

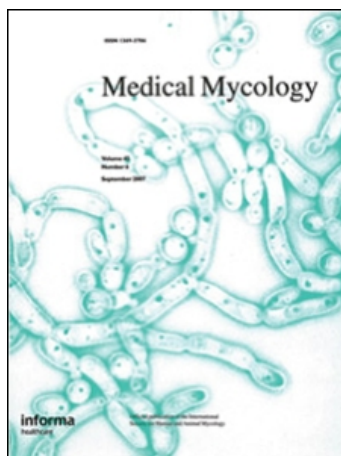
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Review Article

Potential contribution of fungal infection and colonization to the development of allergy

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Fungi have long been recognized as an important source of allergens in patients with atopic disease. In this review, we explore the hypothesis that fungal exposures resulting in colonization or infection directly influence the tendency of an individual to develop allergic disease. According to this hypothesis, fungal exposures especially those early in life may influence the manner in which the immune response handles subsequent responses to antigen exposures. Studies detailing this potential connection between fungi have already provided important insights into the immunology of fungal-human interactions and offer the potential to provide new approaches and targets for the therapy of allergic disease. The first half of this review summarizes the data concerning fungal infections and asthma, including possible connections between fungal infections and urban asthma. The second half explores the potential role of the fungal gastrointestinal microbiota in promoting allergic inflammation.

Keywords Fungi, allergy, asthma, *Candida*, infection

Introduction

Fungi are well recognized allergens for individuals with atopy, a genetic pre-disposition to develop allergic manifestations including asthma, dermatitis and rhinitis. In atopics, these manifestations reflect the effects of antigen-elicited TH2 inflammation and associated IgE production. Fungal sensitization for these individuals is generally thought to occur as a result of repeated, transient inhalational exposures to a variety of fungal antigens. Results from recent epidemiologic studies, however suggest that fungal exposures may also somehow exacerbate allergic diseases, including asthma (reviewed in [1]). In this regard, patients with evidence

of fungal sensitization are more likely to experience life-threatening asthma and die from their asthma [2–5].

In this review, we explore the hypothesis that host-fungal interactions outside the traditional sensitization process contribute to the development of TH2 inflammation and associated allergic disease. We suggest that these fungal-host interactions directly promote the development of a TH2 pre-disposition and atopic disease. The first part of this review will explore evidence regarding the role fungal infections play in allergic diseases, while the second part examines the role of fungal microbiota.

Fungal infections and allergy

Allergic bronchopulmonary aspergillosis

A wide array of clinical and laboratory studies highlight the importance of viral and bacterial respiratory infections in the pathogenesis of allergic asthma [6–8]. The best documented example of asthma related to fungal infection is allergic bronchopulmonary

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aspergillosis (ABPA), which was first recognized as a distinct entity by Hinson in 1952 [9]. In ABPA, airway infection with *Aspergillus* exacerbates asthma symptoms, primarily in patients with chronic asthma and cystic fibrosis (CF). ABPA has been reported to occur in 1–40% of chronic asthmatics [10–12] and 2–10% of patients with CF [13–15]. *Aspergillus fumigatus* is most commonly implicated, though several different species of *Aspergillus* have also been associated with ABPA [16–18]. A role for other fungi in ABPA, including *Candida albicans* has also been hypothesized [19,20]. The clinical features of ABPA include: asthma, eosinophilia, elevated total serum IgE and elevated serum IgE and IgG specific to *Aspergillus*. Affected patients have mucus plugging and may develop central bronchiectasis with pulmonary fibrosis. The importance of *Aspergillus* infection as an immediate cause of ABPA (and not just an indicator of a severely diseased airway) is highlighted by recent studies that reveal improvements in pulmonary function and clinical symptoms in association with anti-fungal therapy [21,22].

The mechanisms by which persistent airway infection with *Aspergillus* spp. leads to ABPA appears to involve dysregulation of both innate and adaptive immunity (reviewed in [23,24]). In this regard, *Aspergillus* directly induces activation of macrophages, granulocytes and dendritic cells via Toll-like receptors 2 and 4 [25,26]. Inhibition of dendritic cell maturation following stimulation with *Aspergillus* protease has recently been demonstrated and hypothesized to contribute to the development of allergic (TH2) response to infection [27].

Both type I (IgE-mediated) and type III (IgG-mediated) hypersensitivity reactions contribute to the pathogenesis of ABPA [28]. The tendency of patients with ABPA to develop allergic (type I hypersensitivity, TH2) responses to *Aspergillus* is reflected in the isolation of antigen specific TH2 T cells from affected individuals [29–31]. Furthermore, antigen specific T cell clones isolated from patients with ABPA secrete a Th2 cytokine profile and proliferate in response to IL-4 [30]. Interestingly, the enhanced allergic responsiveness of patients with ABPA appears to be antigen specific and may not reflect an overall change in TH bias. In this regard, there was no increase in overall frequency of circulating TH2 cells in patients with ABPA compared to CF patients without ABPA. Though, there was a decreased frequency of circulating TH1 cells [24].

Infection-induced airway damage appears to be important in the pathogenesis of ABPA and TH2 skewing. This may occur as a result of the direct effects of infection and or toxin production [32–34]. Epithelial

damage as manifested by cell shrinking and desquamation can be induced *in vitro* by exposure of epithelial cells to *Aspergillus* proteases [35]. Epithelial damage induced by *Aspergillus* has been hypothesized to promote hypersensitivity by allowing for increased antigen exposure [24]. In addition, epithelial damage induced by *Aspergillus* is associated with the release of IL-6 and IL-8, which promote inflammation [35,36]. Furthermore, inflammation-induced lung damage may perpetuate *Aspergillus* infection by hindering normal clearance mechanisms. Neutrophils are thought to play a prominent role in this process. In a murine model of ABPA, genetically enhanced of neutrophil influx was associated with worsening of allergic inflammation and increased airway responsiveness [37]. Likewise, increased neutrophil numbers in the sputum has been correlated with diminished respiratory function in patients with ABPA [38,39].

Both host and pathogen related factors leading to ABPA have been identified. Many *Aspergillus*-associated allergens have proteolytic activity that promote airway damage (i.e., epithelial cell detachment *in vitro*) and subsequent pro-inflammatory cytokine (IL-6) and chemokine (IL-8, MCP-1) production [36,40]. These antigens may also directly activate T and B cells [30] and play a role in promoting a Th2 bias. Antibodies to these proteases are present in patients with ABPA and in one study increased antibody levels correlated with disease exacerbations [41]. Specific host factors that have been implicated in the pathogenesis of ABPA include: HLA type, mutations in the cystic fibrosis transmembrane regulator gene (CFTR), IL-10, both IL-4R and surfactant polymorphisms (reviewed in [42]).

Allergic fungal sinusitis

An allergic response to non-invasive fungal infection of the respiratory sinuses has been hypothesized to produce a condition known as allergic fungal sinusitis (AFS). The diagnostic criteria for AFS were established by Bent and Kuhn and include the use of both major and minor criteria. Major criteria consist of the following: evidence of type I, IgE-mediated hypersensitivity, nasal polyposis, characteristic CT findings; eosinophilic mucus without fungal invasion into sinus tissue and positive fungal smear or culture [43]. The pathogenesis of allergic fungal sinusitis is thought to be similar to ABPA and to involve both type I and type III hyper-sensitivity responses (reviewed in [44]). The role of TH2 inflammation and type I hypersensitivity in the pathogenesis of AFS is supported by studies that document elevated fungal specific IgE in the serum of

patients with suspected AFS [45,46]. In one study, fungal specific IgE was commonly found in the mucin of affected patients [47]. Staining for major basic protein and neutrophil elastase has revealed increased amounts of these proteins in the mucin of patients with AFS suggesting that neutrophil and eosinophil degranulation contribute to pathogenesis of AFS [48]. Nonetheless, the precise immunologic basis of this condition remains poorly understood and several controversies surround both diagnostic criteria and pathogenesis [49,50]. A variety of fungi have been implicated, including *Curvularia*, *Bipolaris*, *Drechslera*, *Exserohilum* and *Aspergillus* species [51–53]. Affected individuals are usually immunocompetent with a history of atopy and present with chronic nasal congestion, nasal obstruction and thick dark nasal secretions. Proptosis may be present. Nasal secretions contain large number of eosinophils and fungal stains are typically positive. Treatment generally involves a combination of surgical (endoscopic removal of sinus contents) and medical therapies (anti-inflammatory corticosteroids). The roles of de-sensitization and anti-fungal therapy require further study.

Dermatomycotic infections

Chronic fungal infections outside the respiratory tract may also exacerbate allergic symptoms. Fungal skin infections have been reported to worsen symptoms of both atopic dermatitis (AD) [54] and asthma [55] and also produce chronic urticaria [56,57]. A link between fungal infections of the skin, especially *Malassezia* spp., and AD especially in the head and neck area has been suggested by several groups [58,59]. Evidence to support a causal association between *Malassezia* infection and AD include the following: isolation of *Malassezia* from patients with AD [60], a correlation between a positive skin test to *Malassezia* and atopic dermatitis [59], and an association between high IgE titers to *Malassezia* and AD [61,62]. The basis by which skin infection with *Malassezia* could lead to AD is unknown. *In vitro* experiments indicate that *Malassezia* induces pro-inflammatory and TH2 cytokine production by keratinocytes [63,64]. *Malassezia* is ingested by dendritic cells and induces dendritic cell maturation without IL-12 production, which may in turn promote Th2 inflammation [65]. Studies to define the role of anti-fungal therapy in AD associated with *Malassezia* have yielded mixed results [66–68].

A role for dermatophytic infections in asthma pathogenesis has also been hypothesized on more limited data. This hypothesis is based on the following observations: (i) asthmatic patients with dermatophytic

infections exhibit immediate hypersensitivity reactions and elevated serum IgE levels to *Tricophyton* spp. [69,70], and (ii) anti-fungal treatment of asthmatic patients with dermatophytic infections has been reported to improve asthma symptoms in affected patients [71–73]. The basis by which fungal infections of the skin can lead to an exacerbation of allergic symptoms in the respiratory tract or skin is unclear. It has been suggested that fungal infection of the skin leads to allergen exposure within the lung, either as a result of unrecognized, associated pulmonary exposure or the migration of fungal antigen loaded dendritic cells from the skin to the lung [74]. Alternatively, it is possible that skin fungal infection actively modifies the immune response to subsequent antigen exposures.

Asthma, an urban fungal infection?

The prevalence and severity of asthma in children from urban areas are disproportionately high when compared with the national average leading some to term the problem an epidemic [75,76]. In NYC asthma is the leading cause of visits to the Pediatric Emergency Department and hospitalization among children (New York City Department of Health: www.nyc.gov/html/doh). The basis for the high prevalence of asthma in urban areas is unknown, but likely to be multifactorial, involving both host and environmental elements, including: substandard healthcare [77], sedentary lifestyle leading to obesity [78] and air pollution [79]. Residential exposures (such as cockroaches [80]) have also been hypothesized to contribute significantly to the problem of urban asthma.

Given the high prevalence of asthma in urban areas and the potential for fungal infections to modify allergic inflammation, including asthma we have investigated a potential connection between fungal infections, especially those due to *C. neoformans* and asthma. *C. neoformans* is typically isolated from sites contaminated with avian excreta, especially pigeon excreta. Pigeons have adapted particularly well to living in metropolitan area. It is estimated that pigeon populations are roughly the size of 2% of the human population in large cities [81]. Pigeons are so ubiquitous in urban environments that exposure to pigeon antigens has been hypothesized to produce respiratory disease (hypersensitivity pneumonitis) in some urban residents [82]. Pigeon guano serves as an excellent environmental reservoir for *C. neoformans*. The acidic nature and high nitrogen content of pigeon excreta provides excellent growth conditions for *C. neoformans* and densities of greater than 10 million organisms per gram of excreta can be reached. Furthermore,

C. neoformans can survive in desiccated pigeon excreta for prolonged intervals (almost two years in one study) [83].

C. neoformans infection is acquired through the inhalation of infectious forms in the environment. While cryptococcal disease is recognized primarily in immunocompromised individuals, the vast majority of infections in immunocompetent individuals are likely asymptomatic or incorrectly diagnosed as viral illnesses. Furthermore, it is likely that infection persists for prolonged periods. This hypothesis is supported by a variety of clinical observations, including: (i) inadvertent detection of pulmonary cryptococcosis as a result of chest radiographs done for other reasons [84,85]; (ii) autopsy studies demonstrating a primary cryptococcal complex [86]; (iii) molecular typing studies showing discrepancies between clinical and environmental isolates [87]; (iv) serology studies showing the presence of antibodies to *C. neoformans* prior to clinical presentation of disease [88,89] and (v) transplant-associated infections in which the donor lung was the source of infection [90]. Using an immunoblot assay we found that a large proportion of children living in the Bronx, an area with an extraordinarily high rate of asthma, have serologic evidence of subclinical cryptococcal infection [91]. In contrast, we found a low prevalence of subclinical infection as inferred by serology in children living in a northern suburb of New York, where the prevalence of asthma is low [92].

A potential role for *C. neoformans* in the pathogenesis of allergic inflammation is further supported by animal experimentation which demonstrates the tendency of *C. neoformans* to elicit allergic inflammation. The tendency of *C. neoformans* to induce allergic inflammation in mice has led one of the authors of this review (GH) to label this experimental system as a model of allergic bronchopulmonary mycosis [93]. Using a rat model, we (DG *et al.*) demonstrated that pulmonary *C. neoformans* infection enhances allergic inflammation to aerosolized antigen as manifested by increased specific IgE and eosinophil infiltration [94]. Pulmonary infection was also associated with an increased number of airway goblet cells and non-specific airway hyper-reactivity, both features of asthma. Anti-fungal treatment in rats with pulmonary cryptococcosis partially ameliorated the enhancement of allergic inflammation induced by infection.

The mechanisms by which *C. neoformans* elicits allergic inflammation have not been fully defined. In mice, cryptococcal virulence is linked to the ability to promote Th2 polarization [95,96]. Infection-related IL-12 suppression and enhancement of IL-10 production have been observed [97,98]. Experimental cryptococcal

infection also inhibits the production of interferon-gamma, TNF- α , and IL-18 [99]. Cryptococcal polysaccharide (CNPS), the main component of which is glucuronoxylomannan (GXM) promotes Th2 cytokine production. GXM is a potent down regulator of macrophage TNF- α release after LPS stimulation [100]. Apart from reducing secretion of the pro-inflammatory cytokines, soluble CNPS induces secretion of IL-10 [101]. CNPS also promotes proliferation of murine CD4⁺ T cells that produce high levels of IL-4 [102]. Other components of *C. neoformans* may contribute to enhanced Th2 inflammation. *C. neoformans* produces both prostaglandins and leukotrienes (see below).

Fungi, chitinase and asthma

An alternative mechanism by which fungal pulmonary infections including cryptococcosis may promote allergic inflammation relates to the presence of chitin and the induction of host chitinases. Chitin is a significant component of fungal cell walls, including *C. neoformans*. In a murine model, chitin has recently been shown to induce the accumulation of eosinophils and basophils via the production of leukotriene B4 [103]. Mammalian chitinases consist of a family of enzymes known as Glycoside Hydrolase 18. Some of these enzymes have chitinolytic activity, while others do not. The potential connection between chitinase and asthma was first suggested by the Elias group [104]. This group demonstrated that acidic mammalian chitinase (AMCase) acts as a down-stream modulator of IL-13 in a murine model of asthma. In this system, over-expression of IL-13 induced endogenous chitinase activity and resulted in airway hyperreactivity, while blockage of chitinase activity attenuated this response [104]. Subsequent studies have suggested an association between certain AMCase polymorphisms and asthma [105]. More recently, YKL-40, another member of the Glycoside Hydrolase family has been found to be elevated in the serum and lungs of asthmatics when compared to controls [106]. In these studies, YKL-40 levels were found to correlate with the asthma severity.

A potential connection between fungal infection and the induction of host chitinase was first observed in guinea pig model of *Aspergillus* infection. In these studies, serum levels of chitinase were found to be elevated in animals experimentally infected with *Aspergillus* [107]. Our studies indicate that pulmonary but not systemic infection with *C. neoformans* in the rat induces chitinase activity within the lung and that this correlates with increased AMCase expression within airway epithelial cells and alveolar macrophages [108]. These findings suggest a previously unrecognized

mechanism by which pulmonary fungal infections, including cryptococcosis can directly alter asthma pathogenesis.

Fungal colonization and allergy

Candida albicans colonization

Candida albicans is a normal part of the human microbiota and resides in low numbers in the mouth, vagina, and gastrointestinal tract of healthy individuals (reviewed in [109]). The factors affecting *C. albicans* numbers on mucosal surfaces is multi-faceted and includes the composition of the microbiota, hormones, stress, innate immunity and adaptive immunity. However, this fungus can also be pathogenic and cause life-threatening systemic infections. It is a frequent cause of morbidity and mortality in immunosuppressed and critically ill patients and those with indwelling catheters. In addition treatment with antibiotics also increases the risk of complications due to *C. albicans* overgrowth. Thus, control of *C. albicans* by the normal microbiota is very important.

Control of *Candida* colonization by the immune system

One mechanism to control over-exuberant inflammatory responses on the mucosa is via regulatory T (Treg) cells. Through the production of various anti-inflammatory mediators, these cells can down-regulate effector mechanisms of both innate and adaptive immunity, protecting the host mucosa from damage and allowing colonization by non-pathogenic microbes, including *C. albicans* (and probably other far less numerous fungi in the microbiota). There are a number of subsets of Treg cells, with the major distinction being: (i) Tregs that are induced in the periphery following antigen encounter (inducible Tregs), and (ii) Tregs that arise from normal T-cell thymic development (naturally occurring Tregs) [110,111].

Nave CD4 T-cells can be induced to differentiate into inducible Treg cells both *in vivo* and *in vitro*. Inducible Tregs can be further divided into two subsets based on differential cytokine secretion and function: Th3 and Tr1. Culture of nave T-cells in the presence of TGF β results in the generation of a Treg population termed Th3 cells (reviewed in [112]). Th3 cells secrete TGF β and to a lesser extent IL-4 and IL-10 and induce IgA production from Peyer's Patch B-cells. Deficiencies in TGF α -producing Th3 cells have been implicated in human food allergies. For example, fewer TFG β -secreting lymphocytes were isolated from the duodenum and lamina propria of children with food allergies [113]. Tr1 cells are generated when nave T-cells are

antigenically stimulated under the influence of IL-10 and IFN α . Tr1 cells proliferate poorly when stimulated; however they secrete high levels of IL-10 and TGF β . Differentiation of Tr1 cells appears to be controlled by intestinal dendritic cells that secrete high levels of IL-10 and have been isolated from both PP and MLN cells following oral allergen exposure [114–117].

Naturally occurring Treg cells arise during normal thymic T-cell maturation and comprise 5–10% of the peripheral CD4 T-cell population. These cells are most commonly identified by their constitutive expression of CD25 and thus are referred to as CD4⁺ CD25⁺ Treg cells. These cells also can be identified by expression of CTLA-4, GITR, and FoxP3. Studies of oral feeding of ovalbumin have implicated CD4⁺ CD25⁺ Tregs as key mediators of oral tolerance [118]. The clinical relevance of CD4⁺ CD25⁺ Treg cells can be seen in patients with X-linked autoimmunity-allergic dysregulation syndrome [119]. These patients have a mutation in their FoxP3 gene resulting in heightened IgE production and food allergies [120]. Additionally, children who have outgrown milk allergies have higher frequencies of allergen-specific CD4⁺ CD25⁺ T-cells in their peripheral blood [121]. In mouse models of *C. albicans* infection or colonization, CD4⁺CD25⁺Treg cells prevent excessive inflammation and promote colonization/persistence in the gastrointestinal tract [122].

Romani and colleagues have also shown that fungal growth, inflammation, and tolerance are controlled by the coordinate activation of naturally occurring Treg cells and induced Treg cells [123]. Activation via the TRIF pathway is critical for migration of both types of Treg cells to sites of *C. albicans* colonization or infection and the upregulation of MyD88-mediated signaling pathways can dampen the suppressive activity of the naturally occurring Treg cells. Th1-mediated inflammation can be modulated by induced Treg cells through activation of the enzyme indoleamine 2,3-dioxygenase (IDO) in dendritic cells and antagonism of Th17 cell activity antagonism. A mediator of tryptophan catabolism, IDO is induced at sites of *C. albicans* infection, including in dendritic cells and neutrophils, via IFN- γ . Treatment of mice with an IDO inhibitor can greatly exacerbate inflammatory pathology [124]. Blocking IDO accelerates hyphal formation and this is partially reversed by the addition of tryptophan, implicating the potential of host metabolic control of *C. albicans* morphogenesis. Thus, the regulatory response of the host can control both *Candida* morphology as well as the inflammatory and adaptive responses to this organism and promote the generation of adaptive Th1 immunity in secondary lymphoid organs.

Therefore, immunity to *C. albicans* can be characterized as an 'immune paradox'. Namely, hosts normally develop strong Th1 responses systemically (as measured by skin DTH responses to *Candida* extracts and serum IgG antibodies against *C. albicans*) but Treg responses in the mucosa. Thus, our immune response against *C. albicans* is normally set in a balance between inflammatory and anti-inflammatory reactions. Therefore, it is easy to imagine how environmental factors that affect either side of this balance might tip the reaction toward hypersensitivity reactions to *C. albicans*.

Control of Candida colonization by the microbiota

The major effects of antibiotic treatment on the microbiota are the direct killing of a large proportion of the microbiota and decreased colonization resistance within the GI-tract [125]. Colonization resistance results when obligate anaerobic microbiota inhibit the overgrowth of potentially harmful microbes. In humans, yeast infections (including *C. albicans*), at mucosal sites are one of the most common side effects of antibiotic therapy [126–128]. Interestingly, changes in the microbiota population can persist for months after completion of antibiotic treatment and can result in long-term decreases in beneficial anaerobic organisms (*Bifidobacterium*, *Lactobacillus*, *Bacteroides*) [129–131] and increases in potentially harmful microbes (gram negative aerobic enteric bacteria, anaerobic *Clostridium difficile*, and *C. albicans*) [128,132–134].

Changes in the fungal and bacterial microbiota as a factor in allergic disease?

The increase in the incidence of allergic diseases over the past 20–30 years, coupled with the dichotomy in the rate of allergic disease between industrialized and developing countries, suggests that environmental changes are a major factor in the development of allergies. These observations have led researchers to propose the 'hygiene hypothesis' for allergies and asthma, which states that a lack of early microbial stimulation results in aberrant immune responses to innocuous antigens later in life [135,136]. However, an alternative interpretation of the hygiene hypothesis is the 'microflora hypothesis', which proposes that perturbations in gastrointestinal microbiota composition due to antibiotic use and poor diet (low fiber, high sugar) in westernized areas have disrupted mechanisms involved in the development of immunological tolerance. Data supporting this interpretation include the correlation between asthma/allergies and antibiotic use in industrialized countries [137–140] and the

correlation between altered fecal microflora and atopic disease [141–145]. In addition, germ-free animals display numerous defects in immune response generation [146–151], further supporting the hypothesis that antibiotic-induced changes in the gastrointestinal (GI) microbiota can be a predisposing factor for allergic disease.

Our laboratory has tested whether the disturbance of the bacterial microbiota, combined with colonization by *C. albicans*, can promote the development of allergic airway disease [152,153]. Using a brief treatment with the broad-spectrum antibiotic cefoperazone in conjunction with low-level *C. albicans* GI-tract colonization, we were able to eliminate the need for systemic allergen priming (for example, OVA plus alum) in the development of allergen-induced airway inflammation. In the absence of systemic priming with OVA, only mice with altered GI-tract microbiota developed airway allergic responses to intranasally administered OVA. These mice produced: (i) a significant increase in the number of eosinophils in the lungs, (ii) high serum IgE levels, (iii) elevated IL-5 and IL-13 production, and (iv) increased numbers of mast cells in the lungs. The most striking change following microbiota disruption was in the development of a widespread goblet cell metaplasia. Similar changes were seen if conidia from *A. fumigatus* were used as the allergen. In the absence of CD4⁺ T cells or IL-13, the response did not develop. Similar allergic responses could be induced in the airways of both inbred mouse strains tested, C57BL/6 and Balb/c. Finally, these responses were not seen in mice in which only antibiotics or only a gavage of *C. albicans* was given, implicating a need for a change in both the bacterial and fungal microbiota to promote the development of allergic disease.

Other studies by Yamaguchi and colleagues have investigated, using a mouse model, whether gastrointestinal colonization by *C. albicans* can increase antigen permeability across the mucosa, a factor that would promote the development of food allergies and manifest in a variety of allergic symptoms [154]. They first inoculated the GI tract of mice by intragastric delivery of *C. albicans* and then fed the mice a synthetic diet to maintain colonization. They had previously demonstrated that the composition of the diet was key to maintaining *C. albicans* GI colonization in mice in the absence of antibiotics [155]. Mice were then given OVA intragastrically every other day for nine weeks. Extraluminal 'leak' of OVA and OVA-specific IgG and IgE titers were higher in *C. albicans*-colonized mice than in *C. albicans*-free mice. Furthermore, sensitization depended on the presence of mast cells. Thus,

GI colonization by *C. albicans* can alter the GI environment which may promote allergic sensitization.

Our lab and others have reported that *C. albicans*, *C. neoformans*, *A. fumigatus* (and many other fungi) secrete prostaglandins and prostaglandin-like molecules de novo or via conversion of exogenous arachidonic acid [156–159]. PGE₂ and PGE₂-cross reactive compounds can be purified from *C. albicans* that are biologically active on mammalian cells with activity comparable to commercially available PGE₂ [156]. Prostaglandins are potent immunomodulatory molecules that can inhibit Th1 type immune responses, chemokine production, phagocytosis, and lymphocyte proliferation and promote Th2 type responses and tissue eosinophilia [160]. PGD₂ receptor [DP] knockout mice do not develop airway Th2 responses to OVA [161]. Evidence is now accumulating that prostaglandins (especially PGE₂) play a role in overall immune regulation (positive and negative) [162]. Microbe-derived PGD₂ can alter dendritic cell migration and biology [163,164]. Fungal cell wall glucans are also powerful inflammatory stimulants in tissues [165] and may also play a role in the immunomodulatory activity of yeast in the GI tract. Thus, increased levels of fungal microbiota, such as often occurs during antibiotic therapy, may diminish the ability to generate Treg/Th3 responses to swallowed antigens, possibly by interfering with tolerance-inducing antigen presentation via fungal oxylipins and glucans.

Candida albicans and hypersensitivity diseases

While *Candida albicans* is a normal part of the human microbiota, some otherwise healthy individuals develop hypersensitivity diseases to *C. albicans* (both Th1 and Th2 responses) and the mechanism underlying this is not clear. In addition, increased mucosal colonization by *C. albicans* has been implicated for decades in a number of other hypersensitivity diseases, although a definitive understanding (or some would argue, 'proof') has been lacking. For example, it has been hypothesized that *C. albicans* colonization of the gastrointestinal mucosa may exacerbate or promote some forms of atopic dermatitis [166–168]. Due to conserved sequences with α -gliadin and γ -gliadin T-cell epitopes, the hyphal wall protein *Hwp1* has been suggested to be an immune target in patients with celiac disease, an allergic/autoimmune reaction to gluten, thereby contributing to the pathology [169,170]. Patients with Crohn's disease, an inflammatory bowel disease, very often possess high levels of anti-*Saccharomyces cerevisiae* antibodies (ASCA), which have recently been shown to react with a cell wall epitope of *C. albicans*

that is expressed *in vivo* but not in standard culture [171,172].

Perhaps more intriguing is the human condition that has been loosely defined as the following: 'a number of symptoms which improve on a diet low in refined carbohydrates and mold-related foods with or without antifungal therapy. It does not cause death, but its chronicity makes the lives of sufferers a misery...' [173]. As reviewed by Eaton, this collection of symptoms is diverse and complex and includes food sensitivities, allergic responses, digestive problems and psychoneurological manifestations ('brain fog') [173]. The symptoms can last for years and can improve radically in a short period of time. It has been reported in the popular media as 'Yeast Syndrome'. Since there is increasing evidence that changes in the microbiota (dysbiosis) can alter host physiology and there is circumstantial evidence for a role of fungi or fungal products; Eaton and others have proposed the term 'fungal-type dysbiosis' to describe it [173]. However, more research is clearly needed to understand any cause-and-effect relationships ascribed to this perplexing collection of symptoms.

Does *Candida* play a role in these various hypersensitivity diseases? As mentioned above, the data is not definitive, but there is increasing evidence that in some physiologic host states, *C. albicans* may be more than a commensal but less than a pathogen. The immune response to this fungus may promote or exacerbate an already existing hypersensitivity response.

Conclusion

Historically, the focus has been on fungal spores as allergenic triggers or stimuli. Thus, the reason for tracking mold spore counts during high allergy seasons of the year. However, more recent evidence is beginning to accumulate that fungal infection and colonization may play a role in promoting a variety of hypersensitivity diseases, including allergy and asthma. Much work remains to be done but well-characterized animal models are providing new insights into how fungal infection/colonization can stimulate or exacerbate allergies and asthma. With this increased understanding, come opportunities for new therapeutic strategies that treat allergic airway disease by targeting fungal infection/colonization.

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References

- Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006; **27**: 615–626.
- O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, *et al.* Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991; **324**: 359–363.
- Targonski PV, Persky VW, Ramekrishnan V. Effect of environmental molds on risk of death from asthma during the pollen season. *J Allergy Clin Immunol* 1995; **95**: 955–961.
- Zureik M, Neukirch C, Leynaert B, *et al.* Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ* 2002; **325**: 411–414.
- Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 2000; **55**: 501–504.
- Sabato AR, Martin AJ, Marmion BP, Kok TW, Cooper DM. *Mycoplasma pneumoniae*: acute illness, antibiotics, and subsequent pulmonary function. *Arch Dis Child* 1984; **59**: 1034–1037.
- Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *JAMA* 1991; **266**: 225–230.
- Welliver RC. RSV and chronic asthma. *Lancet* 1995; **346**: 789–790.
- Hinkson, Moon AJ, Plummer NS. Broncho-pulmonary aspergillosis; a review and a report of eight new cases. *Thorax* 1952; **7**: 317–333.
- Eaton T, Garrett J, Milne D, Frankel A, Wells AU. Allergic bronchopulmonary aspergillosis in the asthma clinic. A prospective evaluation of CT in the diagnostic algorithm. *Chest* 2000; **118**: 66–72.
- Krasnick J, Greenberger PA, Roberts M, Patterson R. Allergic bronchopulmonary aspergillosis: serologic update for 1995. *J Clin Lab Immunol* 1995; **46**: 137–142.
- Schwartz HJ, Greenberger PA. The prevalence of allergic bronchopulmonary aspergillosis in patients with asthma, determined by serologic and radiologic criteria in patients at risk. *J Lab Clin Med* 1991; **117**: 138–142.
- Laufer P, Fink JN, Bruns WT, Unger GF, Kalbfleisch JH, Greenberger PA, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol* 1984; **73**: 44–48.
- Zeaske R, Bruns WT, Fink JN, Greenberger PA, Colby H, Liotta JL, *et al.* Immune responses to *Aspergillus* in cystic fibrosis. *J Allergy Clin Immunol* 1988; **82**: 73–77.
- Becker JW, Burke W, McDonald G, *et al.* Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest* 1996; **109**: 1536–1540.
- Hoshino H, Tagaki S, Kon H, Shibusa T, Takabatake H, Fujita A, *et al.* Allergic bronchopulmonary aspergillosis due to *Aspergillus niger* without bronchial asthma. *Respiration* 1999; **66**: 369–372.
- Laham MN, Allen RC, Greene JC. Allergic bronchopulmonary aspergillosis (ABPA) caused by *Aspergillus terreus*: specific lymphocyte sensitization and antigen-directed serum opsonic activity. *Ann Allergy* 1981; **46**: 74–80.
- Akiyama K, Takizawa H, Suzuki M, *et al.* Allergic bronchopulmonary aspergillosis due to *Aspergillus oryzae*. *Chest* 1987; **91**: 285–286.
- Sandhu RS, Mehta SK, Khan ZU, Singh MM. Role of *Aspergillus* and *Candida* species in allergic bronchopulmonary mycoses. A comparative study. *Scand J Respir Dis* 1979; **60**: 235–242.
- Lake FR, Tribe AE, McAleer R, Froudast J, Thompson PJ. Mixed allergic bronchopulmonary fungal disease due to *Pseudallescheria boydii* and *Aspergillus*. *Thorax* 1990; **45**: 489–491.
- Wark PA, Gibson PG, Wilson AJ. Azoles for allergic bronchopulmonary aspergillosis associated with asthma. *Cochrane Database Syst Rev* 2004; CD001108.
- Stevens DA, Schwartz HJ, Lee JY, Moskovitz BL, Jerome DC, Catanzaro A, *et al.* A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000; **342**: 756–762.
- Moss RB. Pathophysiology and immunology of allergic bronchopulmonary aspergillosis. *Med Mycol* 2005; **43** (Suppl. 1): S203–S206.
- Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger P, Judson MA, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis – state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003; **37** (Suppl. 3): S225–S264.
- Braedel S, Radsak M, Einsele H, Latge JP, Michan A, Loeffler J, *et al.* *Aspergillus fumigatus* antigens activate innate immune cells via toll-like receptors 2 and 4. *Br J Haematol* 2004; **125**: 392–399.
- Mambula SS, Sau K, Henneke P, Golenbock DT, Levitz SM. Toll-like receptor (TLR) signaling in response to *Aspergillus fumigatus*. *J Biol Chem* 2002; **277**: 39320–3926.
- Lamhamedi-Cherradi SE, Martin RE, Ito T, Kheradmand F, Corry DB, Liu YJ, *et al.* Fungal proteases induce Th2 polarization through limited dendritic cell maturation and reduced production of IL-12. *J Immunol* 2008; **180**: 6000–6009.
- Geha RS. Circulating immune complexes and activation of the complement sequence in acute allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1977; **60**: 357–359.
- Skov M, Poulsen LK, Koch C. Increased antigen-specific Th-2 response in allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis. *Pediatr Pulmonol* 1999; **27**: 74–79.
- Knutsen AP, Mueller KR, Levine AD, *et al.* Asp f I CD4+ TH2-like T-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1994; **94**: 215–221.
- Chauhan B, Knutsen A, Hutcheson PS, Slavin RG, Bellone CJ. T cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. *J Clin Invest* 1996; **97**: 2324–2331.
- Amitani R, Murayama T, Nawada R, Lee WJ, Niimi A, Suzuki K, *et al.* *Aspergillus* culture filtrates and sputum sols from patients with pulmonary aspergillosis cause damage to human respiratory ciliated epithelium *in vitro*. *Eur Respir J* 1995; **8**: 1681–1687.
- Amitani R, Taylor G, Elezis EN, Llewellyn-Jones C, Mitchell J, Kuze F, *et al.* Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect Immun* 1995; **63**: 3266–3271.
- Botterel F, Cordonnier C, Barbier V, Wingerstmann L, Liance M, Coste A, *et al.* *Aspergillus fumigatus* causes *in vitro* electrophysiological and morphological modifications in human nasal epithelial cells. *Histol Histopathol* 2002; **17**: 1095–1101.
- Kauffman HF, Tomee JF, van de Riet MA, Timmerman AJ, Borger P. Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 2000; **105**: 1185–1193.

- 36 Tomee JF, Wierenga AT, Hiemstra PS, Kauffman HK. Proteases from *Aspergillus fumigatus* induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. *J Infect Dis* 1997; **176**: 300–303.
- 37 Park SJ, Wiekowski MT, Lira SA, Mehrad B. Neutrophils regulate airway responses in a model of fungal allergic airways disease. *J Immunol* 2006; **176**: 2538–2545.
- 38 Gibson PG, Wark PA, Simpson JL, Meldrum C, Meldrum S, Saltos N, *et al.* Induced sputum IL-8 gene expression, neutrophil influx and MMP-9 in allergic bronchopulmonary aspergillosis. *Eur Respir J* 2003; **21**: 582–588.
- 39 Wark PA, Saltos N, Simpson J, *et al.* Induced sputum eosinophils and neutrophils and bronchiectasis severity in allergic bronchopulmonary aspergillosis. *Eur Respir J* 2000; **16**: 1095–1101.
- 40 Robinson BW, Venaille TJ, Mendis AH, McAleer R. Allergens as proteases: an *Aspergillus fumigatus* proteinase directly induces human epithelial cell detachment. *J Allergy Clin Immunol* 1990; **86**: 726–731.
- 41 Tomee JF, van der Werf TS, Latge JP, *et al.* Serologic monitoring of disease and treatment in a patient with pulmonary aspergillosis. *Am J Respir Crit Care Med* 1995; **151**: 199–204.
- 42 Gibson PG. Allergic bronchopulmonary aspergillosis. *Semin Respir Crit Care Med* 2006; **27**: 185–191.
- 43 Bent JP, III, Kuhn FA. Diagnosis of allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1994; **111**: 580–588.
- 44 Houser SM, Corey JP. Allergic fungal rhinosinusitis: pathophysiology, epidemiology, and diagnosis. *Otolaryngol Clin North Am* 2000; **33**: 399–409.
- 45 Stewart AE, Hunsaker DH. Fungus-specific IgG and IgE in allergic fungal rhinosinusitis. *Otolaryngol Head Neck Surg* 2002; **127**: 324–332.
- 46 Manning SC, Holman M. Further evidence for allergic pathophysiology in allergic fungal sinusitis. *Laryngoscope* 1998; **108**: 1485–1496.
- 47 Collins M, Nair S, Smith W, *et al.* Role of local immunoglobulin E production in the pathophysiology of noninvasive fungal sinusitis. *Laryngoscope* 2004; **114**: 1242–1246.
- 48 Khan DA, Cody DT, George TJ, Gleich GJ, Leiferman KM. Allergic fungal sinusitis: an immunohistologic analysis. *J Allergy Clin Immunol* 2000; **106**: 1096–1101.
- 49 Schubert MS. Allergic fungal sinusitis: pathogenesis and management strategies. *Drugs* 2004; **64**: 363–374.
- 50 Chakrabarti A, Das A, Panda NK. Controversies surrounding the categorization of fungal sinusitis. *Med Mycol* 2008; **46**: 1–10.
- 51 McCann WA, Cromie M, Chandler F, Ford J, Dolen WK. Sensitization to recombinant *Aspergillus fumigatus* allergens in allergic fungal sinusitis. *Ann Allergy Asthma Immunol* 2002; **89**: 203–208.
- 52 Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. I. Demographics and diagnosis. *J Allergy Clin Immunol* 1998; **102**: 387–394.
- 53 deShazo RD, Swain RE. Diagnostic criteria for allergic fungal sinusitis. *J Allergy Clin Immunol* 1995; **96**: 24–35.
- 54 Faergemann J. Atopic dermatitis and fungi. *Clin Microbiol Rev* 2002; **15**: 545–563.
- 55 Woodfolk JA. Allergy and dermatophytes. *Clin Microbiol Rev* 2005; **18**: 30–43.
- 56 Platts-Mills TA, Fiocco GP, Hayden ML, *et al.* Serum IgE antibodies to *Trichophyton* in patients with urticaria, angioedema, asthma, and rhinitis: development of a radioallergen sorbent test. *J Allergy Clin Immunol* 1987; **79**: 40–45.
- 57 Weary PE, Guerrant JL. Chronic urticaria in association with dermatophytosis. Response to the administration of griseofulvin. *Arch Dermatol* 1967; **95**: 400–401.
- 58 Waersted A, Hjorth N. *Pityrosporum orbiculare* - a pathogenic factor in atopic dermatitis of the face, scalp and neck? *Acta Derm Venereol* 1985; **114** (Suppl.): 146–148.
- 59 Kim TY, Jang IG, Park YM, Kim HO, Kim CW. Head and neck dermatitis: the role of *Malassezia furfur*, topical steroid use and environmental factors in its causation. *Clin Exp Dermatol* 1999; **24**: 226–231.
- 60 Takahata Y, Sugita T, Kato H, *et al.* Cutaneous *Malassezia* flora in atopic dermatitis differs between adults and children. *Br J Dermatol* 2007; **157**: 1178–1182.
- 61 Kato H, Sugita T, Ishibashi Y, Nishikawa A. Detection and quantification of specific IgE antibodies against eight *Malassezia* species in sera of patients with atopic dermatitis by using an enzyme-linked immunosorbent assay. *Microbiol Immunol* 2006; **50**: 851–856.
- 62 Wessels MW, Doekes G, Van Ieperen-Van Kijk AG, Koers WJ, Young E. IgE antibodies to *Pityrosporum ovale* in atopic dermatitis. *Br J Dermatol* 1991; **125**: 227–232.
- 63 Watanabe S, Kano R, Sato H, Nakamura Y, Hasegawa A. The effects of *Malassezia* yeasts on cytokine production by human keratinocytes. *J Invest Dermatol* 2001; **116**: 769–773.
- 64 Ishibashi Y, Sugita T, Nishikawa A. Cytokine secretion profile of human keratinocytes exposed to *Malassezia* yeasts. *FEM Immun Med Microbiol* 2006; **48**: 400–409.
- 65 Buentke E, Heffler LC, Wallin RP, *et al.* The allergenic yeast *Malassezia furfur* induces maturation of human dendritic cells. *Clin Exp Allergy* 2001; **31**: 1583–1593.
- 66 Mayser P, Kupfer J, Nemetz D, Schafer U, Nilles M, Hort W, *et al.* Treatment of head and neck dermatitis with ciclopiroxolamine cream – results of a double-blind, placebo-controlled study. *Skin Pharmacol Physiol* 2006; **19**: 153–158.
- 67 Svejgaard E, Larsen PO, Deleuran M, *et al.* Treatment of head and neck dermatitis comparing itraconazole 200 mg and 400 mg daily for 1 week with placebo. *J Eur Acad Dermatol Venereol* 2004; **18**: 445–449.
- 68 Broberg A, Faergemann J. Topical antimycotic treatment of atopic dermatitis in the head/neck area. A double-blind randomised study. *Acta Derm Venereol* 1995; **75**: 46–49.
- 69 Schwartz HJ, Ward GW. Onychomycosis, *Trichophyton* allergy and asthma - a causal relationship? *Ann Allergy Asthma Immunol* 1995; **74**: 523–524.
- 70 Gumowski P, Lech B, Chaves I, Girard JP. Chronic asthma and rhinitis due to *Candida albicans*, *Epidermophyton*, and *Trichophyton*. *Ann Allergy* 1987; **59**: 48–51.
- 71 Ward GW, Jr, Karlsson G, Rose G, Platts-Mills TA. *Trichophyton* asthma: sensitisation of bronchi and upper airways to dermatophyte antigen. *Lancet* 1989; **1**: 859–862.
- 72 Schwartz HJ, Ward GW. Onychomycosis, *Trichophyton* allergy and asthma - a causal relationship? *Ann Allergy Asthma Immunol* 1995; **74**: 523–524.
- 73 Ward GW, Jr, Woodfolk JA, Hayden ML, Jackson S, Platts-Mills TA. Treatment of late-onset asthma with fluconazole. *J Allergy Clin. Immunol* 1999; **104**: 541–546.
- 74 Woodfolk JA. Allergy and dermatophytes. *Clin Microbiol Rev* 2005; **18**: 30–43.
- 75 Crain EF, Weiss KB, Bijur PE, *et al.* An estimate of the prevalence of asthma and wheezing among inner-city children. *Pediatrics* 1994; **94**: 356–362.
- 76 Grant EN, Daugherty SR, Moy JN, *et al.* Prevalence and burden of illness for asthma and related symptoms among

- kindergartners in Chicago public schools. *Ann Allergy Asthma Immunol* 1999; **83**: 113–120.
- 77 Newacheck PW, Halfon N. Access to ambulatory care services for economically disadvantaged children. *Pediatrics* 1986; **78**: 813–819.
 - 78 Belamarich PF, Luder E, Kattan M, Mitchell H, Islam S, Lynn H, *et al.* Do obese inner-city children with asthma have more symptoms than nonobese children with asthma? *Pediatrics* 2000; **106**: 1436–1441.
 - 79 Kunzli N, Kaiser R, Medina S, Studnicka M, Chanel O, Filliger P, *et al.* Public-health impact of outdoor and traffic-related air pollution: a European assessment. *Lancet* 2000; **356**: 795–801.
 - 80 Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, *et al.* Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol* 1998; **102**: 563–570.
 - 81 Merkus PJ, van Toorenenbergen AW. Birds or zebras? *Allergy* 2002; **57**: 566–569.
 - 82 Curtis L, Lee BS, Cai D, Morozova I, Fan JL, Scheff P, *et al.* Pigeon allergens in indoor environments: a preliminary study. *Allergy* 2002; **57**: 627–631.
 - 83 Littman ML, Schneerson SS. *Cryptococcus neoformans* in pigeon excreta in New York City. *Am J Hyg* 1959; **69**: 49–59.
 - 84 Allende M, Pizzo PA, Horowitz M, Pass HI, Walsh TJ. Pulmonary cryptococcosis presenting as metastases in children with sarcomas. *Pediatr Infect Dis J* 1993; **12**: 240–243.
 - 85 Campbell GD. Primary pulmonary cryptococcosis. *Am Rev Resp Dis* 1966; **94**: 236–243.
 - 86 Salyer WR, Salyer DC, Baker RD. Primary complex of *Cryptococcus* and pulmonary lymph nodes. *J Infect Dis* 1974; **130**: 74–77.
 - 87 Garcia-Hermoso D, Janbon G, Dromer F. Epidemiological evidence for dormant *Cryptococcus neoformans* infection. *J Clin Microbiol* 1999; **37**: 3204–3209.
 - 88 Fleuridor R, Lyles RH, Pirofski L. Quantitative and qualitative differences in the serum antibody profiles of human immunodeficiency virus-infected persons with and without *Cryptococcus neoformans* meningitis. *J Infect Dis* 1999; **180**: 1526–1535.
 - 89 Saha DC, Goldman DL, Shao X, Casadevall A, Husain S, Limaye AP, *et al.* Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin. Vaccine Immunol.* 2007; **14**: 1550–1554.
 - 90 Kanj SS, Welty-Woolf K, Madden J, Tapson V, Baez MA, Davis RD, *et al.* Fungal Infections in lung and heart-lung transplant recipients. *Medicine* 1996; **75**: 142–156.
 - 91 Goldman DL, Khine H, Abadi J, Lindenberg DJ, Pirofski L, Niang R, *et al.* Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 2001; **107**: E66.
 - 92 Davis J, Zheng WY, Glatman-Freedman A, Ng JA, Pagcatipunan MR, Lessin H, *et al.* Serologic evidence for regional differences in pediatric cryptococcal infection. *Pediatr Infect Dis J* 2007; **26**: 549–551.
 - 93 Chen GH, McDonald RA, Wells JC, *et al.* The gamma interferon receptor is required for the protective pulmonary inflammatory response to *Cryptococcus neoformans*. *Infect Immun* 2005; **73**: 1788–1796.
 - 94 Goldman DL, Davis J, Bommarito F, Shao X, Casadevall A. Enhanced allergic inflammation and airway responsiveness in rats with chronic *Cryptococcus neoformans* infection: potential role for fungal pulmonary infection in the pathogenesis of asthma. *J Infect Dis* 2006; **193**: 1178–1186.
 - 95 Abe K, Kadota J, Ishimatsu Y, *et al.* Th1-Th2 cytokine kinetics in the bronchoalveolar lavage fluid of mice infected with *Cryptococcus neoformans* of different virulences. *Microbiol Immunol* 2000; **44**: 849–855.
 - 96 Blackstock R, Buchanan KL, Adesina AM, Murphy JW. Differential regulation of immune responses by highly and weakly virulent *Cryptococcus neoformans* isolates. *Infect Immun* 1999; **67**: 3601–3609.
 - 97 Kawakami K, Qureshi MH, Koguchi Y, Nakajima K, Saito A. Differential effect of *Cryptococcus neoformans* on the production of IL-12p40 and IL-10 by murine macrophages stimulated with lipopolysaccharide and gamma interferon. *FEMS Microbiol Lett* 1999; **175**: 87–94.
 - 98 Blackstock R, Buchanan KL, Adesina AM, Murphy JW. Differential regulation of immune responses by highly and weakly virulent *Cryptococcus neoformans* isolates. *Infect Immun* 1999; **67**: 3601–3609.
 - 99 Huffnagle GB, Chen G-H, Curtis JL, *et al.* Down-regulation of the afferent phase of T cell-mediated pulmonary inflammation and immunity by a high melanin-producing strain of *Cryptococcus neoformans*. *J Immunol* 1995; **155**: 3507–3516.
 - 100 Vecchiarelli A, Retini C, Pietrella D, Monari C, Tascini C, Beccari T, *et al.* Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1beta secretion from human monocytes. *Infect Immun* 1995; **63**: 2919–2923.
 - 101 Vecchiarelli A, Retini C, Monari C, Bistoni F, Kozel TR. Purified capsular polysaccharide of *Cryptococcus neoformans* induces interleukin-10 secretion by human monocytes. *Infect Immun* 1996; **64**: 2846–2849.
 - 102 Almeida GM, Andrade RM, Bento CA. The capsular polysaccharides of *Cryptococcus neoformans* activate normal CD4(+) T cells in a dominant Th2 pattern. *J Immunol* 2001; **167**: 5845–5851.
 - 103 Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, *et al.* Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* 2007; **447**: 92–96.
 - 104 Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, *et al.* Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004; **304**: 1678–1682.
 - 105 Bierbaum S, Nickel R, Koch A, Lau S, Deichmann KA, Wahn U, *et al.* Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. *Am J Respir Crit Care Med* 2005; **172**: 1505–1509.
 - 106 Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, *et al.* A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007; **357**: 2016–2027.
 - 107 Overdijk B, Van Steijn GJ, Odds FC. Chitinase levels in guinea pig blood are increased after systemic infection with *Aspergillus fumigatus*. *Glycobiology* 1996; **6**: 627–634.
 - 108 Vicencio AG, Narain S, Du Z, Zeng WY, Ritch J, Casadevall A, *et al.* Pulmonary cryptococcosis induces chitinase in the rat. *Respir Res* 2008; **9**: 40.
 - 109 Calderone RA (ed.). *Candida and Candidiasis*. Washington, DC: ASM Press, 2001: 472.
 - 110 O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. *Nature Medicine* 2004; **10**: 801–805.
 - 111 Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. *Immunity* 2006; **25**: 195–201.
 - 112 Weiner HL. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect* 2001; **3**: 947–954.

- 113 Perez-Machado MA, Ashwood P, Thomson MA, Latcham F, Sim R, Walker-Smith JA, *et al.* Reduced transforming growth factor-beta1-producing T cells in the duodenal mucosa of children with food allergy. *Eur J Immunol* 2003; **33**: 2307–2315.
- 114 Frossard CP, Tropia L, Hauser C, Eigenmann PA. Lymphocytes in Peyer patches regulate clinical tolerance in a murine model of food allergy. *J Allergy Clin Immunol* 2004; **113**: 958–964.
- 115 Tsuji NM, Mizumachi K, Kurisaki J. Interleukin-10-secreting Peyer's patch cells are responsible for active suppression in low-dose oral tolerance. *Immunology* 2001; **103**: 458–464.
- 116 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994; **265**: 1237–1240.
- 117 Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 1999; **190**: 229–239.
- 118 Zhang X, Izikson L, Liu L, Weiner HL. Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration. *J Immunol* 2001; **167**: 4245–4253.
- 119 Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, *et al.* The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001; **27**: 20–21.
- 120 Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J Ped* 1982; **100**: 731–737.
- 121 Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; **199**: 1679–1688.
- 122 Montagnoli C, Bacci A, Bozza S, Gaziano R, Mosci P, Sharpe AH, *et al.* B7/CD28-dependent CD4+CD25+ regulatory T cells are essential components of the memory-protective immunity to *Candida albicans*. *J Immunol* 2002; **169**: 6298–308.
- 123 de Luca A, Montagnoli C, Zelante T, Bonifazi P, Bozza S, Moretti S, *et al.* Functional yet balanced reactivity to *Candida albicans* requires TRIF, MyD88, and IDO-dependent inhibition of Rorc. *J Immunol* 2007; **179**: 5999–6008.
- 124 Bozza S, Fallarino F, Pitzurra L, Zelante T, Montagnoli C, Bellocchio S, *et al.* A crucial role for tryptophan catabolism at the host/*Candida albicans* interface. *J Immunol* 2005; **174**: 2910–2918.
- 125 van Ogtrop ML, Guiot HF, Mattie H, van Furth R. Modulation of the intestinal flora of mice by parenteral treatment with broad-spectrum cephalosporins. *Antimicrob Agents Chemother* 1991; **35**: 976–982.
- 126 Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet - Inf Dis* 2001; **1**: 101–114.
- 127 Giuliano M, Barza M, Jacobus NV, Gorbach SL. Effect of broad-spectrum parenteral antibiotics on composition of intestinal microflora of humans. *Antimicrob Agents Chemother* 1987; **31**: 202–206.
- 128 Samonis G, Gikas A, Anaissie EJ, Vrenzos G, Maraki S, Tselentis Y, *et al.* Prospective evaluation of effects of broad-spectrum antibiotics on gastrointestinal yeast colonization of humans. *Antimicrob Agents Chemother* 1993; **37**: 51–53.
- 129 Orrhage K, Nord CE. *Bifidobacteria* and lactobacilli in human health. *Drugs Exp Clin Res* 2000; **26**: 95–111.
- 130 Sjoval J, Huitfeldt B, Magni L, Nord CE. Effect of beta-lactam prodrugs on human intestinal microflora. *Scand J Infect Dis Suppl* 1986; **49**: 73–84.
- 131 Lidbeck A, Nord CE. Lactobacilli and the normal human anaerobic microflora. *Clin Infect Dis* 1993; **16** (Suppl. 4): S181–S187.
- 132 Payne S, Gibson G, Wynne A, *et al.* In vitro studies on colonization resistance of the human gut microbiota to *Candida albicans* and the effects of tetracycline and Lactobacillus plantarum LPK. *Curr Issues Intest Microbiol* 2003; **4**: 1–8.
- 133 van der Waaij D. The ecology of the human intestine and its consequences for overgrowth by pathogens such as *Clostridium difficile*. *Annu Rev Microbiol* 1989; **43**: 69–87.
- 134 Guggenbichler JP, Kofler J, Allerberger F. The influence of third-generation cephalosporins on the aerobic intestinal flora. *Infection* 1985; **13** (Suppl. 1): S137–S139.
- 135 Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol* 2002; **3**: 715–720.
- 136 Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001; **1**: 69–75.
- 137 Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. *Lancet* 1999; **353**: 1485–1488.
- 138 McKeever TM, *et al.* Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. *J Allergy Clin Immunol* 2002; **109**: 43–50.
- 139 Wjst M, Hoelscher B, Frye C, *et al.* Early antibiotic treatment and later asthma. *Eur J Med Res* 2001; **6**: 263–271.
- 140 Droste JH, Wieringa MH, Weyler JJ, *et al.* Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? *Clin Exp Allergy* 2000; **30**: 1547–1553.
- 141 Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999; **29**: 342–346.
- 142 Bottcher MF, Nordin EK, Sandin A, Midtvedt T, Bjorksten B. Microflora-associated characteristics in faeces from allergic and nonallergic infants. *Clin Exp Allergy* 2000; **30**: 1590–1596.
- 143 Kalliomaki M, Kirjavainen P, Eerola E, *et al.* Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; **107**: 129–134.
- 144 Kirjavainen PV, Apostolou E, Arvola T, *et al.* Characterizing the composition of intestinal microflora as a prospective treatment target in infant allergic disease. *FEMS Immunol Med Microbiol* 2001; **32**: 1–7.
- 145 Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; **108**: 516–520.
- 146 Bandeira A, Mota-Santos T, Itoharu S, Degermann S, Heusser C, Tonegawa S, *et al.* Localization of gamma/delta T cells to the intestinal epithelium is independent of normal microbial colonization. *J Exp Med* 1990; **172**: 239–244.
- 147 Lefrancois L, Goodman T. *In vivo* modulation of cytolytic activity and Thy-1 expression in TCR-gamma delta+ intraepithelial lymphocytes. *Science* 1989; **243**: 1716–1718.
- 148 Yamanaka T, Helgeland L, Farstad IN, *et al.* Microbial colonization drives lymphocyte accumulation and differentiation in the follicle-associated epithelium of Peyer's patches. *J Immunol* 2003; **170**: 816–822.
- 149 Cooper MD, Kincade PW, Bockman DE, Lawton AR. Origin, distribution and differentiation of IgA-producing cells. *Adv Exp Med Biol* 1974; **45**: 13–22.

- 150 Bos NA, Cebra JJ, Kroese FG. B-1 cells and the intestinal microflora. *Curr Top Microbiol Immunol* 2000; **252**: 211–220.
- 151 Umesaki Y, Setoyama H. Structure of the intestinal flora responsible for development of the gut immune system in a rodent model. *Microbes Infect* 2000; **2**: 1343–1351.
- 152 Noverr MC, Falkowski NR, McDonald RA, Huffnagle GB. The development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen and IL-13. *Infect Immun* 2005; **73**: 30–38.
- 153 Noverr MC, Noggle RM, Toews GB, Huffnagle GB. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect Immun* 2004; **72**: 4996–5003.
- 154 Yamaguchi N, Sugita R, Miki A, Takemura N, Kawabata J, Watanabe J, *et al.* Gastrointestinal *Candida* colonisation promotes sensitisation against food antigens by affecting the mucosal barrier in mice. *Gut* 2006; **55**: 954–960.
- 155 Yamaguchi N, Sonoyama K, Kikuchi H, *et al.* Gastric colonization of *Candida albicans* differs in mice fed commercial and purified diets. *J Nutr* 2005; **135**: 109–115.
- 156 Noverr MC, Phare SM, Toews GB, Coffey MJ, Huffnagle GB. Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect Immun* 2001; **69**: 2957–2963.
- 157 Tsitsigiannis DI, Bok JW, Andes D, *et al.* *Aspergillus* cyclooxygenase-like enzymes are associated with prostaglandin production and virulence. *Infect Immun* 2005; **73**: 4548–4559.
- 158 Erb-Downward JR, Huffnagle GB. *Cryptococcus neoformans* produces authentic prostaglandin E2 without a cyclooxygenase. *Eukaryot Cell* 2007; **6**: 346–350.
- 159 Erb-Downward JR, Noverr MC. Characterization of prostaglandin E2 production by *Candida albicans*. *Infect Immun* 2007; **75**: 3498–3505.
- 160 Noverr MC, Erb-Downward JR, Huffnagle GB. Production of eicosanoids and other oxylipins by pathogenic eukaryotic microbes. *Clin Microbiol Rev* 2003; **16**: 517–533.
- 161 Matsuoka T, *et al.* Prostaglandin D2 as a mediator of allergic asthma. *Science* 2000; **287**: 2013–2017.
- 162 Peters-Golden M. Open mind, open airways: broadening the paradigm of prostaglandins and allergic airway inflammation. *Am J Respir Crit Care Med* 2002; **165**: 1035–1036.
- 163 Angeli V, *et al.* Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J Exp Med* 2001; **193**: 1135–1147.
- 164 Herve M, *et al.* Pivotal roles of the parasite PGD2 synthase and of the host D prostanoid receptor 1 in schistosome immune evasion. *Eur J Immunol* 2003; **33**: 2764–2772.
- 165 Fogelmark B, Thorn J, Rylander R. Inhalation of (1- >3)-beta-D-glucan causes airway eosinophilia. *Mediators Inflamm* 2001; **10**: 13–19.
- 166 Faergemann J. Atopic dermatitis and fungi. *Clin Microbiol Rev* 2002; **15**: 545–563.
- 167 Lacour M, Zunder T, Huber R, Sander A, Daschner F, Frank U. The pathogenetic significance of intestinal *Candida* colonization - a systematic review from an interdisciplinary and environmental medical point of view. *Int J Hyg Environ Health* 2002; **205**: 257–268.
- 168 Nikkels AF, Pierard GE. Framing the future of antifungals in atopic dermatitis. *Dermatology* 2003; **206**: 398–400.
- 169 Garcia YH, Diez SG, Aizpun LT, Oliva NP. Antigliadin antibodies associated with chronic mucocutaneous candidiasis. *Pediatr Dermatol* 2002; **19**: 415–418.
- 170 Nieuwenhuizen WF, Pieters RH, Knippels LM, Jansen MC, Koppelman SJ. Is *Candida albicans* a trigger in the onset of coeliac disease? *Lancet* 2003; **361**: 2152–154.
- 171 Peyrin-Biroulet L, Standaert-Vitse A, Branche J, Chamaillard M. IBD serological panels: facts and perspectives. *Inflamm Bowel Dis* 2007; **13**: 1561–1566.
- 172 Schaffer T, Muller S, Flogerzi B, *et al.* Anti-*Saccharomyces cerevisiae* mannan antibodies (ASCA) of Crohn's patients cross-react with mannan from other yeast strains, and murine ASCA IgM can be experimentally induced with *Candida albicans*. *Inflamm Bowel Dis* 2007; **13**: 1339–1346.
- 173 Eaton K. Is there an allergic and fermentative gut condition, and does it relate to *Candida*? In: Brostoff J, Challacombe SJ (eds). *Food Allergy and Intolerance*. London: Saunders, 2002: 351–364.