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11.1 Introduction

The kingdom Fungi is comprised of biologically distinct, single or multicellular eukaryotic organisms and stands alongside the kingdoms Animalia and Plantae in complexity. Fungi serve as major decomposers of organic material, digesting large polysaccharide, protein, or lipid molecules via efficient exoenzymatic activity. As a result of fungal metabolism, large quantities of nitrogen, phosphorus, and carbon are released into the surrounding environment, enabling plants and animals to utilize previously inaccessible nutrients. As such, fungi play a vital role in supporting a sustainable ecosystem [1].

There are an estimated 2.2 to 5.1 million species of fungi in total, but only approximately 300 species are known to be pathogenic in humans [2]. Although fungi have often been regarded as mainly causing nuisance diseases, including unpleasant but harmless ailments such as athlete's foot, ringworm, or mucosal candidiasis, the true pathogenic potential of fungi is much more serious and far-reaching. The Global Action Fund for Fungal Infections (GAFFI) has estimated that over 300 million people are affected by serious fungal infections annually and 1.6 million people succumb to fungal disease every year, a staggering death toll that is on par with that of tuberculosis and surpasses that of malaria [3]. The rise in the global burden of serious fungal disease has been closely associated with an increasingly large at-risk population of hosts that is particularly susceptible to invasive fungal infection. The advent of the HIV/AIDS pandemic in the 1980s and more recently the widespread use of life-saving solid-organ and hematopoietic stem cell transplantation, cytotoxic chemotherapy for malignancies, and immunomodulating medications for various autoimmune disorders have culminated in an at-risk population of many millions worldwide, including over ten million patients who are at risk of invasive aspergillosis in Europe, the USA, and Japan alone [3]. Although many pathogenic fungi cause deep-seated disease only in highly immunocompromised individuals, some can infect fully immunocompetent hosts or those with chronic disease or with specific risk factors such as intravascular devices or other foreign body implants. Infection with *Candida* species, a pathogenic yeast, is the fourth most common cause of bloodstream infection in hospitalized patients and the fifth most common nosocomial pathogen overall [4]. In areas endemic for *Histoplasma capsulatum*, such as the Ohio and Mississippi River valleys, up to 90% of the population has been exposed to this fun-

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gus during their lifetime. In the year 2012, there were more than 5000 histoplasmosis-related hospital admissions [5].

The magnitude of the public health impact of fungal infections has prompted a surge in mycology research aiming to better understand fungal pathogenesis and immunology, spanning from molecular and genetic to translational and clinical investigation. The study of fungal immunoepidemiology aims to characterize the range of host immune responses in populations to various fungal infections, to understand how genetic and environment factors affect these responses, and to examine how specific innate and acquired immune dysfunction predisposes to particular types of fungal infections. Insights gained from immunoepidemiology are key to defining the geographic distribution of fungi and serve as the building blocks for targeted therapeutic and preventative modalities that may one day revolutionize the treatment of fungal infections.

11.2 Fungal Classification and Morphology

As with other life forms, fungi are grouped using a phylogenetic classification scheme. Seven phyla currently exist, although the current classification is in flux. Medical mycology has adopted a more pragmatic approach to classification, dividing medically important fungi into yeasts, dimorphic fungi, and molds [6]. Yeasts are unicellular organisms that most commonly divide by budding, while molds are multicellular and produce elongated structures called hyphae that are either septated (contain cross-walls) or aseptated. Septated molds include the nonpigmented hyaline molds (such as *Aspergillus*) and the dematiaceous molds, whose hyphae are melanized. Aseptate molds, also known as coenocytic molds (such as *Rhizopus*), contain few or no septae. Molds exist predominantly in asexual form but produce sexual structures including conidia in order to reproduce. Dimorphic fungi can exist in both yeast and mold forms depending on the ambient temperature. These fungi are often endemic to the terrain of certain parts of the USA and the world.

Fungi possess a thick cell wall composed of glycoproteins and polysaccharides located outside the plasma cell membrane (Fig. 11.1) [7]. The inner cell wall typically consists of a chitin layer, with a thick middle layer of glucan components and an outer layer of extensively mannosylated proteins. In some fungi such as *Cryptococcus*, an additional thick outer polysaccharide layer, mainly composed of glucuronoxylomannan, is present. The biologic makeup of the cell wall has important implications for fungal immunology and virulence as well as the development of diagnostic [8], therapeutic [9], and preventative [10] modalities for fungal infections. The cryptococcal capsule, for example, has been shown to act as a potent anti-phagocytic barrier. By virtue of its thickness, the fungal cell wall is resistant to damage by antibody-mediated complement activation. Assays that detect circulating cell wall components (such as 1,3 β -D glucan and galactomannan) are now widely used in the diagnosis of invasive fungal infections. The three main classes of antifungal medications inhibit fungal growth by interfering with specific components of the fungal cell wall (ergosterol: azoles and polyenes; glucan: echinocandins).

11.3 The Innate Immune Response to Fungi

11.3.1 Fungal Recognition by Antigen-Presenting Cells

In immunocompetent hosts, the innate immune system responds to, and effectively eliminates, the vast majority of fungal pathogens. If fungi succeed in breaching primary protective skin and mucosal barriers, they are met and recognized by cells of the innate immune system, including dendritic cells, macrophages, and monocytes. Innate immune cells carry mammalian signaling receptors, also known

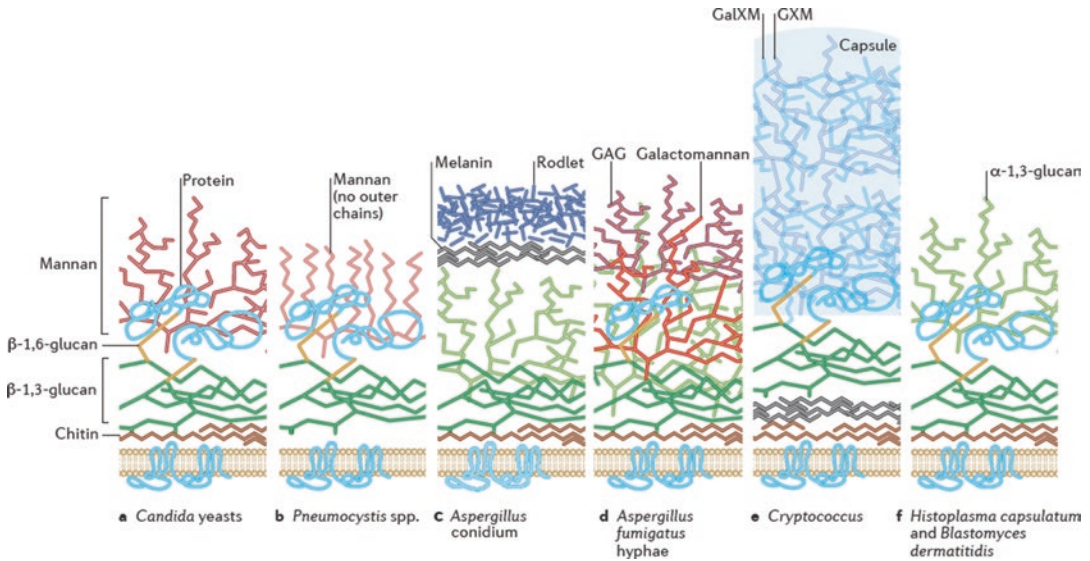


Fig. 11.1 Components of the fungal cell wall. (Adapted from Ewing and Gow [7])

as fungal pattern recognition receptors (PRRs) that bind fungal ligands, also known as fungal pathogen-associated molecular patterns (PAMPs) [11]. Fungal PAMPs are often fungal cell wall components, including glucans, α -mannans, and O-linked and N-linked mannans. Nucleic acid ligands (fungal DNA and RNA) have also been described (Table 11.1). The process of fungal recognition is mediated by binding of PAMPs to PRRs on innate immune cells. Once this interaction occurs, downstream intracellular-signaling pathways are activated within the immune cell. Multiple PRRs that mediate fungal recognition have been described, including C-lectin-type receptors (CLR), toll-like receptors (TLRs), NOD-like receptors, and others. Different families or types of PRRs can recognize the same fungal ligand. For example, both TLR-2 and CD14 can recognize α -(1,4)-Glucans. Alternatively, a PRR can recognize several different fungal ligands. For example, Dectin-2 is cognate with both α -Mannans and O-linked mannoproteins. There are fungal strain-specific PAMPs that are differentially recognized by PRRs, and absence of those PRRs has been shown to lead to strain-specific susceptibility. Sensing by multiple PRRs at once can lead to synergistic downstream signaling. The CLR are the most important PRR family for the detection of fungi. Inherited disorders in CLR lead to particular vulnerability to fungal infections. The CLR family is comprised of two groups based on intracellular-signaling motifs: (i) CLR with immunoreceptor tyrosine-based activation motifs (ITAMs) or ITAM-like domains such as Dectin-1 and (ii) CLR containing non-immunoreceptor tyrosine-based motifs including DC-SIGN and MR. Among the CLR, Dectin-1 mediates recognition of multiple clinically important fungi including *Candida*, *Aspergillus*, and *Histoplasma* by binding to B-glucan on the fungal cell wall [12]. An example of CLR-mediated signal transduction via Dectin-1 is shown in Fig. 11.2.

11.3.2 Fungal Killing by Innate Immune Cells

After recruitment to inflammatory sites, effector innate immune cells including monocytes, neutrophils, and natural killer (NK) cells engage fungal pathogens and kill them [13]. Monocytes and macrophages produce cytokines and chemokines that are directly fungicidal but also enhance neutrophil

Table 11.1 Pathogen-associated molecular patterns (PAMPs), pattern recognition receptors (PRRs), and immune deficiencies associated with medically important fungi

Fungal pathogen	Fungal PAMP	Fungi-specific PRR	Selected associated immunodeficiencies [immunologic defect]
<i>Candida</i>	<i>Glucans</i> : β -glucan, α -glucans <i>Mannans</i> : Mannans, α -mannans, O-linked mannans, N-linked mannans, β -mannosides, α -mannose, galactomannan, phospholipomannan, Glucuronoxylomannan <i>Other</i> : N-acetyl-D glucosamine, glyceroglycolipids, Glycoprotein A, chitin, HSP60, BAD-1, DNA, RNA	<i>CLR</i> : Dectin-1, Dectin-2, Dectin-3, Mincle, DC-SIGN, mannose receptor <i>TLR</i> : TLR-2, TLR-4, TLR-6, TLR-7, TLR-9 <i>NOD</i> -like receptors: NOD2, NLRC4, NLRP3, NLRP10 <i>Integrins</i> : CR3 <i>Other</i> : CD23, CD36, surfactant proteins A and D, galectin-3, Fc γ R	<i>CMC</i> [IL17F, IL17RA, IL17RC, ACT1, STK4, IRF8, CARD9, STAT1, STAT3, RORC, AIRE, anti-IL-17 auto-antibodies] <i>Invasive candidiasis/CMC</i> [CARD9, MPO] <i>Chronic granulomatous disease</i> [NADPH oxidase] <i>Severe congenital neutropenia</i> [ELA2, HAX1]
<i>Cryptococcus</i>	<i>Glucans</i> : β -glucan, α -glucans <i>Mannans</i> : Mannans, glucuronoxylomannan, phospholipomannan, O-linked mannans, rhamnomannans <i>Other</i> : Glycoprotein A	<i>CLR</i> : Dectin-1, mincle, mannose receptor <i>TLR</i> : TLR-1 TLR-2, TLR-4, TLR-6, TLR-9 <i>Other</i> : CD14, CD36, surfactant proteins A and D, Fc γ R	<i>IL-12 receptor deficiency</i> [IL-12R] <i>Autoantibodies</i> [to GM-CSF or IFN- γ] <i>Job's syndrome/CMC</i> [STAT3] <i>MonoMAC syndrome</i> [GATA2] <i>Humoral deficiencies</i> [Fc γ receptor polymorphisms]
<i>Pneumocystis</i>	<i>Glucans</i> : β -glucan <i>Mannans</i> : Mannans, α -mannose, N-linked mannans <i>Other</i> : Glyceroglycolipids, N-acetyl-D glucosamine, Glycoprotein A, chitin	<i>CLR</i> : Dectin-1, mincle, mannose receptor <i>Other</i> : Lactosylceramine	<i>Severe lymphopenia</i> [IL2RG, IL7RA, ADA, RAG1, RAG2, JAK3, ZAP70, ARTEMIS, NEMO/IKKBKG, IKBA] <i>T-cell dysfunction</i> [CD40L, IL21R] <i>Job's syndrome/CMC</i> [STAT3] <i>Wiskott-Aldrich syndrome</i> [WAS] <i>B-cell dysfunction</i> [BTK]
<i>Aspergillus</i>	<i>Glucans</i> : β -glucan <i>Mannans</i> : Mannans, α -mannans, O-linked mannans, galactomannan, glucuronoxylomannan <i>Other</i> : Glycoprotein A, HSP60, BAD-1, DNA	<i>CLR</i> : Dectin-1, Dectin-2, DC-SIGN, MBL <i>TLR</i> : TLR-2, TLR-4, TLR-9 <i>NOD</i> -like receptors: NOD1, NLRP3 <i>Integrins</i> : CR3 <i>Other</i> : Pentraxin-3, surfactant proteins A and D	<i>Chronic granulomatous disease</i> [NADPH oxidase] <i>Job's syndrome/CMC</i> [STAT3] <i>Extrapulmonary aspergillosis/CMC</i> [CARD9] <i>MonoMAC syndrome</i> [GATA2] <i>Leukocyte adhesion deficiency</i> [CD18] <i>Severe congenital neutropenia</i> [ELA2, HAX1]
<i>Histoplasma</i>	<i>Glucans</i> : β -glucan <i>Mannans</i> : Mannans, glucuronoxylomannan <i>Other</i> : HSP60, BAD-1	<i>CLR</i> : Dectin-1 <i>Integrins</i> : CR3	<i>IFN-γ deficiency</i> [IFNGR1] <i>IL-12 receptor deficiency</i> [IL-12R] <i>Job's syndrome/CMC</i> [STAT3, DOCK8] <i>CMC</i> [STAT1] <i>MonoMAC syndrome</i> [GATA2] <i>T-cell dysfunction</i> [CD40L]

Abbreviations: *CLR* C-type lectin receptors, *CMC* Chronic mucocutaneous candidiasis, *TLR* Toll-like receptors, *PAMP* pathogen associated molecular patterns, *PRR* pattern recognition receptors

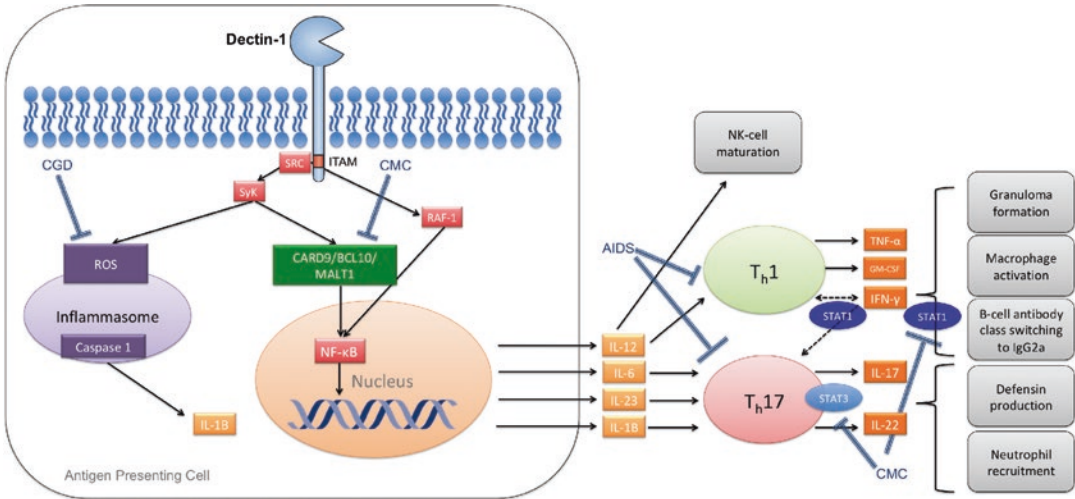


Fig. 11.2 Dectin-1 signaling pathway and associated immunodeficiencies. β -glucan binding to Dectin-1 receptor induces displacement of CD45 and CD148 phosphatases promoting SRC-dependent phosphorylation of the intracellular ITAM-like motif, which leads to Syk kinase recruitment then PKC δ phosphorylation of CARD9. CARD9 then complexes with BCL10 and MALT1 producing a CARD9/BCL10/MALT1 complex that activates NF- κ B (subunits p65 and c-REL), a protein, and possibly complex that controls DNA transcription and cytokine production. Syk-dependent signaling also leads to production of reactive oxygen species (ROS) that induce caspase-1 activation then assembly of the NLRP3 inflammasome. The inflammasome, a multiprotein intracellular complex, promotes maturation and secretion of the pro-inflammatory cytokine Interleukin 1 β (IL-1 β). Dectin-1 binding also activates NF- κ B through a Syk-independent pathway, mediated by RAF-1 kinase phosphorylation and deacetylation. Activation of NF- κ B via both pathways ultimately leads to the production of inflammatory cytokines such as IL-1 β , IL-6, IL-12, and IL-23. IL-1 β and IL-23 promote T-helper 17 cell differentiation. Th17 cells then secrete IL-17A that along with chemokines such as CXCL1 and CXCL2 stimulates neutrophil recruitment to sites of inflammation and activates T and B cells against the fungal insult. IL-12 induces Th1 cell differentiation then IFN- γ release, which activates macrophages, promoting fungal eradication

fungal killing. Activated monocytes can differentiate into macrophages capable of phagocytosing yeasts and also into dendritic cells that transport fungal antigens to regional lymph nodes where they stimulate adaptive immune cell responses to fungal invasion. Dendritic cells secrete IFN- α and IL-23 that activate NK cells to produce GM-CSF, prompting the production of neutrophils in the bone marrow. Various chemokine receptor/ligand combinations regulate monocyte recruitment during different fungal infections. For example, CCL20-CCR6 activation leads to recruitment of monocytes in the setting of invasive pulmonary aspergillosis, while CX3CL1-CX3CR1 signaling mediates kidney macrophages killing of *Candida* yeasts in a mouse model [14]. Monocytes play an important role in mucosal immunity. After fungal recognition, monocytes secrete inflammatory cytokines (IL-1 β , IL-6, IL-23, and TNF- α) that promote T-cell differentiation to Th17 lymphocytes. Th17 cells secrete IL-17 and IL-22 that direct production of antifungal molecules such as defensins. Monocytes and macrophages can build lymphocyte-independent immunologic memory (or trained immunity) against fungi and other pathogens via epigenetic reprogramming that involve histone modifications, DNA methylation, and other mechanisms. For example, trained immunity ensues from β -glucan-Dectin-1 interaction and is associated with a shift of cellular metabolism from oxidative phosphorylation to aerobic glycolysis, which is thought to enhance the capacity of monocytes to respond to fungal rechallenge [15].

Neutrophils clear fungal cells differentially according to pathogen size. Neutrophils are able to ingest yeast cells and other small fungal structures such as conidia and then exert fungicidal activity via NADPH oxidase-mediated intracellular ROS production within phagolysosomes. ROS production

also downregulates IL-1 β production to limit disproportionate neutrophil recruitment and reduces detrimental effects of an excessive inflammatory response. In response to larger microbes such as mold hyphae that are too big for phagocytosis, neutrophils release neutrophil extracellular traps (NET) and produce ROS extracellularly to induce killing. NET are extracellular filaments composed of neutrophil nucleic acids and antimicrobial granule proteins (including neutrophil elastase, cathepsin G, myeloperoxidase, and calprotectin) that trap and kill fungal elements and may also act as physical barriers that impede pathogen movement. NET may reduce collateral tissue damage by diffusing cytotoxic granule proteins away from host cells. In a mouse model of *A. fumigatus* infection, neutrophil calprotectin was crucial in effective killing of hyphae but nonessential for control of smaller *A. fumigatus* conidia [16].

In addition to activating neutrophils through GM-CSF production, NK cells exhibit direct antifungal activity. Activation of NK cell natural cytotoxicity receptors (NCR) such as NKp30 (by an as yet unidentified fungal PAMP) triggers release of perforin, a cytotoxic molecule that leads to fungal death by forming pores in the fungal plasma cell membrane [17]. Downregulation of NKp30 via blocking antibodies or siRNA results in decreased release of perforin and impaired fungal killing. In addition, NK cells directly damage fungal cells by inducing apoptosis via the Fas-FasL or the TNF pathway. NK cells recruited via the CCL2-CCR2 chemokine axis produce IFN- γ , which is protective in some fungal infections such as aspergillosis but has been shown to be detrimental during infection with *Candida* and *Cryptococcus* [18].

Other innate immune cells have important roles in antifungal immunity. Gamma-delta ($\gamma\delta$)T cells play an important role in mucocutaneous immunity by producing IL-17 and inducing defensin production by epithelial cells. Epithelial and endothelial cells often serve as the first line of antifungal defense. After fungal recognition, epithelial cells produce inflammatory cytokines such as IL-1 that promote neutrophil activation and influx. Eosinophils kill fungi by releasing cytotoxic granule proteins, such as eosinophil-derived neurotoxin and major basic protein, but are also implicated in maladaptive allergic responses to environmental fungi as in allergic bronchopulmonary aspergillosis (ABPA) [19].

11.4 The Adaptive Immune Response to Fungi

11.4.1 T-Cell Response: Proinflammatory Responses

Adaptive immune responses to fungi come into play when initial defenses of the innate immune system have been overcome or dampened by immunosuppression [20]. The T-cell-mediated response is essential for adequate control of invasive fungal infections. After recognizing fungal ligands via PRRs, dendritic cells migrate to local lymph nodes where they orchestrate a subsequent phase of immunity, governed by pathogen-specific effector B and T cells. Dendritic cells prime naïve T cells through presentation of pathogen-associated antigens on MHC class I or class II molecules combined with co-stimulatory molecule expression. Several subsets of specialized dendritic cells that differ based on anatomical location and surface marker expression cooperate to stimulate T-cell responses. Dendritic cells stimulate naïve CD8+ T cells via MHC class I and CD4+ T cells via MHC class II. CD8+ T cells, especially the Th1 and Th17 subsets, contribute to fungal eradication, but it is not yet clear if they are indispensable to antifungal immunity. Dendritic cell and macrophage-secreted IL-12 promote development into Th1 cells, a T-cell subset that is highly effective in antifungal defense. Th1 cells produce TNF- α , GM-CSF, and especially the critical IFN- γ . IFN- γ binds to the IFN- γ receptor on macrophages and leads to JAK1-/JAK2-mediated activation of STAT1, a signal transducer and transcription protein that recruits NRAMP1 to the phagolysosome. This allows for more effective killing of fungi within the phagolysosome so that macrophages are turned into voracious phagocytes

capable of abrogating fungal invasion. IFN- γ also prompts B-cell antibody class switching to IgG2a, which is more effective at neutralizing fungi, and augments antigen presentation in antigen-presenting cells. A dominant Th1 adaptive response is associated with protective immunity against fungi. Th17 cells are generated after TGF- β and IL-6 priming of naïve CD4⁺ T cells. IL-6 activates STAT3, which is important for ROR γ t-dependent Th17 cell development, whereas IL-23 is critical for adequate expansion and functioning. Th17 cells operate in concert with Th1 cells, secreting IL-17 and IL-22 that promote defending production and are especially important in antifungal mucosal immunity. They also upregulate Th1 response and dampen Th2 suppressive responses.

11.4.2 T-Cell Response: Regulatory Responses

Secretion of IL-4 and IL-13 induces differentiation of naïve CD4 T cells into Th2 cells. Th2-based immunity serves to dampen Th1 response and counteract excessive inflammation that is associated with tissue damage. Th2 cells play a critical role in response to extracellular parasitic infections such as helminths by regulating B-cell class switching to IgE and recruiting antiparasitic eosinophils and mast cells by secretion of IL-4, IL-5, and IL-13. However, a Th2-dominant response is detrimental in the setting of fungal infection, favoring progression of fungal infection and decreased pathogen clearance. Th2 cells are also involved in development of non-protective inflammatory allergic responses. As with Th2 cells, regulatory T cells (Treg) suppress the pro-inflammatory cascade through multiple pathways in order to control collateral tissue damage, but Th1 suppression can lead to loss of fungal control. Conversely, Treg may be involved in pro-inflammatory responses. Promotion of Th17 cell differentiation via IL-2 sequestration enhances mucosal immunity against oral candidiasis. Although these regulatory responses may be detrimental in some cases of fungal infection, they serve a useful purpose during the late phase of fungal infections by allowing the immune response to return to baseline after fungi have been cleared.

11.4.3 B-Cell Response

Humoral immunity is less central to control of fungal infections. In fact, most patients with inherited and acquired hypogammaglobulinemia are not at significantly increased risk for fungal infections. However, B-cell-depleting therapies such as rituximab (monoclonal antibody to CD20) have been associated with increased risk for invasive fungal disease, indicating some protective role for B cells that is not necessarily antibody-mediated. Moreover, studies in mouse models have demonstrated that monoclonal antibodies are protective against otherwise devastating *Cryptococcus* and *Pneumocystis* infections [21]. Additional investigation has shown that antibodies can be protective, non-protective, or even disease-enhancing, perhaps explaining the conflicting findings on the role of antibodies in antifungal immunity [22]. Antibodies clearly exhibit several antifungal properties. Antibodies enhance phagocytosis via opsonization. However, the fungal cell wall is resistant to complement so antibody-mediated complement activation does not result in cell wall damage. Antibody-mediated opsonization can enhance phagosome activation in macrophages and result in more efficient antigen presentation to T cells. Some antibodies mediate direct antifungal activity, including antibody-mediated iron starvation and aspartyl proteinase inhibition in *Candida* infections. Similarly anti- β -glucan antibodies interfere with cell wall remodeling and adherence in *Candida* and *Cryptococcus* infections leading to fungal death, while yeast killer toxin-like antibodies can bind killer toxin receptors on the fungal cell wall and result in damage [23]. Antibodies, particularly of the IgE class, have been implicated in allergic responses to fungi, including allergic bronchopulmonary aspergilliosis (ABPA).

11.5 Immunoepidemiology of Fungal Infections

11.5.1 Introduction

The immune response to fungal infection described above occurs in the great majority of people even though every person has a unique immune response. The study of immunoepidemiology examines subpopulations that have distinctly different responses to fungal infection from the general population and from each other. These consist of people with well-recognized immune deficiencies. Immunoepidemiology also includes the analysis of people who are relatively immunocompetent but have greater susceptibility to fungal infection based upon race, ethnicity, age, or geographical location. The remainder of the chapter will review these subpopulations.

11.5.2 Susceptibility to Fungal Infections in the Context of Immunodeficiencies

Our understanding of fungal immunology has been greatly enhanced by the study of individuals and populations at increased risk for fungal infections due to inborn or acquired immunodeficiencies. A thorough assessment of the genotypic and phenotypic profile of these populations has led to the discovery of molecular pathways critical to antifungal immunity as well as inheritable mutations and acquired susceptibilities to fungal infections. Furthermore, patients with these disorders are at risk for particular types of mycoses and exhibit certain predictable clinical manifestations, reflective of the specific underlying immune deficiency. In the section below, we examine pathways involved in immune defense against certain clinically important fungal infections by reviewing classic immunodeficiencies linked to increased disease susceptibility.

11.5.3 Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis (CMC) consists of a heterogeneous group of syndromes that usually manifest at a young age, with the common feature of chronic, protracted, noninvasive *Candida* infections of the mucous membranes, skin, nails, and hair refractory to normally effective treatments. Syndromes of CMC are caused by inherited mutations in genes involved in antifungal immunity, particularly those associated with IL-17. A subset of individuals with CMC has been found to carry autosomal dominant mutations in STAT1 [24]. As noted above, STAT1 is an important transcription factor downstream of IFN- γ signaling that primes macrophages for phagocytosis of *Candida* and that primes naïve T cells for maturation into Th17 cells. Gain-of-function STAT1 mutations lead to impaired nuclear dephosphorylation of STAT-1 resulting in decreased Th17 activation and decreased production of IL-17 and IL-22. Impaired Th17 function is thought to be the basis of increased susceptibility to mucocutaneous *Candida* infections. In Job's syndrome (also known as hyperimmunoglobulin E syndrome), a loss-of-function mutation in STAT3 impairs ROR γ t-dependent Th17 cell development, leading to CMC among other clinical manifestations. The syndrome of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also referred to as autoimmune polyendocrine syndrome type I (APS-1), accounts for the majority of CMC cases in some populations. These include cases in Finland and Sardinia, Italy, because of the high prevalence of mutations in the *AIRE* gene, present on chromosome 21q 22.3, in those populations [25]. In the context of APECED, CMC is caused by production of autoantibodies against IL-17 and IL-22. Autoantibodies to IL-17 may also present in patients with thymoma, leading to CMC. Mutations in genes for IL-17 cytokines (IL-17F) and IL-17 receptors (IL-17 RA and RC) are associated with CMC. Many other inborn errors of IL-17 immunity have been linked to development of CMC.

In contradistinction to other forms of CMC, CARD9 deficiency is caused by loss-of-function mutations and predisposes to both mucocutaneous and invasive candidiasis, particularly CNS tropism, but not bacterial or viral infections [26]. As noted above, CARD9 activation leads to activation of NF- κ B, a canonical protein complex that controls DNA transcription and cytokine production. The precise immunologic impairment associated with CARD9 deficiency is yet to be defined but are thought to affect separate pathways that lead to susceptibility to mucocutaneous disease (likely via IL-17 or IL-22 impairment) or to invasive disease (via impaired neutrophil function and chemotaxis).

11.5.4 Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) consists of a heterogeneous group of disorders characterized by recurrent, invasive bacterial and fungal infections with formation of tissue granulomas [27]. Aspergillosis is the most common infection in patients with CGD, with an incidence of 2.6 cases per 100 patient-years and a prevalence of 40%. *Aspergillus nidulans*, an otherwise low-virulence species, almost exclusively affects patients with this syndrome. CGD is caused by inherited mutations in NADPH oxidase. The NADPH oxidase complex consists of five proteins (gp91phox, p22pho, p47phox, p67phox, and p40phox), all of which are necessary for adequate generation of superoxide. Conversion of NADPH to NADP⁺ produces superoxide that is catalyzed into hydrogen peroxide via the action of superoxide dismutase then into hypochlorite and hydroxyl radicals. These reactive oxygen species activate granule proteases, including elastase and cathepsin G that are directly toxic to *Aspergillus*. Mutations in genes for all five components lead to impaired superoxide production and to CGD, with the most common form being X-linked (mutation in gp91phox), while other forms are later in onset and autosomal recessive. The absence of an NADPH-based respiratory burst is associated with decreased killing of catalase positive organisms including fungi, setting the stage for invasive and recurrent forms of aspergillosis.

11.5.5 Acquired Immunodeficiency Syndrome with HIV Infection

If left untreated, infection with HIV leads to gradual depletion of CD4⁺ T cells and the development of an acquired cellular immune deficiency syndrome. AIDS is defined as peripheral CD4⁺ T cell count <200 cells/ μ L or the presence of AIDS-defining conditions, one of which is extrapulmonary cryptococcosis (especially CNS involvement). People living with HIV/AIDS are at significant risk for cryptococcal infection. During the height of the AIDS epidemic in the early 1990s, the incidence of cryptococcosis was approximately 5 cases per 100,000 persons per year in some large urban centers [28]. The risk of infection strongly correlates with the absolute number of circulating CD4⁺ T cells and a dramatic increase in susceptibility at levels below 100 cells/ μ L. The absence of sufficient circulating CD4⁺ T cells leads to decreased secretion of TNF- α , GM-CSF, and IFN- γ , decreased recruitment of phagocytes and lymphocytes, and decreased granuloma formation. This allows the fungus to disseminate most commonly from alveoli where it is first inhaled into the blood then the CNS. *C. neoformans* is thought to escape phagolysosomal killing within macrophages via phagosomal extrusion, after which it is transported across the blood-brain-barrier as a “Trojan horse” where it causes meningoencephalitis [29]. The cryptococcal capsule has been shown to enhance HIV-1 replication and infectivity by promoting increased cellular NF- κ B activation, a key step in the antifungal response that also results in stimulation of the latent HIV-infected pro-virus. If immune function recovers rapidly, as with the initiation of effective antiretroviral therapy, a reinvigorated CD4⁺ T-cell population can orchestrate an influx of inflammatory cells into sites of infection. This leads to the immune reconstitution inflammatory syndrome (IRIS), a paradoxical clinical worsening that can sometimes be life-threatening.

11.6 Susceptibility to Fungal Infections in the Context of Immunocompetent Populations

Increased susceptibility to fungal infections has clearly been documented in certain immunocompetent populations, most often based on ethnicity. A racial predisposition toward severe infection is most evident with *Coccidioides immitis/Coccidioides posadasii*, a dimorphic fungus endemic to the arid landscapes of the Southwestern USA and the agent of coccidioidomycosis [30]. Based on cases occurring in Kern County, California, between 1901 and 1936, researchers noted that persons of Filipino, African American, and Mexican American descent were significantly more likely (176, 14, and 3 times, respectively) than Caucasians to develop disseminated coccidioidomycosis [30]. More recent studies have replicated these findings, described increased predilections in other populations including Asians, and excluded occupational exposure as the sole underlying driver of risk. The genetic basis of increased susceptibility is unknown but may be associated with ABO blood group B and HLA class II antigens (HLA-A9 and HLA-B9 antigens) that are more common in Filipinos and African Americans, although this association may not be causal. Some markers, such as the HLA class II-DRB1*1301 allele, have been associated with severe coccidioidomycosis regardless of ethnicity [31]. An immunologic basis for increased susceptibility has been entertained, possibly due to an intrinsic inability to produce an effective cellular immune response to *Coccidioides*. Indeed, mutations in IFN- γ and IL-12 receptor β 1 deficiency have been reported in some patients with disseminated coccidioidomycosis. However, in a study of patients with active coccidioidomycosis, skin reactivity after inoculation with *Coccidioides* antigen was similar in Caucasians and African Americans. Likewise, Filipinos and African Americans developed comparable T-cell reactivity to Caucasians after immunization with a formalin-killed spherule (FKS) *Coccidioides* vaccine [32]. Studies have also indicated that males exhibit increased risk for infection, but the effect is much less pronounced than that of race. Asian ethnicity may also be associated with increased risk for blastomycosis, an endemic fungal infection caused by *Blastomyces dermatitidis* and endemic to the Midwestern USA. In a 2009 outbreak of blastomycosis in Wisconsin, Asian residents, most of whom were of Hmong ethnicity, developed symptomatic infection at a rate 12 times higher than non-Asians [33]. Though the underlying mechanisms remain a mystery at present, it is likely that future immunoepidemiologic studies will unravel the genetic basis of these predilections.

11.7 Immunoepidemiologic Tools for Characterization of Fungal Geographic Distribution

Immunoepidemiologic tools have strongly informed our knowledge of the global distribution of medically important fungi. These include measures of cellular immunity via testing for skin reactivity, lymphocyte stimulation assays, and measures of humoral immunity via detection of specific antibodies. Skin-testing studies for *H. capsulatum* performed in the 1950s–1970s were key in defining the geographic distribution of this fungus [34]. They documented a high prevalence of histoplasmin skin sensitivity of more than 20% in the Midwestern USA, Central America, and parts of South America, some positivity in northern India (10–20%), and very low prevalence in Africa and Europe (<2%), except for Italy. In the USA, subsequent skin test surveys of newly recruited individuals to the US Navy mapped the endemic area with added precision. More recent studies have described areas of high endemicity in Asia; a study from China reported a prevalence of positive histoplasmin skin test of up to 50%. In highly endemic areas, skin and serologic reactivity testing has demonstrated that around 90% of the population acquire for *H. capsulatum* by age 18, though the vast majority of these infections are subclinical. Conversely, a seroprevalence study of Burmese, Hmong, and Somali refugee

immigrants to the USA found an anti-*H. capsulatum* IgG positivity rate of $\leq 1\%$, suggesting that histoplasmosis is rare in these populations [35]. Skin-reactivity surveys for *C. neoformans* have indicated that it is widely present in nature and global in distribution. *C. neoformans* is also frequently isolated from habitats replete with bird excreta, suggesting an avian environmental reservoir and a possible risk factor for exposure. These findings were substantiated by serological surveys that found a significantly higher prevalence of *C. neoformans* antibodies in pigeon fanciers as compared to control groups [36]. Immunologic surveys have also contributed to defining areas of endemicity for *B. dermatitidis* [37]. In a study of forestry workers in the USA, a *B. dermatitidis* antigen-specific lymphocyte stimulation assay was positive in 30% of workers in endemic areas of Northern Wisconsin and Northern Minnesota, as compared to 0% in Washington State. None of the individuals in the study reported symptomatic disease, indicating that the majority of cases of blastomycosis are subclinical. Seroepidemiologic studies of animal populations have shed light on the geographic distribution and life cycles of fungi, including avian *Aspergillus* exposure and canine paracoccidioidomycosis and blastomycosis. As the effects of global warming begin to take effect, fungal immunoepidemiology will be instrumental in defining evolving geographic ranges of known pathogenic fungi and those of emerging pathogenic species [38].

11.8 Vaccines Against Fungi

The advent of immunization in the late eighteenth century led to a marked decline of several devastating infectious diseases, revolutionized the practice of medicine, and made great contributions to global health. Despite their widespread geographic distribution and the significant morbidity and mortality associated with mycoses, there are no currently available commercial vaccines against fungi [10]. An important barrier to the development of such vaccines is related to the population at risk. Whereas some invasive mycoses can affect fully immunocompetent persons such as those caused by endemic fungi and some dematiaceous molds, the majority infections occur in highly immunocompromised individuals. Because intact immunity is required for an appropriate response to vaccination and building of immunological memory, vaccination is often ineffective in this population [39]. Additionally, although live attenuated vaccines often elicit the strongest and longest lasting immune responses, they are with few exceptions contraindicated in immunocompromised persons because of the risk of disease reactivation. If immunosuppression is planned as part of solid-organ and hematopoietic stem cell transplantation, vaccination can be administered beforehand in order to circumvent this problem. Another strategy is to employ vaccines that act on components of the immune system that are intact. A requirement for most effective antifungal vaccines is the ability to trigger Th1 and/or Th17 immune responses that are needed for phagocyte activation and granuloma formation, critical steps in abrogating fungal infections. Most currently available vaccines for non-fungal pathogens elicit antibody responses, important in neutralizing viruses and bacteria but less effective in controlling fungi. Despite the lack of commercially available vaccines for humans, proof of concept has been successfully demonstrated in animal models. Immunization with 1,3 β -D glucan conjugated to diphtheria toxoid stimulated robust antibody responses and protected mice against inoculations of *Candida*, *Cryptococcus*, and *Aspergillus*. 1,3 β -D glucan is highly immunogenic and can serve as an effective adjuvant in triggering protective Th1 and/or Th17 immune responses [40]. In a newer vaccine model, antigens have been embedded within β -glucan particles, eliciting strong T_h1 and Th17 responses. Targeting pan-fungal structures like 1,3 β -D glucan also carries the potential of protection against a wide range of fungal infections. Live-attenuated vaccination of CD4+ T-cell-deficient mice against *B. dermatitidis* was effective in preventing blastomycosis and was associated with development of IL-17 secreting CD8+ T cells [41, 42]. These and other similar data are highly encouraging for vaccine

development in immunocompromised persons. Some candidate vaccines have already been shown to be successful in Phase I clinical trials in humans and are on a likely path toward development for commercial use. An adjuvant vaccine against *Candida* targeting the N-terminal portion of the agglutinin like sequence 3 protein (Als3p) was tested in 73 patients [43]. All subjects developed T-cell-dependent and T-cell-independent responses, including generation of anti-Als3p antibodies, and vaccination was associated with protection against candidiasis. Extensive N-linked and/or O-linked mannosylation of antigens naturally occurs on fungal cell walls and is associated with more efficient processing by dendritic cells. Vaccines that incorporate antigen mannosylation have been shown to augment fungal vaccine immunogenicity.

11.9 Conclusion

Invasive mycoses are responsible for staggering levels of morbidity and mortality worldwide. The public health impact of fungal infections is on track to expand as the population at risk for pathogenic fungi continues to grow. A warming world is likely to result in evolving geographic ranges of known medically important fungi and in the emergence of newly pathogenic species. By characterizing antifungal host immune responses in ethnically, geographically, and immunologically diverse populations, fungal immunoepidemiology provides critical insights that can be utilized for global mapping of existing and emerging fungal pathogens and for the development of effective diagnostic, therapeutic, and preventative modalities. These will include development of new diagnostic fungal markers, new classes of antifungal agents, and first-generation antifungal vaccines.

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