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Fungal glycan interactions with epithelial cells in allergic airway disease

René M. Roy^{1,4,5} and Bruce S. Klein^{1,2,3}

¹Departments of Pediatrics, at the University of Wisconsin School of Medicine and Public Health; University of Wisconsin-Madison, Madison, WI, 53792

²Internal Medicine, at the University of Wisconsin School of Medicine and Public Health; University of Wisconsin-Madison, Madison, WI, 53792

³Medical Microbiology and Immunology at the University of Wisconsin School of Medicine and Public Health; University of Wisconsin-Madison, Madison, WI, 53792

⁴Cell and Molecular Biology Graduate Training Program at the University of Wisconsin School of Medicine and Public Health; University of Wisconsin-Madison, Madison, WI, 53792

⁵Medical Scientist Training Program at the University of Wisconsin School of Medicine and Public Health; University of Wisconsin-Madison, Madison, WI, 53792

Abstract

Human exposure to fungi results in a wide range of health outcomes, from invasive disease or allergy to immune tolerance. Inhaled fungi contact airway epithelial cells as an early event, and this host:fungal interaction can shape the eventual immunological outcome. Emerging evidence points to exposure to fungal cell wall carbohydrates in the development of allergic airway disease. Herein, we describe determinants of fungal allergenicity, and review the responses of airway epithelial cells to fungal carbohydrates. A greater understanding of the recognition of and response to fungal carbohydrates by airway epithelial cells may lead to the development of targeted therapies that ameliorate allergic airway disease.

Introduction

Human fungal pathogens represent a serious and growing health challenge due to an increase in the numbers of immunocompromised patients, and the emergence of new and virulent fungal pathogens. Set against the backdrop of a limited pharmacological armamentarium of antifungal drugs, emerging resistance to multiple antifungal compounds, poor diagnostic capabilities, and limited research investment compared to other infectious diseases [1], the rising incidence of fungal infections represents a daunting challenge to health care systems worldwide.

Adding to the fungal burden on health care systems is the prominent role of sensitization to environmental fungi in the development, maintenance, and exacerbation of allergic airway

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Reprints and correspondence to: Bruce Klein, University of Wisconsin-Madison, Microbial Sciences Building, 1550 Linden Drive, Madison, WI, 53706 608-263-9217 (telephone), 608-262-8418 (Fax). bsklein@wisc.edu.

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disease. At least 20%, but possibly as high as 80%, of the 25 million Americans with asthma have a positive skin test to at least one fungal allergen [2, 3]. More than 50 genera from across the fungal kingdom are capable of sensitizing exposed individuals and most fungal species possess multiple allergens. While most humans are exposed to millions of fungal spores daily with little effect, increases in atmospheric fungal spore levels are associated with increases in asthma exacerbations and hospital admissions in patients sensitized to fungi. Ongoing climate change linked to increased temperature and atmospheric CO₂ levels further increases atmospheric fungal spore concentrations with a parallel increase in allergic airway disease [4]. When economic losses, infections, and allergy are considered together, the growing impact of fungi on the human condition is cannot be overstated.

Airway epithelial cells line the conducting airways of the lung and form an active barrier that provides a robust defense against inhaled fungi. This active barrier represents the initial point of contact for inhaled fungal allergens and toxins and triggers the inflammatory response to inhaled fungi. Moreover, dysregulation of airway epithelial cell responses to inhaled allergens is central to allergic airway pathology, including in asthma [5, 6]. This review will focus on recognition of fungi by airway epithelial cells and the role of airway epithelial cells in mediating allergic sensitization to fungi.

Determinants of fungal allergenicity

Fungi are well suited to incite airway disease due to their size, their ubiquitous airborne presence, and their particular repertoire of carbohydrates and proteins that interact with cells in the airways (Figure 1). The size of fungal spores from so-called “allergenic” genera varies from 1µm to 100µm although most are smaller than 10µm [7]. Along with other particulates of this size range that are associated with allergy such as diesel exhaust particles, fungal spores can penetrate deeply into the airways depositing near the terminal bronchioles. The composition of the outer shell of fungal spores also facilitates their ability to penetrate deeply into the airways. For example, the rodlet protein, RodA, provides a hydrophobic covering to *Aspergillus fumigatus* spores [8], which allows for increased deposition in the small airways compared to hygroscopic particles, which swell and deposit in larger airways [9].

The ubiquity of fungal spores in the environment ensures that the airway is exposed to fungal spores with each breath. Upon germination, fungi secrete proteases, which have direct effects on the airway epithelial layer (Figure 2). First, the proteases act on and degrade proteins of the tight junctions between airway epithelial cells leading to dysregulated barrier function [10]. Bronchial biopsies from asthmatic patients demonstrate irregular tight junctions between airway epithelial cells with increased permeability of the epithelium pointing to a pathogenic role of epithelial dysregulation in asthma [11]. Following allergen induced epithelial cell apoptosis, neighboring epithelial cells engulf the dying cell and secrete anti-inflammatory cytokines, which limit allergic inflammation and maintain barrier integrity. Inhibition of this Rac-1 dependent process results in breakdown of epithelial integrity, increased IL-33 secretion, and enhanced neutrophil recruitment to the lung following allergen exposure [12]. In addition, breakdown of the epithelial barrier hinders the maintenance of an effective anti-fungal fluid layer comprised of surfactants, complement, defensins, cathelicidins, lactoferrin, and mucins at the apical surface of the epithelium. Second, fungal proteases cleave and activate protease-activated receptors (PAR), a critical requirement for the allergenicity of purified fungal allergens. Exposure of human epithelial cells *in vitro* to purified allergens from *Alternaria* leads to production of inflammatory cytokines in a PAR-2 dependent manner [13]. Activation of PAR can also exacerbate existing allergic inflammation and break inhalational tolerance to otherwise innocuous antigens in the airway [14]. Finally, damage induced by fungal proteases results in the

release of endogenous danger signals such as free ATP and uric acid that further enhance inflammation in the airway.

Recognition of fungal carbohydrates by airway epithelial cells

While hundreds of protein fungal allergens have been characterized, little is known about the role of polysaccharides in the fungal cell wall in mediating allergic sensitization to fungi. Over 90% of the fungal cell wall is comprised of polysaccharides linked together in a three dimensional matrix that undergoes constant modification in response to environmental cues. Because fungal cell wall carbohydrates are widely conserved across the fungal kingdom but absent in humans, these structural components of the cell wall are convenient targets for immune recognition by airway epithelial cells. Airway epithelial cells express a broad repertoire of PRR, including toll like receptors (TLR), c-type lectins (CLR), PAR, and NOD-like receptors which can sense both fungal pathogen associated molecular patterns (PAMP) and damage associated molecular patterns (DAMP) produced by local cellular death or stress [15] (Figure 2). The central core of the fungal cell wall is comprised of branched β -1,3-glucan cross-linked to chitin, a linear polysaccharide of β -1,4 N-acetyl D-glucosamine (GlcNAc). Both carbohydrates trigger inflammatory responses from airway epithelial cells.

Fungal β -glucans

Fungal β -glucans robustly induce IL-8 and IL-6 from airway epithelial cells *in vitro* and in animal models in a manner partially dependent on Dectin-1 [16, 17]. Similarly, β -glucan exposure promotes allergic sensitization to co-administered antigens in the lung [17]. β -glucans in house dust mite (HDM) extract, present due to the consumption of fungi by the insects, also potentially induce inflammatory responses from a human airway epithelial cell line [18]. Following exposure to HDM extract, 16HBE14o- cells rapidly secrete CCL20 in a β -glucan- but not protease- or LPS-dependent manner. This suggests that airway epithelial cells are primed to recruit dendritic cells to the airway mucosa via CCL20 secretion upon recognition of the carbohydrate. Recently, HDM extract-associated β -glucans were shown to differentially affect inflammatory responses from nasal and bronchial epithelial cells [19]. Nasal epithelial cells secreted CCL20 in a β -glucan and TLR2 dependent fashion, whereas bronchial epithelial cells secreted CCL20 in a LPS and TLR4 dependent fashion, which did not depend on β -glucan. Interestingly, dectin-1 mediated recognition of β -glucan appears to be largely dispensable in nasal epithelial cells. These findings are consistent with earlier work demonstrating a clear role for TLR4 on lung stromal cells in a HDM model of allergic airway disease [20]. Studies assessing the impact of β -glucan on allergic airway disease have yielded conflicting results depending on the route of β -glucan exposure and the particular allergen used [21-24], however the role of epithelial cells in these studies remained unexplored. These findings highlight the remarkable diversity of mucosal immune responses driven by β -glucan, which continue to be elucidated.

Fungal chitin

In contrast to β -glucan, recognition of and responses to chitin by mammalian cells are less defined. In *Arabidopsis*, recognition of chitin oligosaccharides of at least eight sugars triggers robust anti-fungal immune responses via dimerization of a LysM domain containing receptor, CERK1 [25]. However in mammals, little information is available concerning the chitin structure that is recognized. The receptor FIBCD1, which is highly expressed in intestinal epithelial cells recognizes acetylated structures including chitin [26] while, more broadly, galectins have an affinity for terminal GlcNAc residues. While dectin-1 and TLR2 do not bind to chitin directly, these receptors have been implicated in macrophage and

keratinocyte responses to chitin particles [27, 28]. In response to chitin exposure, murine airway epithelial cells produced CCL2 [29] as part of the early innate immune response in the lung. When exposed to chitin particles *in vitro*, human nasal epithelial cells expressed the allergy-associated products eotaxin-3 and acidic mammalian chitinase (AMCase) [30]. Because chitin rests mainly in the inner fungal cell wall, it may be largely shielded from epithelial cells by other fungal cell wall components during the initial host-fungus encounter [31]. However, through the action of anti-fungal compounds at the epithelial surface and the germination of the fungal organism, direct recognition of fungal chitin by airway epithelial cells may still occur.

Other fungal glycans

While chitin and β -glucan form the core of the fungal cell wall, mannans and α -glucans decorate the fungal cell wall surface. α -glucan serves to mask inflammatory polysaccharides and to downregulate TLR-mediated cytokine production following fungal exposure [32, 33]. Similarly, the polysaccharide capsule of *Cryptococcus* comprised of glucuronoxylomannan (GXM) and galactoxylomannan (GalXM), completely shields the cryptococcal cell wall while mediating attachment to epithelial cells in the lung. Indeed, capsular *C. neoformans* strains meagerly induced IL-8 production from human airway epithelial cells *in vitro* when compared with acapsular strains [34]. N-linked and O-linked mannans in the fungal cell wall may also shield β -glucans from recognition. At low fungal burdens, *Candida* generates a limited response from oral epithelial cells, but upon germination, and an increase in fungal numbers triggers the release of inflammatory cytokines [35]. At the same time, recognition of *Candida* fungal mannans by dectin-2 on myeloid cells is critical in mounting an anti-fungal Th17 immune response [36]. While dectin-2 on dendritic cells has been implicated in the development of allergic inflammation in a HDM model of asthma [37], little is known about the role of dectin-2 in the development of fungal-associated allergic airway disease or the role of the receptor in epithelial recognition of fungi.

Concluding remarks

Rapid and significant strides are currently being made to understand the pathophysiology of allergic airway disease and the immune recognition of fungi. Yet, comparatively little is known about the role of the airway epithelium in the recognition and response to fungi particularly as it relates to allergic airway disease. Because fungi possess a broad array of PAMP, including cell wall carbohydrates, airway epithelial cells must integrate the information and initiate an appropriate immune response through the secretion of anti-fungal compounds and the recruitment of innate and adaptive immune cells to the site of the encounter. A greater understanding of the dynamic integration of these signals in airway epithelial cells and the effects of epithelial cell products on the evolving immune response may lead to targeted interventions that alter the progress of the allergic response and reestablish tolerance to innocuous fungi in the airway.

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Highlights

Many patients with asthma have a positive skin test to at least one fungal allergen.

Airway epithelium initially senses inhaled fungi and triggers inflammatory responses.

Fungal glycans interact with airway epithelial cell receptors initiating inflammation.

Figure 1. Fungal determinants of allergenicity

Inhaled fungal spores penetrate to the terminal bronchioles due to their size and hydrophobic coat. Upon germination, fungal spores expose their polysaccharide cell wall and release proteases which damage the epithelial cells and allow for penetration of the epithelial cell barrier.

Figure 2. Epithelial cell interactions with fungi

Epithelial cells secrete antifungal compounds that line the airways. Epithelial cells damaged by fungal proteases release DAMP, which contribute to the inflammatory response. Fungal proteases also activate PAR. Fungal spores are recognized by PRR, which trigger release of inflammatory cytokines.