C., & Denning, D. W. (2005). Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulmonary Medicine*, 5(1), 4–14.
- Ogawa, H., Fujimura, M., Amaike, S., Matsumoto, Y., Kitagawa, M., & Matsuda, T. (1997). Eosinophilic pneumonia caused by *Alternaria alternata*. *Allergy*, 52(10), 1005–1008.
- Ogawa, H., Fujimura, M., Ohkura, N., & Makimura, K. (2014). It is time to call attention to the clinical significance of fungal colonization in chronic cough. *Allergology International*, 63(4), 611–612.
- Ogawa, H., Fujimura, M., Ohkura, N., & Makimura, K. (2015). Effects of nebulized amphotericin B and budesonide inhalation for chronic cough-related laryngeal sensations. *Auris, Nasus, Larynx*, 42(3), 221–225.
- Ogawa, H., Fujimura, M., Takeuchi, Y., & Makimura, K. (2009). The importance of basidiomycetous fungi cultured from the sputum of chronic idiopathic cough: A study to determine the existence of recognizable clinical patterns to distinguish CIC from non-CIC. *Respiratory Medicine*, 103(10), 1492–1497.
- Ogawa, H., Fujimura, M., Takeuchi, Y., & Makimura, K. (2013). Clinical experience with low-dose itraconazole in chronic idiopathic cough. *Cough*, 9(1), 1.
- Orlandi, R. R., Kingdom, T. T., Hwang, P. H., Smith, T. L., Alt, J. A., Baroody, F. M., et al. (2016). International consensus statement on allergy and rhinology: Rhinosinusitis. *International Forum of Allergy and Rhinology*, 6(Suppl. 1), S22–209.

- Ortega, H. G., Liu, M. C., Pavord, I. D., Brusselle, G. G., FitzGerald, J. M., Chetta, A., et al. (2014). Mepolizumab treatment in patients with severe eosinophilic asthma. *The New England Journal of Medicine*, 371(13), 1198–1207.
- Oshikata, C., Watanabe, M., Saito, A., Ishida, M., Kobayashi, S., Konuma, R., et al. (2017). Allergic Bronchopulmonary Mycosis due to Exposure to Eurotium herbariorum after the Great East Japan Earthquake. *Prehospital and Disaster Medicine*, 32(6), 688–690.
- Ozcan, C., Metin, A., Erkocoglu, M., & Kocabas, C. N. (2014). Allergic diseases in children with primary immunodeficiencies. *The Turkish Journal of Pediatrics*, 56(1), 41–47.
- Pacheco, A., Cuevas, M., Carbelo, B., Maiz, L., Pavon, M. J., Perez, I., et al. (1998). Eosinophilic lung disease associated with *Candida albicans* allergy. *The European Respiratory Journal*, 12(2), 502–504.
- Pakdaman, M. N., Corry, D. B., & Luong, A. (2011). Fungi linking the pathophysiology of chronic rhinosinusitis with nasal polyps and allergic asthma. *Immunological Investigations*, 40(7–8), 767–785.
- Pashley, C. H. (2014). Fungal culture and sensitisation in asthma, cystic fibrosis and chronic obstructive pulmonary disorder: What does it tell us? *Mycopathologia*, 178(5–6), 457–463.
- Pashley, C. H., Fairs, A., Morley, J. P., Tabor, S., Agbetele, J., Bafadhel, M., et al. (2012). Routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence. *Medical Mycology*, 50(4), 433–438.
- Pasqualotto, A. C., Powell, G., Niven, R., & Denning, D. W. (2009). The effects of antifungal therapy on severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis. *Respirology*, 14(8), 1121–1127.
- Patiroglu, T., Gungor, H. E., Lazaroski, S., & Unal, E. (2013). Chronic granulomatous disease with markedly elevated IgE levels mimicking hyperimmunoglobulin E syndrome. *Acta Microbiologica et Immunologica Hungarica*, 60(2), 155–162.
- Patro, S. K., Verma, R. K., Panda, N. K., Chakrabarti, A., & Singh, P. (2015). Efficacy of preoperative itraconazole in allergic fungal rhinosinusitis. *American Journal of Rhinology & Allergy*, 29(4), 299–304.
- Patterson, R., Rosenberg, M., & Roberts, M. (1977). Evidence that *Aspergillus fumigatus* growing in the airway of man can be a potent stimulus of specific and nonspecific IgE formation. *The American Journal of Medicine*, 63(2), 257–262.
- Patterson, T. F., Thompson, G. R., 3rd, Denning, D. W., Fishman, J. A., Hadley, S., Herbrecht, R., et al. (2016). Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the infectious diseases society of America. *Clinical Infectious Diseases*, 63(4), e1–e60.
- Pei, Y., Geng, S., Liu, L., Yan, F., Guan, H., Hou, J., et al. (2013). Fel d 1-airway inflammation prevention and treatment by co-immunization vaccine via induction of CD4+ CD25-Foxp3+ Treg cells. *Human Vaccines & Immunotherapeutics*, 9(5), 1019–1031.
- Pepys, J. (1973). Immunological and clinical findings in workers and consumers exposed to enzymes of *Bacillus subtilis*. *Proceedings of the Royal Society of Medicine*, 66(9), 930–932.
- Pepys, J., Longbottom, J. L., Hargreave, F. E., & Faux, J. (1969). Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet*, 1(7607), 1181–1184.
- Philit, F., Etienne-Mastroianni, B., Parrot, A., Guerin, C., Robert, D., & Cordier, J. F. (2002). Idiopathic acute eosinophilic pneumonia: A study of 22 patients. *American Journal of Respiratory and Critical Care Medicine*, 166(9), 1235–1239.
- Pilmis, B., Coignard-Biehler, H., Rouzaud, C., Jullien, V., Lanternier, F., & Lortholary, O. (2017). Acute reversible pancreatitis induced by posaconazole. *The Journal of Antimicrobial Chemotherapy*, 72(2), 628–630.
- Ponikau, J. U., Sherris, D. A., Kern, E. B., Homburger, H. A., Frigas, E., Gaffey, T. A., et al. (1999). The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clinic Proceedings*, 74(9), 877–884.

- Porter, P., Fields, A., Qian, Y., Abramson, S., Delclos, G. L., Kheradmand, F., et al. (2011). Necessary and sufficient role for T helper cells to prevent fungal dissemination during mucosal airway infection. *Infection and Immunity*, 79, 4459–4471.
- Porter, P. C., Lim, D. J., Maskatia, Z. K., Mak, G., Tsai, C. L., Citardi, M. J., et al. (2014). Airway surface mycosis in chronic TH2-associated airway disease. *The Journal of Allergy and Clinical Immunology*, 134(2), 325–331.
- Porter, P. C., Onger, V., Luong, A., Kheradmand, F., & Corry, D. B. (2011). Seeking common pathophysiology in asthma, atopy and sinusitis. *Trends in Immunology*, 32(2), 43–49.
- Porter, P., Susarla, S. C., Polikepahad, S., Qian, Y., Hampton, J., Kiss, A., et al. (2009). Link between allergic asthma and airway mucosal infection suggested by proteinase-secreting household fungi. *Mucosal Immunology*, 2(6), 504–517.
- Postma, D. S., & Rabe, K. F. (2015). The asthma-COPD overlap syndrome. *The New England Journal of Medicine*, 373(13), 1241–1249.
- Promsopa, C., Kansara, S., Citardi, M. J., Fakhri, S., Porter, P., & Luong, A. (2016). Prevalence of confirmed asthma varies in chronic rhinosinusitis subtypes. *International Forum of Allergy and Rhinology*, 6(4), 373–377.
- Ragab, A., Clement, P., & Vincken, W. (2004). Objective assessment of lower airway involvement in chronic rhinosinusitis. *American Journal of Rhinology*, 18(1), 15–21.
- Rai, S. P., Panda, B. N., & Bhargava, S. (2004). Treatment of allergic bronchopulmonary aspergillosis with fluconazole and itraconazole. *Medical Journal Armed Forces India*, 60(2), 128–130.
- Rains, B. M., 3rd, & Mineck, C. W. (2003). Treatment of allergic fungal sinusitis with high-dose itraconazole. *American Journal of Rhinology*, 17(1), 1–8.
- Ram, B., Aggarwal, A. N., Dhooria, S., Sehgal, I. S., Garg, M., Behera, D., et al. (2016). A pilot randomized trial of nebulized amphotericin in patients with allergic bronchopulmonary aspergillosis. *The Journal of Asthma*, 53(5), 517–524.
- Ramirez-Vargas, N., Arabin-Oropeza, S. E., Mojica-Martinez, D., Yamazaki-Nakashimada, M. A., de la Luz Garcia-Cruz, M., Teran-Juarez, L. M., et al. (2014). Clinical and immunological features of common variable immunodeficiency in Mexican patients. *Allergologia et Immunopathologia*, 42(3), 235–240.
- Rautemaa, V., Green, H. D., Jones, A. M., & Rautemaa-Richardson, R. (2017). High level of beta-(1,3)-d-glucan antigenaemia in cystic fibrosis in the absence of invasive fungal disease. *Diagnostic Microbiology and Infectious Disease*, 88(4), 316–321.
- Reece, E., Segurado, R., Jackson, A., McClean, S., Renwick, J., & Grealley, P. (2017). Co-colonisation with *Aspergillus fumigatus* and *Pseudomonas aeruginosa* is associated with poorer health in cystic fibrosis patients: An Irish registry analysis. *BMC Pulmonary Medicine*, 17(1), 70.
- Reese, T. A., Liang, H.-E., Tager, A. M., Luster, A. D., Van Rooijen, N., Voehringer, D., et al. (2007). Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature*, 447(7140), 92–96.
- Riley, D. J., Mackenzie, J. W., Uhlman, W. E., & Edelman, N. H. (1975). Allergic bronchopulmonary aspergillosis: Evidence of limited tissue invasion. *The American Review of Respiratory Disease*, 111(2), 232–236.
- Rodrigues, J., Caruthers, C., Azmeh, R., Dykewicz, M. S., Slavin, R. G., & Knutsen, A. P. (2016). The spectrum of allergic fungal diseases of the upper and lower airways. *Expert Review of Clinical Immunology*, 12(5), 531–550.
- Rodriguez-Goncer, I., Niven, R., & Denning, D. W. (2017). Successful long-term terbinafine therapy in an asthmatic patient with *Aspergillus* sensitization and bronchiectasis. *Medical Mycology Case Reports*, 16, 31–33.
- Rosenberg, M., Patterson, R., Roberts, M., & Wang, J. (1978). The assessment of immunologic and clinical changes occurring during corticosteroid therapy for allergic bronchopulmonary aspergillosis. *The American Journal of Medicine*, 64(4), 599–606.

- Roth, R., & Schatz, M. (2013). Allergic bronchopulmonary aspergillosis presenting as chronic cough in an elderly woman without previously documented asthma. *The Permanente Journal*, 17(2), e103–e108.
- Sade, K., Mysels, A., Levo, Y., & Kivity, S. (2007). Eosinophilia: A study of 100 hospitalized patients. *European Journal of Internal Medicine*, 18(3), 196–201.
- Saini, S. K., Boas, S. R., Jerath, A., Roberts, M., & Greenberger, P. A. (1998). Allergic bronchopulmonary mycosis to *Fusarium vasinfectum* in a child. *Annals of Allergy, Asthma & Immunology*, 80(5), 377–380.
- Salez, F., Bricchet, A., Desurmont, S., Grosbois, J. M., Wallaert, B., & Tonnel, A. B. (1999). Effects of itraconazole therapy in allergic bronchopulmonary aspergillosis. *Chest*, 116(6), 1665–1668.
- Sandhu, R. S., Mehta, S. K., Khan, Z. U., & Singh, M. M. (1979). Role of *Aspergillus* and *Candida* species in allergic bronchopulmonary mycoses. A comparative study. *Scandinavian Journal of Respiratory Diseases*, 60(5), 235–242.
- Santos, C. B., Davidson, J., Covar, R. A., & Spahn, J. D. (2014). The chitinase-like protein YKL-40 is not a useful biomarker for severe persistent asthma in children. *Annals of Allergy, Asthma & Immunology*, 113(3), 263–266.
- Sehgal, I. S., Choudhary, H., Dhooia, S., Aggarwal, A. N., Garg, M., Chakrabarti, A., et al. (2019). Is there an overlap in immune response between allergic bronchopulmonary and chronic pulmonary aspergillosis? *The Journal of Allergy and Clinical Immunology: In Practice*, 7(3), 969–974.
- Seiberling, K., & Wormald, P. J. (2009). The role of itraconazole in recalcitrant fungal sinusitis. *American Journal of Rhinology & Allergy*, 23(3), 303–306.
- Seidegard, J., Nyberg, L., & Borga, O. (2008). Presystemic elimination of budesonide in man when administered locally at different levels in the gut, with and without local inhibition by ketoconazole. *European Journal of Pharmaceutical Sciences*, 35(4), 264–270.
- Seidegard, J., Nyberg, L., & Borga, O. (2012). Differentiating mucosal and hepatic metabolism of budesonide by local pretreatment with increasing doses of ketoconazole in the proximal jejunum. *European Journal of Pharmaceutical Sciences*, 46(5), 530–536.
- Seki, M., Ohno, H., Gotoh, K., Motooka, D., Nakamura, S., Iida, T., et al. (2014). Allergic bronchopulmonary mycosis due to co-infection with *Aspergillus fumigatus* and *Schizophyllum commune*. *IDCases*, 1(1), 5–8.
- Seymour, B. W., Gershwin, L. J., & Coffman, R. L. (1998). Aerosol-induced immunoglobulin (Ig)-E unresponsiveness to ovalbumin does not require CD8+ or T cell receptor (TCR)-gamma/delta+ T cells or interferon (IFN)-gamma in a murine model of allergen sensitization. *The Journal of Experimental Medicine*, 187(5), 721–731.
- Shaw, J. L., Fakhri, S., Citardi, M. J., Porter, P. C., Corry, D. B., Kheradmand, F., et al. (2013). IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *American Journal of Respiratory and Critical Care Medicine*, 188(4), 432–439.
- Shin, S.-H., Ponikau, J. U., Sherris, D. A., Congdon, D., Frigas, E., Homburger, H. A., et al. (2004). Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi. *The Journal of Allergy and Clinical Immunology*, 114(6), 1369–1375.
- Shoseyov, D., Brownlee, K. G., Conway, S. P., & Kerem, E. (2006). *Aspergillus* bronchitis in cystic fibrosis. *Chest*, 130(1), 222–226.
- Slavin, R. G., Million, L., & Cherry, J. (1970). Allergic bronchopulmonary aspergillosis: Characterization of antibodies and results of treatment. *The Journal of Allergy*, 46(3), 150–155.
- Smith, N. L., & Denning, D. W. (2011). Underlying conditions in chronic pulmonary aspergillosis including simple aspergilloma. *The European Respiratory Journal*, 37(4), 865–872.
- Smith, D., & Spanel, P. (2015). SIFT-MS and FA-MS methods for ambient gas phase analysis: Developments and applications in the UK. *The Analyst*, 140(8), 2573–2591.

- Snapper, C. M., Finkelman, F. D., & Paul, W. E. (1988). Differential regulation of IgG1 and IgE synthesis by interleukin 4. *The Journal of Experimental Medicine*, 167(1), 183–196.
- Soeria-Atmadja, D., Onell, A., & Borga, A. (2010). IgE sensitization to fungi mirrors fungal phylogenetic systematics. *The Journal of Allergy and Clinical Immunology*, 125(6), 1379–1386.
- Sokol, C. L., Barton, G. M., Farr, A. G., & Medzhitov, R. (2008). A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nature Immunology*, 9(3), 310–318.
- Soubani, A. O., & Chandrasekar, P. H. (2002). The clinical spectrum of pulmonary aspergillosis. *Chest*, 121(6), 1988–1999.
- Spector, S. L., Bernstein, I. L., Li, J. T., Berger, W. E., Kaliner, M. A., Schuller, D. E., et al. (1998). Parameters for the diagnosis and management of sinusitis. *The Journal of Allergy and Clinical Immunology*, 102(6 Pt 2), S107–S144.
- Spergel, J. M., & Paller, A. S. (2003). Atopic dermatitis and the atopic march. *The Journal of Allergy and Clinical Immunology*, 112(6 Suppl), S118–S127.
- Sun, W. K., Lu, X., Li, X., Sun, Q. Y., Su, X., Song, Y., et al. (2012). Dectin-1 is inducible and plays a crucial role in Aspergillus-induced innate immune responses in human bronchial epithelial cells. *European Journal of Clinical Microbiology & Infectious Diseases*, 31(10), 2755–2764.
- Svenningsen, S., & Nair, P. (2017). Asthma Endotypes and an Overview of Targeted Therapy for Asthma. *Frontiers in Medicine*, 4, 158.
- Swartz, J., & Stoller, J. K. (2009). Acute eosinophilic pneumonia complicating *Coccidioides immitis* pneumonia: A case report and literature review. *Respiration*, 77(1), 102–106.
- Sweerus, K., Lachowicz-Scroggins, M., Gordon, E., LaFemina, M., Huang, X., Parikh, M., et al. (2016). Claudin-18 deficiency is associated with airway epithelial barrier dysfunction and asthma. *The Journal of Allergy and Clinical Immunology*, 139, 72–81.e1.
- Tay, T. R., Bosco, J., Gillman, A., Aumann, H., Stirling, R., O’Hehir, R., et al. (2016). Coexisting atopic conditions influence the likelihood of allergic bronchopulmonary aspergillosis in asthma. *Annals of Allergy, Asthma & Immunology*, 117(1), 29–32.e1.
- Tercelj, M., Rott, T., & Rylander, R. (2007). Antifungal treatment in sarcoidosis—A pilot intervention trial. *Respiratory Medicine*, 101(4), 774–778.
- Tercelj, M., Salobir, B., Zupancic, M., & Rylander, R. (2011). Antifungal medication is efficient in the treatment of sarcoidosis. *Therapeutic Advances in Respiratory Disease*, 5(3), 157–162.
- Tercelj, M., Salobir, B., Zupancic, M., & Rylander, R. (2014). Sarcoidosis treatment with antifungal medication: A follow-up. *Pulmonary Medicine*, 2014, 739673.
- Tomassen, P., Vandeplas, G., Van Zele, T., Cardell, L. O., Arebro, J., Olze, H., et al. (2016). Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *The Journal of Allergy and Clinical Immunology*, 137(5), 1449–1456.e4.
- Tong, X., Cheng, A., Xu, H., Jin, J., Yang, Y., Zhu, S., et al. (2018). Aspergillus fumigatus during COPD exacerbation: A pair-matched retrospective study. *BMC Pulmonary Medicine*, 18(1), 55.
- Tracy, M. C., & Moss, R. B. (2018). The myriad challenges of respiratory fungal infection in cystic fibrosis. *Pediatric Pulmonology*, 53(S3), S75–S85.
- Tsao, C. H., Chen, L. C., Yeh, K. W., & Huang, J. L. (2003). Concomitant chronic sinusitis treatment in children with mild asthma: The effect on bronchial hyperresponsiveness. *Chest*, 123(3), 757–764.
- Urm, S. H., Yun, H. D., Fenta, Y. A., Yoo, K. H., Abraham, R. S., Hagan, J., et al. (2013). Asthma and risk of selective IgA deficiency or common variable immunodeficiency: A population-based case-control study. *Mayo Clinic Proceedings*, 88(8), 813–821.
- Uzunhan, Y., Nunes, H., Jeny, F., Lacroix, M., Brun, S., Brillet, P. Y., et al. (2017). Chronic pulmonary aspergillosis complicating sarcoidosis. *The European Respiratory Journal*, 49(6), 1–13.

- Valladao, A. C., Frevert, C. W., Koch, L. K., Campbell, D. J., & Ziegler, S. F. (2016). STAT6 regulates the development of eosinophilic versus neutrophilic asthma in response to *Alternaria alternata*. *Journal of Immunology*, 197(12), 4541–4551.
- Van Dyken, S. J., Garcia, D., Porter, P., Huang, X., Quinlan, P. J., Blanc, P. D., et al. (2011). Fungal chitin from asthma-associated home environments induces eosinophilic lung infiltration. *Journal of Immunology*, 187(5), 2261–2267.
- Van Dyken, S. J., Mohapatra, A., Nussbaum, J. C., Molofsky, A. B., Thornton, E. E., Ziegler, S. F., et al. (2014). Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and delta T cells. *Immunity*, 40(3), 414–424.
- van Woerden, H. C., Gregory, C., Brown, R., Marchesi, J. R., Hoogendoorn, B., & Matthews, I. P. (2013). Differences in fungi present in induced sputum samples from asthma patients and non-atopic controls: A community based case control study. *BMC Infectious Diseases*, 13, 69.
- Vannella, K. M., Ramalingam, T. R., Borthwick, L. A., Barron, L., Hart, K. M., Thompson, R. W., et al. (2016). Combinatorial targeting of TSLP, IL-25, and IL-33 in type 2 cytokine-driven inflammation and fibrosis. *Science Translational Medicine*, 8(337), 337ra65.
- Varis, T., Kivisto, K. T., Backman, J. T., & Neuvonen, P. J. (1999). Itraconazole decreases the clearance and enhances the effects of intravenously administered methylprednisolone in healthy volunteers. *Pharmacology & Toxicology*, 85(1), 29–32.
- Varis, T., Kivisto, K. T., Backman, J. T., & Neuvonen, P. J. (2000). The cytochrome P450 3A4 inhibitor itraconazole markedly increases the plasma concentrations of dexamethasone and enhances its adrenal-suppressant effect. *Clinical Pharmacology and Therapeutics*, 68(5), 487–494.
- Varis, T., Kivisto, K. T., & Neuvonen, P. J. (2000). The effect of itraconazole on the pharmacokinetics and pharmacodynamics of oral prednisolone. *European Journal of Clinical Pharmacology*, 56(1), 57–60.
- Vicencio, A. G., Chupp, G. L., Tsirilakis, K., He, X., Kessel, A., Nandalike, K., et al. (2010). CHIT1 mutations: Genetic risk factor for severe asthma with fungal sensitization? *Pediatrics*, 126, e982–e985.
- Wan, H., Winton, H. L., Soeller, C., Tovey, E. R., Gruenert, D. C., Thompson, P. J., et al. (1999). Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *The Journal of Clinical Investigation*, 104(1), 123–133.
- Wang, J., Lv, H., Luo, Z., Mou, S., Liu, J., Liu, C., et al. (2018). Plasma YKL-40 and NGAL are useful in distinguishing ACO from asthma and COPD. *Respiratory Research*, 19(1), 47.
- Ward, G. W., Jr., Woodfolk, J. A., Hayden, M. L., Jackson, S., & Platts-Mills, T. A. (1999). Treatment of late-onset asthma with fluconazole. *The Journal of Allergy and Clinical Immunology*, 104(3), 541–546.
- Werner, J. L., Gessner, M. A., Lilly, L. M., Nelson, M. P., Metz, A. E., Horn, D., et al. (2011). Neutrophils produce interleukin 17A (IL-17A) in a dectin-1- and IL-23-dependent manner during invasive fungal infection. *Infection and Immunity*, 79(10), 3966–3977.
- Werner, J. L., Metz, A. E., Horn, D., Schoeb, T. R., Hewitt, M. M., Schwiebert, L. M., et al. (2009). Requisite role for the dectin-1 beta-glucan receptor in pulmonary defense against *Aspergillus fumigatus*. *Journal of Immunology*, 182(8), 4938–4946.
- Wiesner, D. L., & Klein, B. S. (2017). Lung epithelium: Barrier immunity to inhaled fungi and driver of fungal-associated allergic asthma. *Current Opinion in Microbiology*, 40, 8–13.
- Wills-Karp, M. (1999). Immunologic basis of antigen-induced airway hyperresponsiveness. *Annual Review of Immunology*, 17, 255–281.
- Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T. Y., Karp, C. L., et al. (1998). Interleukin-13: Central mediator of allergic asthma. *Science*, 282(5397), 2258–2261.

- Wise, S. K., Laury, A. M., Katz, E. H., Den Beste, K. A., Parkos, C. A., & Nusrat, A. (2014). Interleukin-4 and interleukin-13 compromise the sinonasal epithelial barrier and perturb intercellular junction protein expression. *International Forum of Allergy and Rhinology*, 4(5), 361–370.
- Wollschlaeger, C., & Khan, F. (1984). Aspergillomas complicating sarcoidosis. A prospective study in 100 patients. *Chest*, 86(4), 585–588.
- Wright, D. N., Nelson, R. P., Jr., Ledford, D. K., Fernandez-Caldas, E., Trudeau, W. L., & Lockey, R. F. (1990). Serum IgE and human immunodeficiency virus (HIV) infection. *The Journal of Allergy and Clinical Immunology*, 85(2), 445–452.
- Wu, Y., Du, S., Johnson, J. L., Tung, H. Y., Landers, C. T., Liu, Y., et al. (2019). Microglia and amyloid precursor protein coordinate control of transient candida cerebritis with memory deficits. *Nature Communications*, 10, 58.
- Yoon, J., Ponikau, J. U., Lawrence, C. B., & Kita, H. (2008). Innate antifungal immunity of human eosinophils mediated by a beta 2 integrin, CD11b. *Journal of Immunology*, 181(4), 2907–2915.
- Yoshida, K., Kurashima, A., Kamei, K., Oritsu, M., Ando, T., Yamamoto, T., et al. (2012). Efficacy and safety of short- and long-term treatment of itraconazole on chronic necrotizing pulmonary aspergillosis in multicenter study. *Journal of Infection and Chemotherapy*, 18(3), 378–385.
- Yoshimura, K., Suzuki, Y., Inoue, Y., Nishimoto, K., Mori, K., Karayama, M., et al. (2018). Utility of serum Aspergillus-galactomannan antigen to evaluate the risk of severe acute exacerbation in chronic obstructive pulmonary disease. *PLoS One*, 13(6), e0198479.
- Zhang, Y., Yu, X., Ichikawa, M., Lyons, J. J., Datta, S., Lamborn, I. T., et al. (2014). Autosomal recessive phosphoglucomutase 3 (PGM3) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment. *The Journal of Allergy and Clinical Immunology*, 133(5), 1400–1409.
- Zhou-Suckow, Z., Duerr, J., Hagner, M., Agrawal, R., & Mall, M. A. (2017). Airway mucus, inflammation and remodeling: Emerging links in the pathogenesis of chronic lung diseases. *Cell and Tissue Research*, 367(3), 537–550.
- Zhu, Z., Zheng, T., Homer, R. J., Kim, Y.-K., Chen, N. Y., Cohn, L., et al. (2004). Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science*, 304(5677), 1678–1682.