

Innate and Adaptive Immune Response to Fungal Products and Allergens



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Exposure to fungi and their products is practically ubiquitous, yet most of this is of little consequence to most healthy individuals. This is because there are a number of elaborate mechanisms to deal with these exposures. Most of these mechanisms are designed to recognize and neutralize such exposures. However, in understanding these mechanisms it has become clear that many of them overlap with our ability to respond to disruptions in tissue function caused by trauma or deterioration. These responses involve the innate and adaptive immune systems usually through the activation of nuclear factor kappa B and the production of cytokines that are considered inflammatory accompanied by other factors that can moderate these reactivities. Depending on different genetic backgrounds and the extent of activation of these mechanisms, various pathologies with resulting symptoms can ensue. Complicating this is the fact that these mechanisms can bias toward type 2 innate and adaptive immune responses. Thus, to understand what we refer to as allergens from fungal sources, we must first understand how they influence these innate mechanisms. In doing so it has become clear that many of the proteins that are described as fungal allergens are essentially homologues of our own proteins that signal or cause tissue disruptions. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2016;4:386-95)

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High-throughput sequencing methods suggest that up to 5.1 million fungal species exist inhabiting practically every niche on Earth.^{1,2} They form a significant portion of the Earth's biomass

(25%)³ and thus exposure to fungi and their products is substantial and ubiquitous. Because of an elaborate collection of human defensive mechanisms, fungi rarely cause infectious diseases in healthy immunocompetent hosts. However, the elaboration of toxic metabolites, cellular injury through competitive metabolism, replication, and overexuberant innate and adoptive immune responses can lead to various pathologies and symptoms.^{3,4} Of particular concern is the connection between symptoms resulting from either sterile or infectious aspects of inflammation.

Besides intact skin and mucosal surfaces that are bathed in various antimicrobial substances (eg, surfactants A and D, β -defensins, cathelicidins, chemokines, lysozyme, lactoferrin, and mucins), multicellular organisms possess a number of mechanisms to recognize and deal with invading organisms and foreign materials. These include innate and adoptive immune mechanisms that also interact synergistically to prevent disruptions in homeostasis. Detection of microorganisms and foreign materials is mediated by an admixture of genetically encoded receptors known as pattern recognition receptors (PRRs).⁵ These receptors (transmembrane and cytoplasmic) recognize and bind to evolutionary conserved structures generally referred to as pathogen-associated molecular patterns (PAMPs). Interestingly, these receptors not only detect microorganism invasions but also are closely tied to the detection of tissue damage, whether by trauma or pathogens, and trigger physiological responses to deal with both. With respect to internal tissue and cell damage, the various substances released are referred to as danger- or damage-associated molecular patterns (DAMPs).⁶ Signals generated through PAMPs and DAMPs induce various patterns of cytokine and chemokine expression, which, in turn, modulate innate and adaptive inflammatory responses. The clinical consequences of this depend on the type, location, extent, and duration of these responses. Often, it is the cumulative effect of these responses interacting with the genetic background involved that determines the outcome. And because these events are not mutually exclusive, difficulties in recognizing specific mechanisms of symptoms elicited by fungal exposures remain.

In this article, we briefly describe the innate system's abilities to recognize fungal components (fungi PRRs), the fungal molecules that are recognized (PAMPs and DAMPs), and the defined antigens to which adaptive immune responses are elicited. In addition, because many of the innate inflammatory responses involve lymphoid cells (innate lymphoid cells) and in particular type 2 innate lymphoid cells (ILCs2), a discussion of the connections between fungal promotion of both innate and adaptive immune responses (particularly T_H2 and T_H17 responses) is presented. How these responses are coordinated and their connections to symptoms and a discussion of interpretive variables of immunological findings will also be presented.

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Abbreviations used

CTLR- C-type lectin-like receptor
DAMP- damage-associated molecular pattern
DC-SIGN- Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin
HSP- heat shock protein
ILC2- type 2 innate lymphoid cell
MR- mannose receptor
NF- κ B- nuclear factor kappa B
NLR- nucleotide oligomerization domain-like receptor
NOD- nucleotide oligomerization domain
PAMP- pathogen-associated molecular pattern
PRR- pattern recognition receptor
RAGE- receptor for altered advanced glycation end product
RLR- retinoic acid-inducible gene 1-like receptor
s-IgE- specific immunoglobulin E
siglec- sialic acid-binding immunoglobulin-like lectins
TLR- Toll-like receptor
Treg- regulatory T

INNATE RECOGNITION OF FUNGAL STRUCTURES AND PRODUCTS

The cell-associated PRRs identified so far have been divided into 4 families: Toll-like receptors (TLRs), nucleotide-oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene 1-like receptors (RLRs), and C-type lectin receptors.⁷ In addition, protease-activated receptors and sialic acid-binding proteins (sialic acid-binding immunoglobulin-like lectins [siglecs]) may be included in this group.^{7,8} Because fungal cell walls are composed of layers of carbohydrates including mannans (polymers of mannose), β -glucans (polymers of glucose linked by β glycosidic bonds), and chitins (polymers of *N*-acetyl-D-glucosamine), a number of PRRs are alerted to their presence.^{9,10} In addition, many of the secreted and intracellular fungal proteins represent or can induce DAMPs as outlined below. Again, the purpose of these PRRs is to collectively alert the innate immune systems to the presence of possible insults to tissue homeostasis and promote and control both innate and adaptive systems responses.

Toll-like receptors

Currently, there are 12 known TLRs in humans that recognize various PAMPs. These receptors are type 1 transmembrane receptors (ie, N terminus is extracellular) with intracellular signaling tails that are homologous to the IL-1 receptor and are located on innate leukocytes such as macrophages and polymorphonuclear cells.^{11,12} TLR1, TLR2, TLR3, TLR4, TLR6, and TLR9 have been implicated in recognition of fungal PAMPs, resulting in possible clearance, inflammation, and tissue injury.¹³

TLR receptor signaling depends on a number of different adaptor molecules that lend some degree of specificity between different TLR members. The outcome depends on which TLR or combination of TLRs is activated. For example, TLR6 seems to be involved in the production of IL-23 and IL-17A, which promote T_H17 responses. A deficiency of a TLR6 response results in an augmented asthma response, presumably by enhancing T_H2 responses.^{10,14} Responses that favor TLR2 activation induce oxidative pathways in polymorphonuclear cells with the release of gelatinases and inflammatory cytokines. The gelatinases are extracellular matrix destructive enzymes that are associated with neutrophil migration. These reactivities seem to

be important in lung pathology in which neutrophilic infiltrates and lung damage occur. TLR2 also seems to play a role in promoting regulatory T (Treg) cells and in limiting some anti-fungal responses.¹⁵ TLR3 responds to double-stranded RNA from conidia, and TLR9 responds to unmethylated CpG motifs from fungal DNA.¹⁶ TLR4 (and cofactor CD14) activation by recognition of fungal conidia ligands seems to enhance TNF- α production and oxidative pathways.¹⁷ Millien et al¹⁸ have demonstrated that allergic airway inflammation can be an anti-fungal defensive strategy that is driven by fibrinogen cleavage and TLR4 activation. TLR2 recognizes fungal conidia and hyphae. A number of TLRs can combine to recognize different fungal structures, for example, as TLR2/1, and TLR1, TLR2, TLR4, and TLR6 are capable of forming heterodimers in different combinations that can recognize fungal structures (and oxidation-specific fungal epitopes).¹⁹ Among other activities, TLR signaling often results in the activation of nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinases, which regulate proinflammatory gene transcription and expression. In addition, the TLRs seem to cooperate with other PRRs such as the C-type lectin-like receptors (CTLRs) (discussed below), which serves to amplify and modify such responses.²⁰

Evidence of the interaction of TLRs and elements of fungi include the demonstration that TLR2 recognizes general fungal β -glucans from pathogenic fungus *Coccidioides*^{21,22} and the demonstration that TLR6 is involved in the recognition of *Candida albicans*.²³ Innate immune detection of *Aspergillus fumigatus* is mediated by TLR4 and TLR2 together with TLR1 or TLR6 in mice and TLR1 but not TLR6 in humans.²⁴

Nucleotide oligomerization domain/nucleotide oligomerization domain-like receptors

The NLRs are a family of intracellular receptors that are capable of recognizing various PAMPs. In humans, 23 of these PAMPs have been identified. The NOD subfamily contains at least 1 caspase-activating and recruiting domain, whereas the NLRs are identified as having a pyrin domain.²⁵ NOD1 and NOD2 are associated with the adaptor molecule receptor-interacting protein 2 (RIP2), which can activate NF- κ B. The NLR subfamily (NLR1-12) forms a multimeric complex known as an inflammasome after ligand recognition. The inflammasome NLRP3 can activate procaspase 1 to caspase 1, which, in turn, cleaves TLR-induced pro IL-1 β into active IL-1 β . It also activates IL-18 in a similar fashion. When secreted, these cytokines have multiple proinflammatory effects. The NLRP3 inflammasome is the best studied and seems to have cross talk with C-type lectin receptors by their ability to stimulate the production of reactive oxygen species.^{26,27} One of the end results is the activation of IL-1 β , known to be an important mediator of inflammatory responses to PAMPs. Evidence for the interaction of fungal components and NLRs includes demonstration that *Aspergillus fumigatus* hyphal fragments induce NLRP3 inflammasome assembly *in vitro* and the illustration that absent in melanoma 2 (AIM2) and NLRP3 form a dual cytoplasmic surveillance system that orchestrates responses against *A fumigatus* infection in mice.²⁸

C-type lectin-like receptors

CTLRs form a family of protein receptors that recognize self and nonself ligands including various carbohydrates, lipids, and proteins. The 1000+ members are organized into 17 groups and,

following binding of their ligands, mediate a number of responses including phagocytosis, clearance of apoptotic cells and materials, modulation of innate and adaptive immune responses, and cell-cell adhesion.²⁹

The best-studied CTLRs involved in fungal recognition include dectin-1, dectin-2, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin (DC-SIGN), macrophage inducible C-type lectin, and the mannose receptor (MR).³⁰ Dectin-1 binds and promotes phagocytosis of particles containing β 1,3 glucans. It also cooperates with TLR2, supporting the activation of NF- κ B and in inducing the production of reactive oxygen species.³¹ Dectin-1 activation can also induce mast cells to produce proinflammatory and T_H2-polarizing cytokines (IL-4 and IL-13).³² Dectin-2 binds mannans and fucose and also activates NF- κ B. In addition, dectin-2 promotes T_H17 polarization by inducing IL-17A, which is crucial in neutralizing some fungi.³³ Activation of dectin-2 receptors also promotes T_H2-mediated lung inflammation by inducing IL-33, a key proinflammatory cytokine.³⁴ DC-SIGN is a C-type lectin that binds to mannans, α -mannosyl, and α fucosyl residues and is involved in the recognition of a large spectrum of pathogens.³⁵ Various allergens interact with DC-SIGN via their fucose moieties and induce TNF- α , a proinflammatory cytokine.³⁶ Macrophage inducible C-type lectin is another C-type lectin that recognizes terminal α mannoses.³⁷ Its engagement has been shown to suppress IL-12 transcription.³⁸ The MR recognizes mannose, fucose, or *N*-acetylglucosamine residues present on glucoconjugates. It promotes phagocytosis and cooperates with DC-SIGN in recognizing N-linked mannans. Together or separately, the CTLRs form important sensors for recognizing fungal surfaces and integrating cellular events such as phagocytosis, microbiocidal activities, and cytokine induction.³⁰ Specific evidence for the involvement of the MR in fungal-related pathogenesis includes studies with PRR-deficient cells that demonstrate that dectin-1, MR, TLR-2, and TLR-4 control lymphocyte proliferation and IL-17 production induced by *Paracoccidioides brasiliensis*-stimulated dendritic cells.³⁴

Retinoic acid-inducible gene 1 receptors

Currently, there are 3 known retinoic acid-inducible gene 1-like receptors including retinoic acid-inducible gene 1, melanoma differentiation-associated protein 5 (MDA-5), and laboratory of genetics and physiology 2 (LGP2). These cytoplasmic receptors recognize foreign RNA and double-stranded RNA and activate downstream signaling cascades and subsequent proinflammatory cytokines. These receptors activate NF- κ B and IFN- β promoter stimulator 1, which induces mitochondrial and peroxisomal responses to infections. Retinoic acid-inducible gene 1-like receptors are thought to be mostly responsive to viral infections. Their activation represses TGF- β and thus Treg-cell responses, which control T_H2 responses.³⁹

Protease-activated receptors

Four distinct protease-activated receptors have been identified that all belong to the 7-transmembrane type of receptors.⁴⁰ They share a common signal pathway that involves heterotrimeric G proteins that regulate important intracellular enzymes such as guanylyl cyclase, adenylyl cyclase, and phospholipase C, which, in turn, regulate important intracellular secondary messages that influence Ca⁺⁺ stores, and transcriptional activation of cytokines, chemokines, and mediators such as prostaglandin E₂.⁴¹

The best studied of these receptors is protease-activated receptor 2, which is expressed in elevated levels in the lungs and nasal epithelium of people with asthma.⁸ The activation of protease receptors on epithelial cells results in proinflammatory cytokine release (IL-1, IL-25, IL-33, thymic stromal lymphopoietin, and GM-CSF and also danger signals such as HMGBBox protein, uric acid, and ATP), which activates the dendritic cell network and other innate immune cells.⁴² Activating proteases can be endogenous (trypsin, tryptase, elastase, cathepsin G) or exogenous such as proteases from allergenic sources (Der p1, Bla g 1, and multiple fungal proteases as listed below).^{43,44} Although there is much to learn about the interactions and regulation of protease receptors, it is clear that protease allergens can directly affect them and be considered exogenous danger signals. It has been demonstrated that *Alternaria* carries intrinsic serine protease activity that can elicit rapid release of IL-33 into murine airways through the activation of protease-activated receptor 2.⁴⁵

Sialic acid-binding immunoglobulin-like lectins

Siglecs are a family of immunoglobulin-like type I transmembrane proteins with immunoglobulin V-like domains that bind to sialic acids attached to the terminus of cell surface glycoconjugates. These have been divided into 2 families on the basis of structure and so far consist of 14 types based on their extracellular domains in humans.⁴⁶ Siglecs are highly expressed on cells of the adaptive immune system (particularly B cells, and to a lesser extent on dendritic cells, myeloid cells, and T cells) and are highly involved in self-discrimination and nonself discrimination.⁴⁷ In addition, they selectively affect innate immune responses to DAMPs but not to PAMPs and are suspected to play a major role in sensing infection versus tissue injuries. Siglecs do respond to pathogen-derived sialoglycoconjugates, are involved in B-cell tolerance to self-antigens, and modulate plasmacytoid dendritic cells and T cells.⁷ Most siglecs have immunoreceptor tyrosine-based inhibitory motifs in their intracellular domain. Activation of immunoreceptor tyrosine-based inhibitory motifs generally attenuates signal transmission and thus the siglec receptors seem to be mainly involved in either inhibition or at least dampening inflammatory responses. Of significance with regard to fungal proteins, heat shock protein 70 (HSP-70) and HSP-90 are DAMPs capable of inducing significant inflammatory responses by their association with CD24 and receptors for altered advanced glycation end products (RAGEs). CD24 molecules are highly sialylated and bind to siglec-10/G.⁴⁶ The interaction of DAMPs such as HSP-70/90, high-mobility group protein B1, and nucleolin with CD24 and siglec 10/G seems to moderate these responses. Other studies have demonstrated that the type of linkage of sialic acid can determine the polarization of the immune response by dendritic cells through siglec binding.

Receptor for altered glycation products

RAGE is a transmembrane receptor of the immunoglobulin super-family with various splice variants. It is responsive to a broad range of ligands (modified proteins or lipids that become glycosylated and/or oxidized after coming into contact with aldose sugars, high-mobility group protein B1, S100B, S100A7, amyloid B protein, membrane attack complex A, phosphotydy ceramides, SR-A, etc),⁴⁸ leading to the activation of NF- κ B and transcription of proinflammatory cytokines, chemokines, and adhesion molecules. T lymphocytes, B lymphocytes, and macrophages express high levels of RAGE.⁴⁹ Positive correlations

in asthma with increased levels of RAGE and its ligand high-mobility group protein B1 have been noted and RAGE deletion has been demonstrated to protect mice from airway remodeling, eosinophilic infiltration, and airway hypersensitivity regardless of the allergen involved.⁵⁰ RAGEs recognize DAMPs, and their activation results in the production of IL-33, a key cytokine in activating local T_H2 cytokine production.^{51,52} Because a number of fungal products that are identified as allergens are involved in sugar metabolism and redox metabolism, their recognition by RAGEs is a likely trigger for T_H2 induction.

Type 2 innate lymphoid cells

Although recently identified receptor groups comprise the bulk of new pathways whereby fungal constituents might interact with the human immune system, there are also cellular mediators on the horizon. ILCs2 have recently been identified as important constituents in the immune process. These cells with limited or no rearranged antigen receptors produce high amounts of type 2 cytokines (IL-5, IL-9, and IL-13) when activated.^{53,54} Epithelial cells when stimulated by endogenous DAMPs (high-mobility group box 1 [HMGB-1], uric acid, ATP, etc) produce cytokines (IL-1, IL-25, IL-33, thymic stromal lymphopoietin, and GM-CSF) that activate the dendritic cell network and ILC2 immune cells.^{50,55} ILC2 cells are found in mucosal surfaces, and their role in experimental asthma and atopic dermatitis supports a role for them in promoting type 2 inflammatory responses.⁵⁶ Because a number of PRRs when activated produce these cytokines (via either DAMPs or PAMPs), ILC2 cells are apt to play a significant role in atopic disease and in linking innate and adaptive immune systems.⁵⁷

ADAPTIVE IMMUNE SYSTEM AND FUNGAL PRODUCTS

Adaptive immune responses result from a complex network involving specific receptors directed toward a wide variety of substances (antigens). The purpose of these receptors is to bind these substances and activate subsequent mechanisms, resulting in antigen neutralization and elimination. These mechanisms often involve various inflammatory pathways that if activated in excess can result in symptoms. With respect to allergic inflammation, the receptors largely belong to the IgE class whose participation results in allergic inflammation through well-known pathways.⁹ IgE antibodies when produced are particularly exhibited at portals of entry such as the skin and mucosal membranes. Here, they are best suited to alert to exogenous danger signals. The mechanisms used to produce IgE involve a complex interplay between dendritic cells, Treg cells, and effector T cells. Signals produced chiefly by dendritic cells report microenvironmental events such as captured antigens, microbial products, and trauma (exogenous and endogenous danger signals). And, along with other genetic determinants, these signals determine whether T_H1, T_H2, T_H17, or Treg-cell-polarized adaptive immune responses occur.⁵⁸ For example, the production of IL-4 and IL-13 biases the adaptive immune system toward T_H2 polarization, resulting in IgE production and possible allergic inflammation.⁵⁹ Here, the type of adaptive immune response depends on whether the antigen itself is a danger signal, or whether it induces danger signals, or is accompanied by something that produces danger signals. All these possibilities are evident with respect to fungal allergens. For example, fungal proteases can induce danger signals via protease-activated

receptors,⁴³ fungal allergens are usually presented with PAMPs recognized by PRRs (β -glucans, chitin, mannans, etc), and a number of fungal proteins that are recognized as allergens are danger signals themselves.

IgE production is complicated by multiple phenomena including targeting⁶⁰ and the creation of a microenvironment rich in IgE-promoting cytokines, allowing the production of IgE in molecules that accompany the original allergen.⁶¹ *Targeting* refers to the ability of specific immunoglobulin E (s-IgE) to present an allergen to other immune cells, thus exposing other epitopes on the same molecule. The IgE system seems to react to preferred epitopes on molecules from different sources.⁶² This creates cross-reactive antibodies that can react with similar molecules from a wide variety of sources. Thus, when s-IgE is present, it is likely that it can react with molecules from a wide variety of different sources. This explains the persistence of s-IgE production to certain allergens and the high amount of cross-reactivity seen with proteins from different sources.⁶³

Only a few (0.5%-1%) of the 6000 to 10,000 gene products (proteins) in fungi seem to be s-IgE-binding proteins. Most of these seem to be minor allergens and perhaps not relevant to disease being the product of bystander sensitizations. Thus, even though more than 189 individual allergens have been defined, not all of these have been proven to be directly involved in producing allergic symptoms. Some allergens from the genera *Alternaria*, *Aspergillus*, and *Cladosporium* are now well characterized, and allergens from several other genera, including some basidiomycetes, have been purified.⁶⁴ Using genome-wide studies of several fungal species, it has been shown that fungi share a highly conserved set of allergen orthologues (proteins from genes in different species that evolved from a common ancestral gene; normally, orthologues retain the same function).⁶⁵ These include proteins involved in proteolysis (vacuolar serine, acid, alkaline, aspartyl, and metalloproteases), stress response proteins (peroxisomal membrane protein, thioredoxin, glutathione reductases, manganese superoxide dismutase, etc), protein involved in synthesis (cyclophilins, HSPs, acid ribosomal proteins P1 and P2), carbohydrate-metabolizing enzymes (enolase, alcohol and aldehyde dehydrogenases, glycosidases, and several multifunctional proteins of the Krebs and Emden-Meyerhof pathways), and a number of proteins with miscellaneous functions such as ribotoxins and nuclear factor 2. These important proteins seem to be highly homologous and widespread throughout the fungal (and animal) kingdom and as such would be expected to exhibit a high degree (but not perfect) of cross-reactivity. Most are intracellular proteins common to eukaryotic organisms that besides having several different distinct functions within cells can represent DAMPs when released into the extracellular milieu. A compilation that relates the occurrence of 82 allergen sequences in 22 fungal genomes with their approximate degree of similarity is available.⁶⁵ However, a few key allergens such as Asp f 1 (ribotoxin-like protein) and Alt a 1 and Alt a 2 (unknown function) appear to be highly specific to only a few species and as such seem to be good markers of specific sensitization to these fungi.⁶⁶

IMPORTANT FUNGAL ALLERGENS

Proteases as allergens

Proteolytic enzymes play important roles in the physiology of most organisms. With respect to fungi, several are secreted and are responsible for the digestion of protein substrates. They can

induce inflammatory responses by altering the permeability of epithelial layers and by the induction of proinflammatory cytokines through protease-activated receptors.⁸ Fungal proteases appear to be essential in eliciting T_H2 responses and also act as adjuvants potentiating responses to themselves and to other allergens (ie, bystanders).⁶⁷ Inflammation can also result from protease activity on protease inhibitors and interactions with the kinin, coagulation, complement, and fibrinolytic cascades.⁶⁸ Examples of fungal proteases that are important allergens include vacuolar serine proteases (Asp f 18, Pen c 2, Pen c 18, Rho m 2), alkaline serine proteases (Asp f 13, Pen b 13, Tri r 2, Cur l 1, Epi p 1, Pen c 1, Tri r 4), metalloproteinase (Asp f 5), and aspartic protease (Asp f 10) (<http://www.allergome.org>). Exogenous proteases appear to be important initiators of allergic responses, can damage airways, enhance bystander-type reactions, and simultaneously act as allergens.

Allergens involved in stress responses

Peroxisomes are subcellular organelles found in all eukaryotic cells having a number of important functions from lipid metabolism (catabolism and biosynthesis), control of metabolic flux, energy homeostasis, scavenging peroxides and reactive oxygen species, and antiviral defense.⁶⁹ A total of 22 peroxisomal membrane proteins have been identified (<http://www.allergome.org>), which seems to be highly conserved throughout eukaryotic cells but which ones are actually allergens has not been identified. Examples of peroxisomal membrane proteins identified as allergens include *Aspergillus* (Asp f 3), *Penicillium* (Pen c 3), and *Malassezia* (Mal f 2 and 3), all of which are intracellular proteins of around 20 kD. These proteins and others participate in oxidation reactions creating oxidation-specific epitopes that indicate cellular damage and during apoptosis represent DAMPs.⁷⁰ These are, in turn, recognized by both cellular and soluble PRRs such as CD36, TLRs, natural antibodies, C-reactive protein (pentraxin), and siglecs and can mediate various immune and inflammatory responses.

Several other fungal proteins that could be included in this section include manganese superoxide dismutase, thioredoxins, flavodoxins, catalase, and glutathione S-reductase. These proteins are involved with thiol-based redox switches that modulate proteins involved in a myriad of different pathways.⁷¹ These proteins are intimately involved in innate immune mechanisms and the release of DAMPs to alert immune signaling cascades.⁷² Identified fungal allergens from this group are listed in Table 1.

HSPs as allergens

HSPs are highly conserved very abundant proteins found in virtually all organisms from bacteria to humans.⁷³ Different HSPs play important roles in the folding and unfolding of proteins, the assembly of multiprotein complexes, protein transport in the cell, cell-cycle control and signaling (maintenance of steroid receptors and transcription factors), and protection against stress and apoptosis. Extracellular and membrane-bound HSPs (especially HSP-70) have been implicated in binding antigens and in presenting them to the immune system. Recently, HSPs have been shown to have a role in immune presentation by stimulating antigen-presenting cells (via CD40 and TLR-2 and 4) and by upregulating costimulatory molecules.⁷⁴ In addition, when antigen peptides are bound to HSP-70, they can be internalized and subsequently presented on MHC molecules.

HSPs while being present at all times as monitors of cellular processes and protein maintenance are highly upregulated during various environmental stress conditions such as infection, inflammation, exposure to toxins, starvation, heat, and hypoxia.⁷⁵ In large part this is protective, yet in some circumstances is deleterious. For example, in *Candida*, HSP-90 is involved in the calcineurin-caspase apoptotic pathway that result in increased reactive oxygen species production and cell death.⁷⁶ HSPs interact with a wide variety of proteins (198 putative physical interactions and 451 putative genetic and chemical interactions) including transcription factors and thus influence a wide variety of physiological processes. Repression of *Candida* HSP-90 production (by geldanamycin) causes decreased spore viability, decreased hyphal growth, and severe defects in germination and thus might represent an attractive antifungal strategy.⁷⁷

Examples of HSPs identified as allergens include *Alternaria* (Alt a 3), *Cladosporium* (Clad a HSP-70), *Penicillium* (Pen c 19), *Malassezia* (Mal s 10), *Dermatophagoides* (Der f HSP-70), and *Toxiplamodium* (Tox g HSP-70) for HSP-70 and *Cladosporium* (Clad a 12) and *Aspergillus* (Asp f 12) for HSP-90 (<http://www.allergome.org>). Again, the high degree of homology of these proteins with those from humans indicates that they represent DAMPs when released from cells.

Acid ribosomal proteins P1 and P2

Acid ribosomal proteins P1 and P2 are unique phosphoproteins of the 60S ribosomal subunit. Of the 80 proteins of ribosomes they are the only acidic ones. They are highly conserved in all eukaryotes and show a high similarity to bacterial L proteins. They form a visible stalk off the 60S ribosomal subunit and are thought to be involved in elongation of proteins and also are suspected to be involved in the regulation of transcription and DNA repair.⁷⁸ They are highly represented in the cytoplasm of all cells. The P proteins are important allergens from fungi, and antibodies to them are present in patients with systemic lupus erythematosus.⁷⁹ Examples of acidic ribosomal proteins that are allergens from fungi include *Alternaria* (Alt a 12), *Cladosporium* (Clad h 12), *Penicillium* (Pen b 26), *Saccromyces* (Sac c P1) for the P1 family and *Alternaria* (Alt a 5), *Cladosporium* (Cla h 5), *Aspergillus* (Asp f 8), *Sarrromyces* (Sac c P2), and *Fusarium* (Fus c 1) for the P2 family (<https://fermi.utmb.edu/>). Hom s P2 protein has been listed as an autoantigen.

An additional allergenic protein from the 60S ribosomal subunit is *Aspergillus* (Asp f 23), an L3 ribosomal protein. This protein confers susceptibility to trichodermin, a trichothecene toxin produced by plant pathogenic fungi. In addition, the P1 and P2 proteins seem to be the target of ricin toxin.

Cyclophilins

Cyclophilins are ubiquitous proteins present in all subcellular compartments. They are multifunctional proteins whose functions include protein trafficking and maturation (protein folding and degradation), receptor complex stabilization, apoptosis, receptor signaling, RNA processing, and spliceosome assembly (<https://fermi.utmb.edu/>). Cyclophilins are composed of a number of isozymes (17 characterized in humans), all characterized by having peptidyl prolyl isomerase activity.⁸⁰ This activity catalyzes the isomerization of peptide bonds from cis to trans at proline residues, which facilitates protein folding where more than 90% of proteins have trans prolyl imide bonds.^{81,82}

TABLE I. Function of characterized fungal allergens

Fungi	Alt	Cla	Asp	Cand	Fus	Cur	Pen	Stem	Tric	Mal	Sac	Rho	Epi	Miscellaneous*
Vacuolar serine protease		Cla a 9	Asp f 18 Asp n 18			Cur l 4	Pen c 18 Pen c 2					Rho m 2		
Serine protease			Asp f 5			Cur l 1			Tri r 4 Tri t 4				Epi p 1	Hom s psa Cuc m 1 Api m 7 Per a 10 Pol d 4 Der f 3, 5, 6, 9 Can f 5
Alkaline serine protease			Asp f 13, 15 Asp fl 5 Asp fl 1, 13 Asp v 13 Asp 0 13				Pen c 13 Pen b13		Tri r 2					
Aspartyl protease			Asp f 10	Cand a CAAP										Bla g 2 Cry j ap Rhiz m ap
Enolase	Alt a 6	Cla h 6	Asp f 22	Cand a E		Cur l 2	Pen c 22				Sac c E	Rho m 1		Hev b 9 Cyn d 22 Gad m 2 Bla g E
Manganese superoxide dismutase	Alt a sod	Cla h 6	Asp f 6	Cand a sod		Cur l sod				Mal s 11	Sac c sod			Hom s sod Ole e 5 Dro m sod Pis v 4
Peroxisomal membrane proteins			Asp f 3	Cand a 2 Cand a 3			Pen c 3			Mal f 2 Mal f 3				
Thioredoxins	Alt a 4		Asp f 28 Asp f 29		Fus c 2	Cur l Trx				Mal s 13				Hom s trx Hev b trx Tri a 25 Zea m 25 Cop c 2
Flavodoxin (YCP4 homologue)	Alt a 7	Cla h 7									Sac YCP4			
Catalase			Asp n 30				Pen c 30							
Gluathione S- transferase	Alt a 13	Cla gst	Asp f gst			Cur l gst	Pen c 24						Epi p gst	Hom s gst Bla g 5 Blo t 8 Der p 8 Sar s gst Tri a gst

(continued)

TABLE I. (Continued)

Fungi	Alt	Cla	Asp	Cand	Fus	Cur	Pen	Stem	Tric	Mal	Sac	Rho	Epi	Miscellaneous*
Cyclophilin-peptidyl prolyl isomerase			Asp f 11 Asp f 27	Can a Cyp						Mal s 6	Sac c Cyp			Hom s cyp Bet v 7 Cuc m cyp Dau c cyp Psi c 2 Der f 29
HSP- 70	Alt a 3	Cla a 70					Pen c 19			Mal s 10				Der f sp70 Tox g sp70
HSP-90		Cla f 12	Asp f 12											
Aldehyde dehydrogenase	Alt a 10	Cla a 10												Hom s apdh Har a 2
Alcohol dehydrogenase		Cla h 3		Can a 1						Mal s 12				
Mannitol dehydrogenase	Alt a 8	Cla h 8												
Translationally controlled tumor protein														
Protein initiation factor	Alt a 2													
Alt a 1 related	Alt a 1													
Ribotoxin-mitigilian			Asp f 1											
Ribosomal protein P1	Alt a 12	Cla h 12	Asp f 23				Pen b 26							
Ribosomal protein P2	Alt a 5	Cla h 5	Asp f 8		Fus c 1						Sac c p2			
Fibrinogen- binding protein			Asp f 2											
Unknown (APBA)			Asp f 4											
Unknown		Cla h 1	Asp f 7											
		Cla h 4	Asp f 17											
Glycosyl hydrolase			Asp f 9 Asp f 16											
Cell wall protein			Asp f 34											

Alt, Alternaria sp; APBA, allergic bronchopulmonary aspergillosis; Asp, Aspergillus sp; Cand, Candida sp; Clad, Cladosporium sp; Cur, Curvularia sp; Fus, Fusarium sp; Epi, Epicoccum sp; Mal, Malassezia sp; Pen, Penicillium sp; Rhiz, Rhizomucor sp; Rhod, Rhodotorula sp; Sac, Sacchromyces sp; Stem, Stemphilium sp; Tri, Trichophytum sp.

Occupational fungal allergens: α amylase (Asp o 21), γ amylase (glucoamylase), cellulase/xylanase (Asp m 14), lactase, lipase, phytase (Asp n 25), biodiatase, flaviatase, aspartic protease, pectinase, and glutathione S-transferase (Alt a 13).

*Nonfungal sources of cross-reacting allergens (Can f = dog, Cry j = Japanese cedar, Pis v = pistachio, Cuc m = muskmelon, Api m = honeybee, Per a & Bla g = cockroach, Pol d = paper wasp, Der f & Blo t = dust mite, Hev b = latex, Cyn d = Bermuda grass, Gad m = cod, Ole e = olive pollen, Dro m = fruit fly, Tri a = wheat, Zea m = corn, Cop c 2 & Psi c = mushroom, Sar s = salmon, Bet v = birch, Dau c = carrot, Tox g = protozoan).

Examples of cyclophilins (peptidyl prolyl isomerases) as allergens include *Aspergillus* (Asp f 11, Asp f 27), *Malassezia* (Mal s 6), and *Psilocybe* (Psi c 2). Other cyclophilins identified as allergens include Hom s cyp, *Betula* (Bet v 7), *Cucur* (Cuc m cyp), *Daucus* (Dau c cyp), and *Dermatophagoides* (Der f 29) (www.allergenome.org). Although these proteins are highly conserved, the antigenic relationships between the different families and species have not been defined. Interestingly, some of the cyclophilins are involved in the ubiquitin and chaperone pathways along with HSP-90 and HSP-70, which have also been recognized as allergens from some mold species. These proteins all have a striking similarity to human counterparts and may represent DAMPs when released from cells.⁸³

Allergens involved in carbohydrate metabolism

Several studies have demonstrated substantial IgE antibody reactivity to fungal glycosidase enzymes including invertase (*Saccharomyces cerevisiae*), cellulase (*Trichoderma viride*), and glucosidase (*S cerevisiae*). In addition, s-IgE directed against a combination of 4 enzymes—glucosidase, maltase, invertase, and invertase V—detected 100% of mold-allergic patients, suggesting an efficient way to identify such individuals.⁸⁴ A number of common nearly ubiquitous enzymes involved in carbohydrate metabolism have been identified as allergens from fungi. These proteins are abundantly expressed in many organisms and include enolase, alcohol dehydrogenase, mannitol dehydrogenase, aldehyde dehydrogenase, malate dehydrogenase, and aldolase to date. Each will be considered separately, and specific allergens are listed in Table 1.

Alpha-enolase is a key enzyme in the metabolism of glucose.⁸⁵ It also plays important roles in several other biological processes.⁸⁵⁻⁸⁷ It is therefore a constituent likely common to all fungi and is a recognized allergen in *Alternaria* (Alt a 6), *Cladosporium* (Clad h 6), *Aspergillus* (Asp f 22), *Candida* (Can a E), *Curvularia* (Cur l 2), *Penicillium* (Pen c 22), *Saccharomycetes* (Sac c E), and *Rhodotorula* (Rho m 1) and is also a recognized allergen from other sources such as latex *Hevea* (Hev b 9), grass (Cyn d 22), cod (salmon and tuna also) fish (Gad m 2), and cockroach (Bla g E). It is most likely that other sources of enolases are also allergenic, making enolase a panallergen (<http://www.allergome.org>).

Alcohol and mannitol dehydrogenases

Alcohol dehydrogenases are a group of dehydrogenase enzymes that occur in many organisms. They catalyze the oxidation of primary and secondary alcohols to aldehydes and ketones. In animals, they serve to break down alcohols that otherwise are toxic and they also participate in the generation of useful aldehyde, ketone, or alcohol groups during the biosynthesis of various metabolites. In yeast, plants, and bacteria, some alcohol dehydrogenases catalyze the opposite reaction as part of fermentation. Alcohol dehydrogenase has been identified as an allergen in *Candida* (Cand a 1) and *Malassezia* (Mal s 12), *Cladosporium* (cla h 3), and *Alternaria* (Alt a 10) (<http://www.allergome.org>).

Miscellaneous fungal allergens

A number of identified fungal allergens that do not fit into the above categories include protein initiation factor (Alt a 2), ribotoxin (Asp f 1), cell wall protein (Asp f 34), glycosyl hydrolase (Asp f 9 and Asp f 16), fibrinogen-binding protein (Asp f 2), and translationally controlled tumor protein. The latter again is a highly conserved protein common to many eukaryotes that is involved in several

important cellular activities.⁸⁸ Of particular interest is that it was first described as an IgE-dependent histamine-releasing factor.⁸⁹

CONCLUSIONS

Fungal cell walls are composed of layers of carbohydrates including mannans (polymers of mannose), β glucans (polymers of glucose linked by β glycosidic bonds), and chitins (polymers of *N*-acetyl-D-glucosamine). These structures are recognized by a number of innate receptors resulting in the production of proinflammatory cytokines, which are designed to neutralize disruptions of normal functions. These cytokines activate other factors, resulting in differential gene responses, which result in signals referred to as alarmins.⁹⁰ Here, nuclear proteins such as high mobility group box protein, calreticulins, extracellular ATP, and other substances collectively referred to as DAMPs are actively produced, which produce inflammation and shape adaptive immune responses. Many of the proteins recognized as fungal allergens seem to be involved in these pathways and as such represent exogenous sources of these DAMPs. Whether the resulting inflammation produces overt symptoms or what is referred to as meta-inflammation depends on the strength and combinations of the signals. The bottom line is that fungal exposures can through a panoply of mechanisms result in inflammation. Interestingly, many of these pathways are also responsible for maintaining homeostasis with respect to tissue damage in which through combined efforts with the adaptive immune system they assist in limiting the dispersal of damaging materials released on cell death. Here again, fungal proteins identified as allergens that are homologues of intracellular proteins seem to be involved in these processes.

Collective theories of immune responses seem to indicate that proteins that are allergens cause cellular damage directly, are accompanied by molecules that cause cellular damage, or mimic endogenous alarm signals. Thus, patterns of inflammation and adaptive immune responses would seem to be actively involved according to their actions in causing symptoms.⁹¹ Although the activation of innate mechanisms seem to bias toward T_H2 adaptive immunity and the production of s-IgE, it is difficult to discern whether symptoms are a result of innate inflammatory or adaptive inflammatory mechanisms or both. Because of this and other factors, positive tests for sensitization to fungal allergens do not always correlate with symptoms.

Allergic fungal sensitivity has long been determined by the use of ill-defined and highly variable extracts of fungal mats or supernatants by skin prick testing or serological tests for s-IgE.⁹² Given the commonality of these proteins among different species and the number of different possible but undefined allergens, the results of these tests and their correlation with clinical history are often misleading.⁹³ A few exceptions to this including tests for either recombinant or purified natural allergens have been used successfully (Alt a 1, Asp f 2 and 6). But a lot of work remains to be done to define the relevancy of these fungal constituents. Treatment for fungal sensitivity via immunotherapy is common but also hampered by similar considerations again with the possible exception of treatment with *Alternaria* extracts for asthma. Here again, because the Alt a 1 content of these extracts has been shown to be highly variable, questions remain.⁹⁴

Multiple allergens to which individuals are sensitized have been identified, yet their involvement in symptom production is

yet to be defined. It is clear that fungal exposures can activate various innate inflammatory mechanisms that encourage T_H2 type of adaptive responses but which are responsible for symptoms remains poorly defined. Although great progress has been seen in this field, it is obvious that significant work remains to be done to understand fungal exposures and their various roles in producing disease.

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