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Adaptive Immunity to Fungi

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Life-threatening fungal infections have risen sharply in recent years, owing to the advances and intensity of medical care that may blunt immunity in patients. This emerging crisis has created the growing need to clarify immune defense mechanisms against fungi with the ultimate goal of therapeutic intervention. We describe recent insights in understanding the mammalian immune defenses that are deployed against pathogenic fungi. We focus on adaptive immunity to the major medically important fungi and emphasize three elements that coordinate the response: (1) dendritic cells and subsets that are mobilized against fungi in various anatomical compartments; (2) fungal molecular patterns and their corresponding receptors that signal responses and shape the differentiation of T-cell subsets and B cells; and, ultimately (3) the effector and regulatory mechanisms that eliminate these invaders while constraining collateral damage to vital tissue. These insights create a foundation for the development of new, immune-based strategies for prevention or enhanced clearance of systemic fungal diseases.

Encounters with fungi require a coordinated host innate and adaptive immune response to successfully eradicate the fungus and promote long-lived immunological memory of the encounter. This review covers three key elements that orchestrate this coordinated response: dendritic cells (DCs), pattern-recognition receptors (PRR), and antigen-specific T and B cells. DCs lie at the intersection of innate and adaptive immunity. These cells are capable of taking up and processing antigen for display by major histocompatibility complex (MHC) class I or MHCII molecules to naïve T cells and of mediating fungicidal activity. Surface and intracellular PRRs enable DCs to sense fungi. On fungal recognition, DCs secrete cytokines and express costimulatory molecules that help drive naïve $CD4^+$ T-cell differentiation into a T-helper (Th) phenotype. In immunocompetent hosts, $CD4^+$ T-cell-mediated clearance of fungi with limited tissue damage requires a finely tuned balance among Th1, Th17, and Treg (regulatory

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T cell) subsets; in CD4-deficient hosts, $CD8^+$ T cells may come into play. A calibrated balance of helper, regulatory, and effector T- and B-cell responses integrate optimal innate and adaptive immunity to fungi.

CHARACTERIZATION AND FUNCTION OF DC AND MONOCYTE SUBSETS

Steinman and Cohn first reported the identification of a cell with "continually elongating, retracting, and reorienting" long cytoplasmic processes in the spleen and lymph nodes of mice (Steinman and Cohn 1973). These cells, termed DCs, are hematopoietic cells that serve as professional antigen (Ag)-presenting cells (APCs) and initiate T-cell responses. When DCs encounter Ag at the boundary of immunological defense sites, such as the skin, airways of the lung, or draining nodes of the lymphatic system, DCs amplify the innate immune response by secreting cytokines that recruit and activate other leukocytes. After uptake, processing and presentation of Ag, DCs initiate and shape adaptive responses by promoting naïve T-cell differentiation into effector or regulatory T cells. Since the discovery of DCs, many subsets have been described based on anatomical location, function, and surface marker expression (Fig. 1).

Plasmacytoid DCs

Plasmacytoid dendritic cells (pDCs) are typified by interferon-α (IFN-α) production in response to nucleic acids sensed by endosomal Toll-like receptors, and are characterized by surface expression of sialic acid binding immunoglobulin-like lectin H (Siglec H). pDCs induce IL-10-producing $CD4^+$ $Foxp3^+$ Treg cells, limit Th1 and Th17 cell polarization at mucosal sites, and activate $CD8^+$ T cells (Takagi et al. 2011). pDCs control viral infection via the induction of $CD8^+$ T cells, but may impair bacterial clearance and contribute to septic shock. Although pDCs mediate antiviral immunity, the role of pDCs in fungal infections is less clear. pDCs recognize *Aspergillus fumigatus* DNA via TLR9 (Ramirez-Ortiz et al. 2008) and inhibit *Aspergillus* growth in vitro. pDCs accumulate in the lungs in a murine model of *Aspergillus* pulmonary infection (Ramirez-Ortiz et al. 2011), and their elimination enhances progression of infection, suggesting that pDCs may recognize and combat fungi directly in vivo.

A subset of pDCs exists that develops in the context of elevated IFN-α and is similar to pDCs found in Peyer's patches (Li et al. 2011). Uncharacteristically, this pDC subset fails to produce IFN-α after stimulation with TLR ligands, but secretes elevated levels of interleukin (IL)-6 and IL-23 and primes Ag-specific Th17 cells in vivo. This finding suggests a potential role for IFN-α-elicited pDCs in the polarization of antifungal Th17 cells. Combined with the recent findings that pDCs are critical mediators of Treg/Th17 balance at mucosal surfaces, recognition of fungi by pDCs or IFN-α-elicited pDCs at mucosal surfaces may tilt the balance toward tolerance or inflammation.

Conventional DCs

Conventional DCs or resident DCs exist in the lymphoid tissue and are comprised of two main subpopulations: $CD8^+$ and $CD4^+CD8^-$ resident DCs. The spleen contains a third, minor population of so-called double-negative DCs, which lack CD4 and CD8 expression and appear to be largely similar in function to $CD4^+CD8^-$ DCs (Luber et al. 2010). $CD8^+$ resident DCs are identified by the surface phenotype $CD8^+$ $CD4^-CD11b^-CD11c^+MHCII^+DEC205^+$ and are located chiefly in the T-cell zone of the spleen and lymph nodes (Idoyaga et al. 2009). A major function of $CD8^+$ DCs is to cross-present Ag via MHCI to $CD8^+$ cytotoxic T lymphocytes (CTLs) (den Haan et al. 2000). $CD8^+$ DCs obtain Ag by engulfment of live or apoptotic cells or Ag-containing apoptotic vesicles.

DCs acquire and cross-present *Histoplasma capsulatum* Ags to CTL by ingestion of live or killed yeasts or uptake of *Histoplasma*-containing apoptotic macrophages (Lin et al. 2005b). Subcutaneous injection of apoptotic phagocytes containing carboxyfluorescein succinimidyl ester (CFSE)-labeled, heat-killed *Histoplasma* results in the accumulation of CFSE in

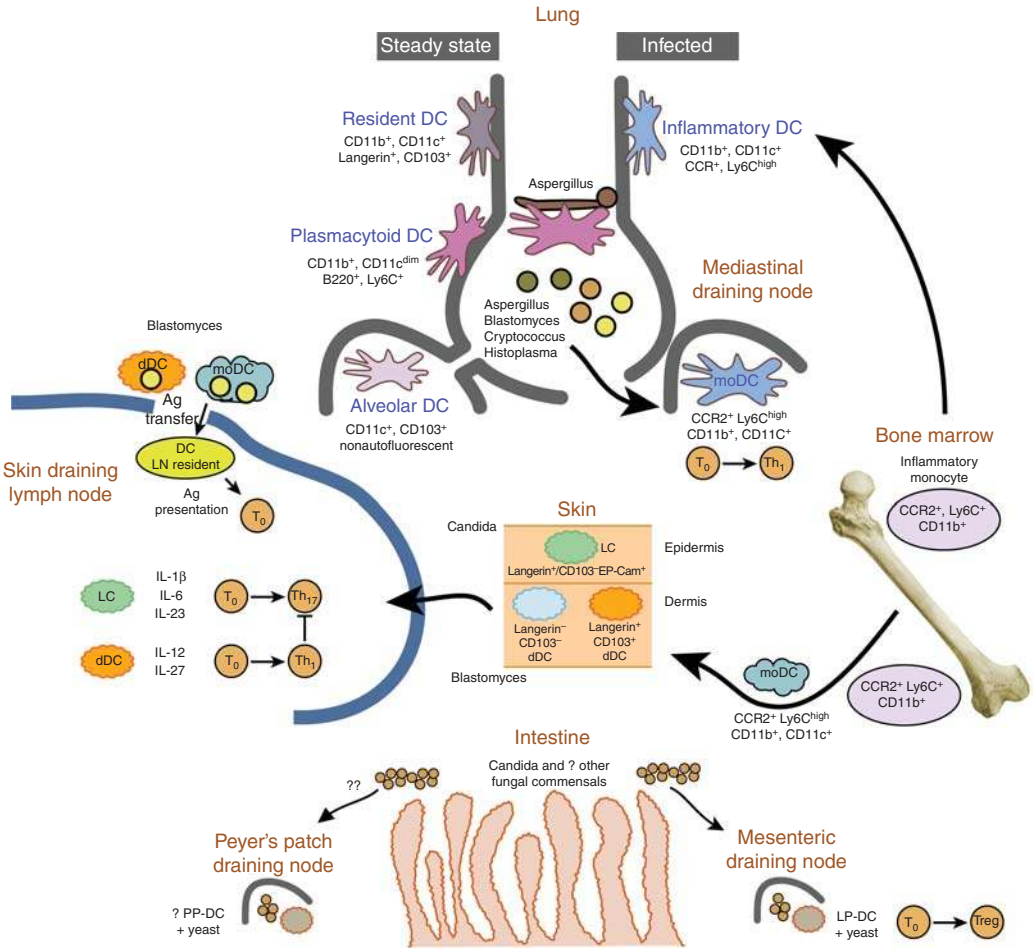

Figure 1. Dendritic cells and priming of adaptive immunity to fungi. There are at least five subsets of DCs that participate in priming T cells during fungal infection. Lung DCs can be divided into $CD11b^{+}$ and $CD11b^{-}$. $CD103^{+}$-resident classical (c)DCs are important in response to viruses, whereas inflammatory DCs participate in response to several fungal pathogens, and plasmacytoid DCs are vital in immunity to *Aspergillus*. Inflammatory monocyte-derived DCs (moDCs) are $CD11b^{+}$ and $Ly6C^{high}$. These cells express the chemokine receptor CCR2, which mediates egress from the marrow chiefly in response to the chemokines CCL2 and CCL7. In the absence of CCR2 ($CCR2^{-/-}$ mice), animals evince a skewed Th response in the lung, dominated by Th2 cytokines. Inflammatory monocyte-derived DCs also deliver subcutaneously injected vaccine yeast into draining lymph nodes, where they collaborate with migratory dermal and Langerhans DCs in priming CD4 T cells on antigen transfer into resident lymph node DCs. Dermal DCs elaborate IL-12 and IL-27 and efficiently prime Th1 cells, whereas Langerhans DCs elaborate IL-1β, IL-6, and IL-23 and skew the response toward Th17. *Candida*, a commensal of the intestinal tract, and other as-yet-unidentified fungi make up the "mycobiome" and modulate host physiology through interaction with C-type lectins, such as Dectin-1, likely displayed on intestinal DCs (Iliev et al. 2012). DCs in the lamina propria (LP-DC) influence the development of Treg, whereas those in the Pyer's patch (PP-DC) have not been investigated with respect to fungi.

$CD11c^+$ cells in skin-draining lymph nodes and $CD11c^+$-dependent CTL-mediated protection against *Histoplasma* challenge (Hsieh et al. 2011). Although these studies show that fungal Ags can be acquired and presented by resident DCs, the resident DC subpopulation(s) involved in vivo remain undefined.

$CD4^+CD8^-$ resident DCs also cross-present fungal Ags. In studies using an OVA-expressing strain of *Saccharomyces cerevisiae*, both $CD8^+$ and $CD4^+CD8^-$ DCs from mouse spleen that were primed with OVA–*S. cerevisiae*–primed robust OVA-specific $CD4^+$ T-cell proliferation ex vivo; however, only $CD4^+CD8^-$ DCs stimulated an OVA-specific $CD8^+$ T-cell response (Backer et al. 2008). Further work will be needed to unravel the relative contributions of resident DC subpopulations to $CD4^+$ T-cell and CTL activation and polarization in vivo.

During vaccine immunity to *Blastomyces dermatitidis*, lymph node–resident DCs primed antifungal T-cell responses after Ag transfer from other cells, although the phenotype of resident DC was not defined. DC acquisition of Ag required ferrying of yeast from the skin to the lymph node by migratory and monocyte-derived DCs, after which resident DCs in the skin-draining lymph nodes acquired and displayed Ag and primed Ag-specific $CD4^+$ T cells (Ersland et al. 2010).

Migratory DCs

Migratory DCs (or tissue DCs) are immature DCs located mainly in peripheral tissues, such as the skin, lung, and gut. Following uptake of Ag, migratory DCs exit the tissue and mature as characterized by (1) enhanced Ag processing and presentation, (2) down-regulation of tissue homing receptors, (3) up-regulation of CCR7, and (4) increased surface display of costimulatory molecules. $CCR7^+$ DCs migrate to the T-cell zone of lymphoid tissue where they can activate naïve T cells or transfer Ag to resident DCs (Banchereau and Steinman 1998; Allan et al. 2006). Migratory DCs have the capacity for division and self-renewal in situ, whereas monocyte subsets also contribute to replenishment of migratory DCs. Migratory DCs line the surfaces of the body exposed to the environment and thus encounter fungi and other pathogens and Ags. Although the migratory DC networks that line the skin, lung, and intestine share similarities, each site has functional differences that are important in antifungal immunity.

Skin

The skin contains a network of DCs that can be divided into the epidermis-associated Langerhans cells (LCs) and a collection of dermis-associated dermal DCs (Henri et al. 2010). In addition to their epidermal location, LCs are characterized by surface expression of langerin (CD207), CD11c, and MHCII. The dermis contains LCs that migrate to the draining lymph node, $CD207^+CD103^+$ dermal DCs, and a group of $CD207^-CD103^-$ DCs (Henri et al. 2010). On subcutaneous injection of *B. dermatitidis* vaccine, $DEC205^+$ skin-derived DCs migrated to the draining lymph nodes in a CCR7-dependent fashion, presented (or transferred) model Ag expressed by the yeast, and activated $CD4^+$ T cells (Ersland et al. 2010).

LCs and $CD8^+$ dermal DCs have been shown to specialize in Ag presentation and T-cell polarization functions in a cutaneous exposure model to *Candida albicans* (Igyarto et al. 2011). LCs are required for the generation of Ag-specific Th17 cells via the production of elevated levels of IL-6, IL-1β, and IL-23. Although LCs are not required for the generation of CTL, $CD207^+$ dermal DCs are required for CTL and also Th1 polarization. Compared with LCs, $CD207^+$ dermal DCs produce more IL-12 and IL-27, and less IL-1β and IL-6, and no IL-23, making them poor promoters of Th17. $CD207^+$ dermal DCs also blunt the ability of LCs and $CD207^-$ dermal DCs to promote Th17 responses. Because IL-12 and IL-27, as well as IFN-γ from Th1 cells, inhibit Th17 differentiation and proliferation, $CD207^+$ dermal DCs likely block *Candida*-specific Th17 cells by promoting Th1 differentiation. Thus, exposure of LCs and $CD207^+$ dermal DCs to *Candida* can promote opposing effects via elaboration of polarizing cytokines that induce development of Th1 or Th17 responses (Igyarto et al. 2011).

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Lung

DCs in the lung and airways confront constant exposure to inhaled spores and hyphal fragments. A network of DCs lines the airways, sampling inhaled Ags and shuttling them to the mediastinal lymph nodes. Besides pDCs, lung DC subsets include two broad subsets: $CD103^+$ DCs and $CD11b^+$ DCs. $CD103^+$ lung DCs also express CD207, making them similar to $CD207^+$ $CD103^+$ dermal DCs. $CD103^+$ DCs extend dendrites into the airway lumen to sample Ag without disturbing the epithelium (Jahnsen et al. 2006; Sung et al. 2006). $CD103^+$ DCs can acquire soluble and apoptotic-cell-associated Ags from the airway and migrate to mediastinal lymph nodes under steady state and inflammatory conditions. At the lymph node, $CD103^+$ DCs cross-present Ag and activate CTL (Desch et al. 2011). $CD11b^+$ DCs differ from monocyte-derived DCs and specialize in cytokine and chemokine production (Beaty et al. 2007) as well as presenting Ag to $CD4^+$ T cells in the mediastinal lymph node after migration (del Rio et al. 2007). Rapid recruitment of $Ly6C^+$ monocyte-derived DCs to the lung on inflammation has clouded the functional analysis of lung resident $CD11b^+$ DCs, which lack Ly6C expression. On lung exposure to *A. fumigatus* conidia, $CD103^+$ DCs failed to take up and transport conidia to the mediastinal lymph node, whereas $CD11b^+$ DCs did transport conidia (Hohl et al. 2009). In this model, lung $CD11b^+$ DCs were reduced in $CCR2^{-/-}$ mice, relative to wild-type mice, following *A. fumigatus* exposure. Conversely, naïve $CCR2^{-/-}$ mice had lung $CD11b^+$ DC numbers similar to wild-type mice, suggesting that recruited monocyte-derived DCs and not lung resident $CD11b^+$ DCs are responsible for conidial uptake and Ag presentation in the setting of *A. fumigatus*–induced inflammation.

Intestine

As in the lung, DCs in the intestine are situated on the basolateral side of the epithelium, and largely isolated from the gut microflora. DCs in the intestine localize to the lamina propria (LP-DCs) and Peyer's patch (PP-DCs); both subsets in each region differentially regulate immune responses. The role of PP-DCs and LP-DCs in generating antifungal immunity in the gut is unclear and relatively unstudied. *C. albicans*, a gut commensal, can cause systemic infection if the gut epithelial/DC barrier is breached or in the setting of broad-spectrum antibiotic use, leading to *Candida* overgrowth. Strong induction of Treg cells by LP-DCs in the mesenteric lymph node highlights the critical role of limiting inflammation in the gut to maintain the epithelial barrier and prevent disseminated infection. Furthermore, heightened Th17 responses in the gut impair protective Th1 responses and worsen *Candida* infection (Zelante et al. 2007). Although bone marrow–derived DCs produce IL-23 in response to *Candida* in vitro and IL-23 neutralization promoted fungal clearance in vivo, the identity of the DC subset recognizing and responding to the fungus in this model was not determined. Nevertheless, DCs in the gut appear to tightly control tolerance and immunity to fungal organisms.

Monocytes, Monocyte-Derived DCs, and Inflammatory DCs

Monocytes are derived from a macrophage-DC progenitor and, in the absence of inflammation, are found in the bone marrow and circulating at low levels in the blood and spleen. Two classes of $CD11b^+CD115^+$ monocytes arise from the progenitor and circulate in the blood, $Ly6C^+$ $CCR2^+$ and $Ly6C^-CX_3CR1^{hi}$ monocytes (Geissmann et al. 2003). Monocytes have broad developmental plasticity, replenish subsets of DCs and LCs in the setting of experimental depletion (Ginhoux et al. 2007), and represent an emergency store of DC precursors that can be rapidly deployed. Under inflammatory conditions, $Ly6C^+CCR2^+$ monocytes migrate to inflammatory sites and acquire expression of the DC markers CD11c and MHCII, while losing expression of Ly6C (Osterholzer et al. 2009), thus becoming "inflammatory DCs." Whereas $Ly6C^-CX_3CR_1^{hi}$ cells do not appear to be involved in innate immunity to fungi (Dominguez and Ardavin 2010), $Ly6C^+CCR2^+$

monocytes play a critical role in responding to many medically important fungi including *A. fumigatus* (Hohl et al. 2009), *Cryptococcus neoformans* (Osterholzer et al. 2009), *H. capsulatum* (Szymczak and Deepe 2009), and *B. dermatitidis* (Ersland et al. 2010).

Monocyte-derived DCs have an outsized role in antifungal immunity, particularly through the induction of Th1 cells. $CCR2^{-/-}$ mice show skewed Th2 responses and poorly controlled *H. capsulatum* infection compared to wild-type mice (Szymczak and Deepe 2009). Similar CCR2-dependent phenotypes are found in experimental infection with *A. fumigatus* or *C. neoformans*; that is, priming Th1 cells in response to fungi requires $CCR2^{+}$ monocyte-derived inflammatory DCs (Traynor et al. 2000; Hohl et al. 2009; Osterholzer et al. 2009; Ersland et al. 2010). The tissue environment has a prominent role in inflammatory DC function, as the defect in $CD4^{+}$ T-cell priming by these DCs during infection with *A. fumigatus* is restricted to the lung in $CCR2^{-/-}$ mice and not to other lymphoid organs, such as the spleen (Hohl et al. 2009). Similarly, although $Ly6C^{+}$ $CCR2^{+}$ monocytes play a major role in delivering *B. dermatitidis* into skin-draining lymph nodes after subcutaneous vaccination, this shuttling function can be compensated by other skin migratory DC subsets in $CCR2^{-/-}$ mice (Ersland et al. 2010). Conversely, $CCR2^{+}$ monocytes and monocyte-derived inflammatory DCs are essential to prime Ag-specific $CD4^{+}$ T cells in the lung during infection with *Blastomyces* or *Histoplasma* (Wuthrich et al. 2012b). Thus, in the lung, which is often the primary route of infection for fungi, but not in the skin or the spleen, monocyte-derived DCs play a critical and indispensable role in antifungal immunity.

PATTERN-RECOGNITION RECEPTORS

In mammals, fungi are detected by germline encoded PRRs that are expressed by innate cells (Medzhitov 2007). The three major pathogen-associated molecular patterns (PAMPs) that are unique to fungi and set them apart from the mammalian host are chitin, α- and β-glucans, and mannans. The innate recognition of these fungal PAMPs activates signaling cascades to induce the expression of MHC, costimulatory molecules and cytokines by APC that influence the development of adaptive immunity. Because Th17 and Th1 cells are the principal T-helper subsets that contribute to protective immunity to several pathogenic fungi, we highlight the most recent literature on the signaling pathways and other factors that influence the differentiation of these two T-helper subsets.

Among the best-characterized PRRs that recognize fungi are the Toll-like receptors (TLR) and C-type lectins (CLR) (Fig 2). TLR1–4, 6, 7, and 9 recognize a variety of fungal species through mostly undefined ligands (Netea et al. 2004b; Biondo et al. 2005, 2011; Chai et al. 2009; Kasperkovitz et al. 2010; Bourgeois et al. 2011). The main TLRs involved in sensing fungal ligands are TLR2, TLR4, and TLR9 that recognize zymosan, phospholipomannan, *O*-linked mannans, glucoronoxylomannan, and fungal DNA (Shoham et al. 2001; Brown 2011; Romani 2011). Mice lacking the signaling adaptor myeloid differentiation primary response protein 88 (Myd88) are more susceptible to infection with *C. neoformans*, *C. albicans*, *A. fumigatus*, *B. dermatitidis*, and *Paracoccidioides brasiliensis* (Bellocchio et al. 2004; Biondo et al. 2005; Bretz et al. 2008; Calich et al. 2008; Wuthrich et al. 2011), emphasizing important roles for TLR signaling in antifungal immunity, but also reflecting the involvement of Myd88 in IL-1 signaling. However, in murine experimental models of infection, there are conflicting reports on individual contributions of multiple TLRs and fungal species (Calich et al. 2008; Netea et al. 2008). In humans, a similar controversy on the role of Myd88 in mediating antifungal immunity has been reported. Whereas fungal infections were not a problem for children with autosomal recessive Myd88 deficiency (von Bernuth et al. 2008), patients with TLR1 and TLR4 polymorphisms were more susceptible to candidemia (Plantinga et al. 2012) and invasive aspergillosis during stem cell transplantation (Bochud et al. 2008).

Besides the TLRs, CLRs expressed by myeloid and mucosal epithelial cells are key PRRs for the recognition of fungi and induction of

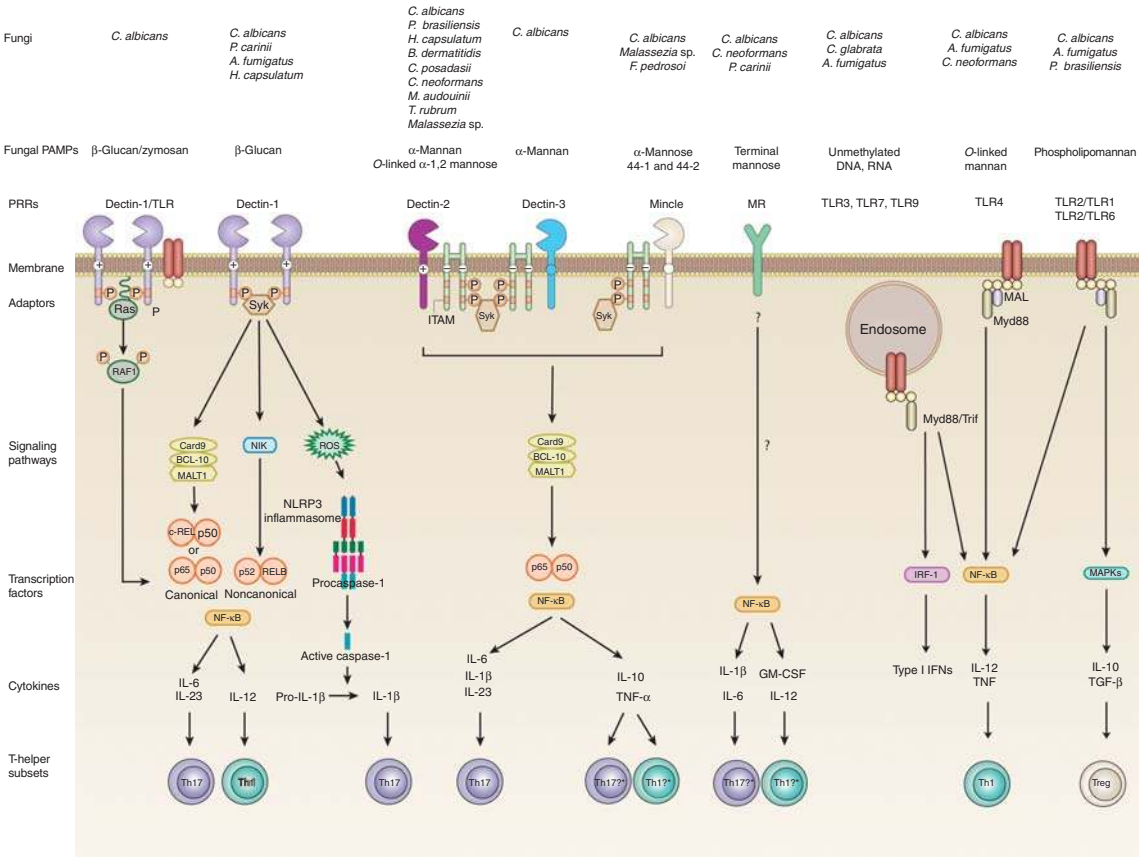

Figure 2. PRR and signaling pathways that lead to differentiation of antifungal T-helper cells. Recognition of fungal pathogen–associated molecular patterns (PAMPs) is mediated by TLRs and CLRs. The binding of fungi or β-glucan to Dectin-1 recruits SYK to the two phosphorylated receptors, which leads to the formation of a complex involving Card9, BCL10, and MALT1 (BCM). This results in the release of NF-κB consisting of either p65–p50 or REL–p50 dimers into the nucleus (Geijtenbeek and Gringhuis 2009). Syk activation also induces the noncanonical NF-κB pathway mediated by NF-κB-inducing kinase (NIK) and the nuclear translocation of p52–RELB dimers. Dectin-1 enhances TLR2 and TLR4-induced cytokines in a Syk-independent manner through the serine/threonine protein kinase RAF1 by Ras proteins, which leads to the phosphorylation of p65 (Gringhuis et al. 2009). Among other cytokines, these pathways lead to the production of IL-6, IL-23, and IL-12 that induce Th17 and Th1 cells, respectively. Dectin-1 recognition of *C. albicans* can also activate the NLRP3 inflammasome through a mechanism that involves Syk, ROS, and potassium efflux (Poeck and Ruland 2010). Fungus-induced pro-IL-1β is cleaved by active caspase-1 to bioactive IL-1β to favor Th17 development. Dectin-2 activation leads to FcR-γ-dependent recruitment and phosphorylation of Syk and activated NF-κB and MAPKs (p38, JNK, and Erk) (Saijo et al. 2010). Card9 is required for the activation of NF-κB and production of cytokines that lead to Th17 cell differentiation. Recognition of α-mannose in *Malassezia* species by Mincle activates the FcR-γ-Syk-Card9 pathway and translocates NF-κB into the nucleus to induce the activation of proinflammatory cytokines (Yamasaki et al. 2009). *Although fungal PAMPs have yet to show the ability to induce a distinct T-helper subset by this pathway, the mycobacterial cord factor and its synthetic analog are potent adjuvants for the differentiation of Mincle-induced Th1 and Th17 cells (Schoenen et al. 2010). Dectin-3 (MCL, Clec4d, or Clecsf8) also recognizes α-mannan from *C. albicans* (Zhu et al. 2013) and TDM from *M. tuberculosis* (Miyake et al. 2013). Dectin-3 can dimerize with Dectin-2 (Zhu et al. 2013) and Mincle (Lobato-Pascual et al. 2013). It is unclear whether the induction of Th17 and Th1 cells requires recognition of fungal PAMPs by homo- versus heterodimers of Dectin-2. The MR lacks a classical signaling motif in its short cytoplasmic tail, but it induces proinflammatory cytokines that have been implicated in Th17 and Th1 differentiation (Willment and Brown 2008). Although MR-dependent triggering of human memory T cells produced IL-17, further studies with naïve T cells will be needed to establish the role of the MR in Th17 cell differentiation (van de Veerdonk et al. 2009). Myd88 is critical for the signaling of TLR2 and TLR4. Phospholipomannans and *O*-linked mannans are recognized by TLRs at the plasma membrane, whereas fungal nucleic acids are sensed by endosomal TLRs and induce NF-κB-, MAPK-, and IRF-dependent cytokine production. TLR2 signaling is thought to generate weaker proinflammatory signals, but induce strong stimulation of TGF-β and IL-10 that induces Treg cells (Netea et al. 2004a; Sutmuller et al. 2006).

protective immunity (Netea et al. 2008; Kerrigan and Brown 2010, 2011; Brown 2011; Romani 2011). CLRs belong to a large family of proteins that recognize ligands in a calcium-dependent manner, which vary in microbes from endogenous to exogenous and are often conserved and carbohydrate based (Kerrigan and Brown 2010). Some CLR contain cytoplasmic signaling motifs that allow direct activation of intracellular signaling cascades; others lacking these motifs make use of adaptor molecules to initiate signal transduction (Kerrigan and Brown 2010). Below, we discuss recent progress on the cell-associated CLRs Dectin-1, Dectin-2, Dectin-3, Mincle, and the mannose receptor.

Dectin-1

Dectin-1 is the archetypical and best-studied non-TLR PRR shown to link innate and adaptive immunity and instruct differentiation of Th1 and Th17 cells (LeibundGut-Landmann et al. 2007; Rivera et al. 2011). Dectin-1 recognizes β-1,3-glucan from fungi, plants and some bacteria (Taylor et al. 2007), and unidentified T cell and mycobacterial ligands (Tsoni and Brown 2008). On ligand activation, Dectin-1 signals through an immunoreceptor tyrosine-based activation-like motif (ITAM-like or HemITAM) (Kerrigan and Brown 2010) to produce cytokines via multiple signaling pathways (reviewed in Vautier et al. 2012). The best characterized Dectin-1 signaling pathway is the Syk/CARD9 pathway leading to activation of the canonical NF-κB subunits p65 and c-Rel and subsequent production of pro-IL-1β, IL-6, IL-10, IL-23, and tumor necrosis factor (TNF)-α (Gringhuis et al. 2011). Cytokine production can be modulated via the formation of inactive RelB–p65 dimers through the noncanonical NF-κB subunit RelB in a NIK-dependent pathway (Gringhuis et al. 2009) and the Syk-independent activation of Raf-1. The former reduces IL-1β and IL-12p40 expression, whereas the latter increases the production of these pro Th1/Th17 cytokines. The balance toward a Th17 response is favored through the collaborative interaction of Dectin-1 with TLR-2 that leads to the production of prostaglandin E2, which will up-regulate the Th17 polarizing cytokines IL-6 and IL-23 (Smeekens et al. 2010).

In addition, recognition of β-glucan, *A. fumigatus*, and *C. albicans* by Dectin-1 and TLR2 activates the NLRP3 inflammasome leading to the production of bioactive IL-1β (Gross et al. 2009; Hise et al. 2009; Kankkunen et al. 2010; Poeck and Ruland 2010; Said-Sadier et al. 2010), thereby enhancing Th17 cell development and antifungal immunity. In a pulmonary model of *A. fumigatus* infection, Dectin-1 decreased the production of IL-12 and IFN-γ in innate cells, which decreased T-bet expression in *A. fumigatus*–specific CD4 T cells and enabled Th17 differentiation (Rivera et al. 2011). Thus, Dectin-1 signaling drives the production of Th17-cell-promoting cytokines that yield resistance to *C. albicans*, *A. fumigatus*, and *Pneumocystis carinii* infection (Saijo et al. 2007; Taylor et al. 2007; Werner et al. 2009).

Some fungi (e.g., *A. fumigatus* and *C. albicans*) have developed mechanisms to mask their β-glucan exposure and limit detection by immune cells (Hohl et al. 2005; Steele et al. 2005; Wheeler et al. 2008). β-glucan shielding can also vary among different strains within a fungal species. For example, *H. capsulatum* strain G186A shields its β-glucan by α-glucan so that yeast are not recognized by Dectin-1 (Rappleye et al. 2007), whereas strain G217B mostly lacks α-(1,3)-glucan on the yeast surface and is recognized by Dectin-1 (Wang et al. 2014). Thus, the induction of Th17 cells, the acquisition of vaccine-induced resistance and primary resistance to the latter strain is blunted in Dectin-$1^{-/-}$ versus wild-type mice (Wang et al. 2014).

Dectin-2 Cluster

The last few years have revealed exciting new insights into the function and role of the Dectin-2 cluster in mediating antifungal immunity. The Dectin-2 family is comprised of Dectin-2, Mincle, MCL, DCIR, DCAR, and BDCA-2. Aside from DCIR, all of the other receptors have short cytoplasmic tails that lack signaling motifs and associate with the FcR-γ chain, an adaptor containing an ITAM motif (Graham

and Brown 2009). Dectin-2 recognizes *C. albicans*, *S. cerevisiae*, *Microsporum audouinii*, *Trichophyton rubrum*, *A. fumigatus*, *H. capsulatum*, *P. brasiliensis*, *Malassezia* sp., *B. dermatitidis*, and *C. posadasii* (Kerscher et al. 2013; Wang et al. 2014). Dectin-2 recognizes *C. albicans* and *Malassezia* via *N*- and *O*-linked α-mannan, respectively, on their surface (McGreal et al. 2006; Sato et al. 2006; Saijo et al. 2010; Ishikawa et al. 2013). It has been proposed that an active Dectin-2 ligand could be a multivalent terminal α-1,2-mannose attached to glycans, proteins, and presumably any kind of scaffold (Ishikawa et al. 2013). Dectin-2-deficient mice are more susceptible to primary *C. albicans* infection (Robinson et al. 2009; Saijo et al. 2010). The Dectin-2/FcR-γ/Syk/Card9 signaling axis is indispensable for the development of vaccine-induced, Ag-specific Th17 cells and immunity to the systemic, endemic, dimorphic fungi *B. dermatitidis*, *H. capsulatum*, and *Coccidioides posadasii* (Wang et al. 2014).

Mincle is another FcR-γ-coupled activating receptor that recognizes pathogenic fungi and *Mycobacteria* (Ishikawa et al. 2009, 2013; Yamasaki et al. 2009). Mincle binds glycolipids, such as trehalose-6,6-dimycolate (TDM) from *Mycobacterium tuberculosis*, and novel glyceroglycolipids from *Malassezia* (Ishikawa et al. 2013). Although not shown yet for fungi, Mincle induces Th1/Th17 adaptive immunity in response to TDM and its synthetic analogue trehalose-6,6-dibehenate (TDB) (Schoenen et al. 2010; Shenderov et al. 2013). Mincle-deficient mice are more susceptible to primary infection with *C. albicans* and *Malassezia* (Wells et al. 2008; Yamasaki et al. 2009). However, Mincle is dispensable for development vaccine-induced immunity and Th17 cells against the three major systemic dimorphic fungi: *B. dermatitidis*, *H. capsulatum*, and *C. posadasii* (Wang et al. 2014).

Similar to Mincle, macrophage C-type lectin (MCL, also called Dectin-3, Clecsf8, and Clec4d) is also an FcR-γ-coupled activating receptor that binds to TDM (Miyake et al. 2013). MCL is constitutively expressed in myeloid cells, whereas Mincle is barely expressed in resting cells, but inducible by TDM (Schoenen et al. 2010). In response to TDM, MCL and Mincle promote the development of Th1/Th17 cells by up-regulating the costimulatory molecules CD80, CD86, and CD40 (Miyake et al. 2013). MCL also contributes to Th17-cell-mediated EAE development. Whether MCL induces antifungal Th17 cell responses is unclear but MCL-deficient mice are highly susceptible to *C. albicans* infection (Zhu et al. 2013).

The multivalent ligands on the fungal surface likely induce multimerization of monomeric and dimeric CLRs. For example, Mincle and MCL form a disulfide-linked heterodimer associated with FcR-γ (Lobato-Pascual et al. 2013) and Dectin-2 forms a heterodimeric PRR with Dectin-3 for sensing and mediating host defense against *C. albicans* (Zhu et al. 2013). The heterodimer showed higher affinity to fungal α-mannan than their respective homodimers and responded effectively to fungal infection, indicating that dimerization may provide different sensitivity and diversity for host cells to detect fungal pathogens and induce adaptive immunity. Because individual and dimeric CLR of the Dectin-2 cluster discussed here signal through the FcR-γ/Syk/Card9 axis, it is noteworthy that Card9 affects the development of Th1/Th17 cells in vivo mostly at the stage of T-cell differentiation and not during the activation, expansion, and survival during the contraction phase (Wang et al. 2014).

Mannose Receptor (MR)

The MR has a short cytoplasmic tail that lacks classical signaling motifs, and its downstream signaling pathway is unknown (Willment and Brown 2008). The MR can induce NF-κB activation and the production of IL-12, GM-CSF, IL-8, IL-1β, and IL-6 (Zhang et al. 2004; Pietrella et al. 2005; Taylor et al. 2005; Tachado et al. 2007). One caveat of studies with MR-deficient mice is that MRC1 expression is coregulated with the macrophage activating miRNA (miR-511-3p) (Squadrito et al. 2012). Although the MR has been reported to induce Th17 cell differentiation of human T cells in response to *C. albicans*, memory and not naïve T cells were the major source of the IL-17 produced (Levitz

2009; van de Veerdonk et al. 2009). Because different sets of cytokines are required to prime naïve T cells and propagate already primed memory T cells, the role of the MR in inducing Th17-cell differentiation remains inconclusive. Because the TLR2/Dectin-1 pathway has a secondary amplification effect on MR-induced IL-17 production (Levitz 2009; van de Veerdonk et al. 2009), it is conceivable that *C. albicans*–derived β-glucan is more important for the differentiation of naïve cells into Th17 cells, whereas the MR might play a more prominent role in triggering IL-17 production by memory cells.

T- AND B-CELL IMMUNITY

It is generally acknowledged that activation of the adaptive arm of the immune system is critical for resolution of fungal infection in the host. The transition from innate to adaptive immunity is facilitated primarily by DCs, although macrophages contribute. These phagocytes process and present fungal Ag to naïve $CD4^+$ T cells in the context of class II MHC. This interaction initiates the commitment to effector Th subsets. DCs also activate $CD8^+$ T cells by Ag presentation via MHCI (Fig. 3). For Ags that enter through the exogenous pathway, engagement of $CD8^+$ proceeds through a mechanism termed cross-presentation in which Ags are shuttled into the class I MHC pathway. In contrast to the requirement of Ag processing for activation of T cells, B cells directly react to fungal Ags and secrete immunoglobulins that may influence the outcome of infection.

Th1 Immunity

The Th1 immune response is instrumental in host defense against most fungal pathogens, and its importance is well established in experimental murine models and in human infections. Following exposure, APCs produce IL-12 that is critical for Th1 lineage commitment. Genetic mutation in the IL-12 signaling pathway is associated with predisposition to a wide variety of fungal diseases, such as cryptococcosis, candidiasis, paracoccidioidomycosis, and coccidioidomycosis (Moraes-Vasconcelos et al. 2005; Aytekin et al. 2011; Vinh et al. 2011; Jirapongsananuruk et al. 2012). Furthermore, increased susceptibility to histoplasmosis has been observed in mice lacking IFN-γ (Allendoerfer and Deepe 1997) and in a patient with a genetic deletion mutation in IFN-γ receptor-1 gene (Zerbe and Holland 2005). Conversely, patients receiving adjunctive IFN-γ immunotherapy display augmented protection against aspergillosis, cryptococcosis, and coccidioidomycosis (Kelleher et al. 2006; Duplessis et al. 2011; Jarvis et al. 2012).

Th1 cells orchestrate antifungal immune responses through the release of proinflammatory cytokines IFN-γ, TNF-α, and GM-CSF (Fig. 3). The signature Th1 cytokine, IFN-γ, manifests pleotropic effects on immune cells during infection. It induces classical activation of macrophages that is critical for arresting growth of intracellular fungal pathogens including *H. capsulatum*, *B. dermatitidis*, *P. brasiliensis*, and *Coccidioides immitis* (Beaman 1987; Brummer and Stevens 1995; Sugar et al. 1995; Calvi et al. 2003). These classically activated phagocytes are speculated to mediate their fungicidal activities through the release of nitric oxide and reactive oxygen intermediates (Wuthrich et al. 2012a). In addition to its effects on macrophages, IFN-γ prompts antibody class switching to IgG2a in B cells (associated with antifungal effects [Snapper and Paul 1987; Lin et al. 2009]) and enhances phagocytosis (Shalaby et al. 1985) and Ag processing/presentation in APCs (Schroder et al. 2004) to combat fungal infections.

TNF-α shares multiple redundant functions with IFN-γ, including classical activation of macrophages (Novak and Koh 2013). Animals deficient in TNF-α are highly vulnerable to a range of fungal infections (Nagai et al. 1995; Huffnagle et al. 1996; Allendoerfer and Deepe 1998; Opata et al. 2013). The protective effects of this cytokine during fungal diseases have also been corroborated in humans. Individuals homozygous for A/A at position -308 in the TNF-α promoter display elevated TNF-α activity and augmented resistance to aspergillosis (Sambatakou et al. 2006). In contrast, patients given TNF-α blockers to treat inflammatory diseases

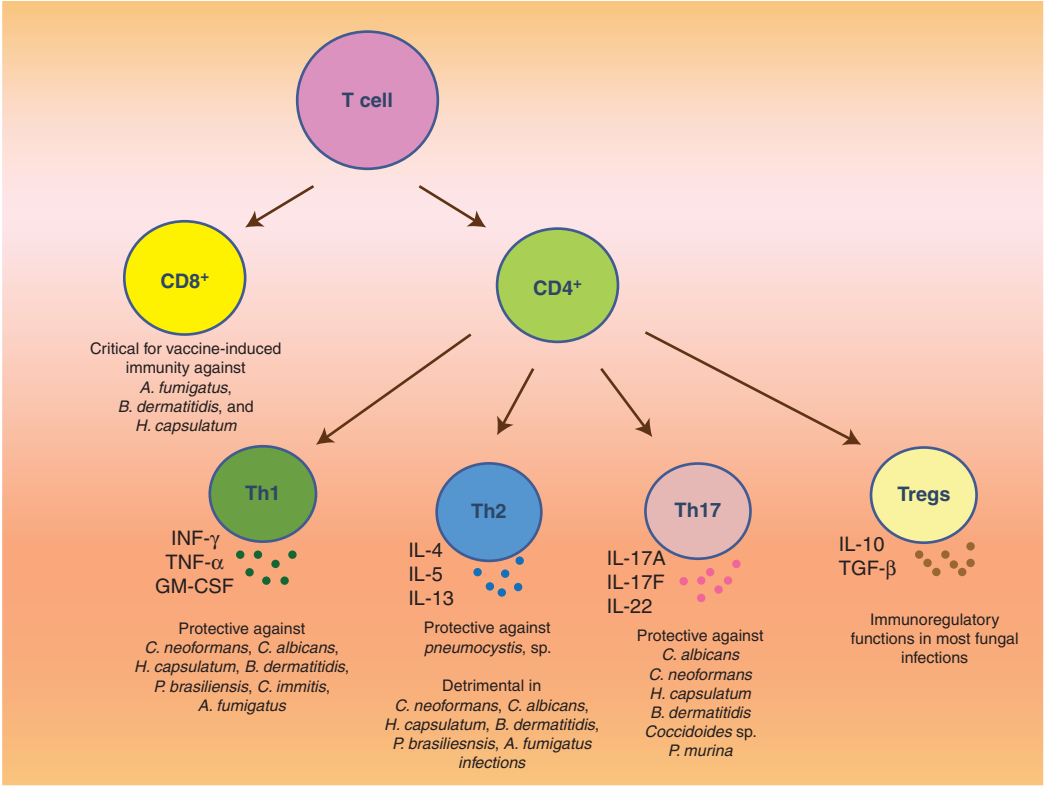

Figure 3. Schematic illustration of different subsets of T lymphocytes and their roles in combating different fungal pathogens. T cells form an integral part of adaptive immunity in vertebrates. They are classified into two distinct lineages: $CD4^+$ (also known as Th cells) and $CD8^+$ cells. The former are further subdivided into Th1, Th2, Th17, and Treg cells based on their effector functions. Th1 cells are the primary source of IFN-γ and are critical for host defense against a majority of fungal pathogens. Similarly, Th17 cells orchestrate potent anti-candidacidal activity by secreting IL-17A, IL-17F, and/or IL-22. In contradistinction, Th2-cell-derived cytokines are associated with exacerbation of most fungal infections, with the exception of pneumocystosis. Treg cells prevent excessive damage to the host during infection through various soluble mediators and contact-dependent mechanisms. $CD8^+$ T cells represent an additional line of defense to combat fungal infections. Two distinct lineages namely Tc1 (IFN-γ-producing $CD8^+$ T cells) and Tc17 (IL-17-producing $CD8^+$ T cells) may exist to bolster Th1 and Th17 immunity, respectively.

suffer from a myriad of fungal infections (Wallis et al. 2004). The importance of GM-CSF in fungal diseases has been elucidated in murine model of pulmonary histoplasmosis. Administration of anti-GM-CSF antibody reduced protective immunity to *H. capsulatum* (Deepe et al. 1999). A recent report has indicated that GM-CSF facilitates its antifungal responses through sequestration of zinc from intracellular yeasts and stimulation of reactive oxygen species in macrophages (Subramanian Vignesh et al. 2013).

Th2 Immunity

For the vast majority of fungal infections, Th2 immunity manifests a detrimental influence on the host. These Th2 responses are comprised of $CD4^+$ T-cell-derived cytokines IL-4, IL-5, and IL-13, and B-cell-secreted IgE. Exaggerated synthesis of these soluble factors in most mycotic diseases interferes with pathogen clearance, and on rare occasions, failure to regulate them can result in a fatal outcome. For example, in mice lacking the chemokine receptor CCR2, infec-

tion with *H. capsulatum* or *C. neoformans* induces a dominant IL-4 response that is associated with impaired host resistance (Traynor et al. 2000; Szymczak and Deepe 2009). In pulmonary aspergillosis and cryptococcosis, undesired activation of Th2 response leads to nonprotective allergic inflammation in mice (Hogaboam et al. 2000; Muller et al. 2013). These animals show signs of eosinophilia, goblet-cell hyperplasia, and heightened susceptibility to the pathogen. In addition to these experimental findings, a single nucleotide polymorphism in the IL-4 promoter region that is linked with amplified IL-4 production occurs with increased frequency in women with recurrent vulvovaginal candidiasis (Babula et al. 2005). This genetic defect is hypothesized to predispose the individuals to *C. albicans* by suppressing the fungicidal activity of macrophages encountering *C. albicans* yeasts (Cenci et al. 1993).

The mechanisms by which Th2 cytokines dampen host immunity are multifactoral. Both IL-4 and IL-13 drive alternative activation of macrophages that is associated with uncontrolled fungal growth. *C. neoformans* and *H. capsulatum* proliferate robustly in macrophages primed with IL-4, as opposed to the ones that are classically activated (Voelz et al. 2009; Winters et al. 2010). These alternatively activated phagocytes display amplified levels of arginase-1, an enzyme that potentially diminishes the amount of nitric oxide required for fungicidal activity (Davis et al. 2013). Additionally, IL-4 modulates fungal access to specific micronutrients in macrophages. This cytokine upregulates the transferrin receptor (Weiss et al. 1997) on the cell surface that results in enhanced iron acquisition. This phenomenon is believed to augment fungal growth within the cells. In addition, IL-4 alters intracellular survival by increasing zinc content that supports the growth of fungal pathogens (Winters et al. 2010).

In sharp contrast to these findings, Th2 responses bestow protection to the host in pneumocystosis. A recent report suggested that alternatively activated macrophages driven by IL-13 show increased fungicidal capacity in *Pneumocystis murina* infection, as opposed to classically activated cells (Nelson et al. 2011). Antibody class switching to a more protective IgG subclass induced by Th2 cytokines also might contribute to the heightened protection against *P. murina* because patients with this defect are vulnerable to *Pneumocystis* pneumonia (Tsai et al. 2012; Al-Saud et al. 2013). Our understanding of why Th2 cytokines are protective in pneumocystis but not other mycotic diseases is incomplete. A probable explanation could be that *Pneumocystis* behaves similarly to helminth parasites by tightly adhering to pulmonary epithelial cells to establish infection (Perez-Nazario et al. 2013). In such a setting, release of Th2 cytokines by effector cells is critical for minimizing the virulence of helminths. Although Th2 immunity significantly contributes to host defense against pneumocystosis, it cannot compensate for the loss of other Th subsets. Patients with autosomal dominant hyper IgE syndrome (HIES) show defects in generation of Th17 responses (but not Th2 responses) and are found to be progressively more susceptible to *Pneumocystis* pneumonia (Grimbacher et al. 2005). Thus, in summary, resolution of pneumocystosis depends on a coordinated action of the Th2 arm of the immune system and other Th subsets.

Th17 Immunity

Th17 cells are a subset of $CD4^+$ T cells that are developmentally distinct from Th1 and Th2 cells and are identified by the expression of cytokines IL-17A, IL-17F, and IL-22. The differentiation of this T-cell lineage requires various cytokines and transcription factors. TGF-β and IL-6 prime the initial differentiation of naïve $CD4^+$ T cells to Th17 cells and IL-23 is critical for maintenance and expansion of these cells (Zuniga et al. 2013). Activation of STAT3 by IL-6 is indispensable for this process because the former directly regulates the transcription of ROR-γt, the master transcription factor controlling Th17 lineage commitment. The influence of Th17 immunity in bolstering host defenses against fungal pathogens has been well substantiated. Indeed, humans with genetic defects in IL-17 signaling axis are severely compromised in their ability to counter mycoses. Individuals with HIES show dominant negative

mutation in STAT3 (Minegishi and Saito 2011). Consequently, these individuals are vulnerable to a spectrum of fungal infections (Grimbacher et al. 2005). Another rare genetic disorder called autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED) is linked with chronic and recurrent mucocutaneous candidiasis (Vautier et al. 2012). This defect is characterized by mutations in the autoimmune regulator (AIRE) gene that result in generation of autoantibodies directed against Th17 cytokines. Furthermore, hereditary mutations in the Dectin-1 signaling that shapes Th17 immunity have been identified in patients with chronic mucocutaneous candidiasis (Ferwerda et al. 2009). The importance of IL-17 responses in antifungal immunity is best studied in diverse models of experimental candidiasis. Mice deficient in IL-17 receptor fail to generate functional Th17 cells and are vulnerable to systemic and oral candidiasis (Conti et al. 2009). The IL-23–IL-17 axis is necessary for development of optimal immunity against *C. albicans*. In models of dermal and oral infection, IL-23-deficient animals develop progressively worse disease (Conti et al. 2009; Kagami et al. 2010). Likewise, in vulvovaginal *C. albicans* infection, impairing Th17 cell differentiation leads to heightened fungal burden at the site of challenge (Pietrella et al. 2011).

Two disparate mechanisms have been described through which Th17 cells manifest antifungal responses. In systemic challenge models, these cells recruit neutrophils by prompting the release of CXC chemokines (Hernandez-Santos and Gaffen 2012). The neutrophils, in turn, show potent anticandidacidal activity and clear the pathogen. In mucosal infection models, IL-17 prompts keratinocytes and epithelial cells to release antimicrobial peptides (AMPs), such as S100A proteins, β-defensins, and histatins that show direct killing activity (Liang et al. 2006; Conti et al. 2011). To overcome the antifungal effects of IL-17 pathway, pathogens have evolved to subvert the activity of this cytokine. Live *Candida* secretes an uncharacterized soluble factor that diminishes the production of IL-17 in human peripheral blood mononuclear cells by inhibiting indoleamine 2,3-dioxygenase (IDO) expression (Cheng et al. 2010). IDO is an enzyme that catalyzes the breakdown of tryptophan, thereby depriving microbes of this essential amino acid (Loughman and Hunstad 2012). Furthermore, IL-17 prompts morphological changes in *C. albicans* and *A. fumigatus*. This cytokine directly binds to the outer surface of these two pathogens and induces transcriptional changes that are associated with augmented hyphal growth and enhanced resistance to host antifungal defenses (Zelante et al. 2012).

Other less-appreciated Th17 cytokines, IL-17F and IL-22, may be involved in anti-*Candida* responses. The latter is fundamental for antifungal resistance in gastric and systemic candidiasis. IL-22 facilitates its protective effects through the elicitation of AMPs and by maintaining the integrity of the mucosal barrier to prevent dissemination of the disease (De Luca et al. 2010; Eyerich et al. 2011). However, contradictory findings have emerged wherein IL-22 is dispensable in murine models of oral and dermal candidiasis (Conti et al. 2009; Kagami et al. 2010). Much less is known about IL-17F and its role in fungal infections. During systemic *Candida* infection, deficiency of this cytokine does not impact host resistance (Saijo et al. 2010). Despite the limited or conflicting literature on IL-17F and IL-22, there is clinical evidence to support their protective effects in mycoses. Self-reacting antibodies against IL-17F and IL-22 are detected in APECED patients, who are vulnerable to mucocutaneous candidiasis (Hernandez-Santos and Gaffen 2012). Moreover, patients with dominant negative mutations in IL-17F are at an increased risk of developing chronic mucocutaneous candidiasis (Puel et al. 2011).

In other experimental models of mycoses, Th17 cells offer protection against several fungal infections, but they are not requisite for host survival. Neutralization of IL-17 during *C. neoformans*, *P. carinii*, or *H. capsulatum* infection results in a delayed clearance of disease but does not impact the lifespan of animals (Rudner et al. 2007; Deepe and Gibbons 2009; Wozniak et al. 2011). In contrast, the Th17 arm is critical for vaccine-induced immunity against the dimorphic fungal pathogens *B. dermatitidis*, *C. posa-*

dasii, and *H. capsulatum* (Wuthrich et al. 2011). In this setting, IL-17-producing cells compensate for the loss of Th1 cells to counter the disease. The proposed mechanism by which these cells orchestrate antifungal activity is through recruitment and activation of neutrophils and macrophages in the lungs.

Contradictory to the above findings, unfavorable aspects of Th17 cells in fungal diseases have been discovered. In mice with gastric candidiasis, IL-23 induces exaggerated activation of Th17 pathway that is linked with deterioration of the disease (Zelante et al. 2007). The mice show heightened tissue damage and defective antifungal activity in neutrophils. Animals deficient in the transmembrane protein Toll IL-1R8 mount amplified Th17 responses and are highly susceptible to candidiasis (Bozza et al. 2008). Parallel findings are reported in murine aspergillosis in which Th17 cells hamper the outcome of infection by impairing the effector functions of neutrophils and exacerbating inflammatory pathology (Zelante et al. 2007). In these scenarios, neutralization of Th17 cytokines enhances resistance to the pathogen. To date, no definite mechanism to clarify the antagonizing function of Th17 cells in different fungal infections has been described.

Regulatory T Cells

Appropriate regulation of proinflammatory immune responses generated against invading infectious agents is necessary to limit collateral damage to the host. Regulatory T cells (Treg cells) contribute significantly to this task. This subset of T cells dampens the immune responses through a multitude of suppressive mechanisms that include secretion of inhibitory cytokines IL-10, TGF-β, or IL-35, repression of IL-2 release, perforin/granzyme-dependent cytolysis of APCs, synthesis of immunosuppressive adenosine, and through contact-dependent downmodulation of APC functions via cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and lymphocyte activation gene 3 (LAG3) (Goodman et al. 2012).

In murine models of fungal infections, accelerated clearance of disease is achieved by altering the Treg cell activity. In candidiasis and paracoccidioidomycosis, signaling through the Toll-like receptor TLR2 and its downstream molecule MyD88 is critical for prolonging survival of Treg cells (Netea et al. 2004a; Loures et al. 2009). $TLR2^{-}/^{-}$ mice express fewer Treg cells under homeostasis and disease state. Moreover, the infected mutant mice show a concomitant increase in Th17 cells. As a consequence, $TLR2^{-}/^{-}$ mice resolve *C. albicans* and *P. brasiliensis* more efficiently than wild-type controls. Similarly, the chemokine receptor CCR5 regulates the equilibrium between Th17 cells and Treg cells. In the absence of this chemokine receptor, mice display reduced influx of the latter to the site of infection and enhanced resistance to *P. brasiliensis* and *H. capsulatum* (Moreira et al. 2008; Kroetz and Deepe 2010).

A common approach to treat inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis, is administration of TNF-α antagonists. However, an undesirable consequence of this therapy is the enhanced frequency and suppressor activity of Treg cells that in turn have been linked with exacerbating fungal diseases (Wallis et al. 2004). These clinical findings are corroborated by experimental evidence. Animals given TNF-α-neutralizing antibody show elevated numbers of $IL\text{-}10^{+}$ Treg cells and are highly susceptible to the disease when infected with *H. capsulatum* (Deepe and Gibbons 2008). Elimination of Treg cells enhances survival of TNF-α-neutralized mice, and, conversely, adoptive transfer of these cells exacerbates infection. Thus, the immunosuppressive functions of Treg cells are deleterious for host immunity in this scenario.

Despite their potent immunosuppressive functions, Treg cells impart a positive impact on host defense by promoting durable antifungal immunity. In murine gastric candidasis, these cells prevent excessive inflammation and allow the fungus to persist in the gastrointestinal tract, thereby generating an effective secondary immune response (Montagnoli et al. 2002). A similar mechanism is in effect in *A. fumigatus* infection wherein Treg cells shape immune responses to be adequate to confer protection against the pathogen, but not cause unwarrant-

ed damage to the host (Montagnoli et al. 2006). In addition to immunosuppressive functions, a remarkable component of Treg cell biology is their ability to promote Th17 cell differentiation and contribute to host defenses against *C. albicans* (Pandiyan et al. 2011). Treg cells manifest such an effect by sequestration of IL-2, a cytokine that inhibits Th17 cell differentiation. As a consequence, the robust Th17 response generated aids in resolution of oral candidiasis. Intriguingly, humans with a genetic defect in genesis of Treg cells are also predisposed to chronic mucocutaneous candidiasis (Kekalainen et al. 2007). Thus, Treg cells represent a double-edged sword; they are crucial for subduing inflammatory responses, but their suppressive functions may be undesirable in certain settings of mycoses.

$CD8^+$ T Cells

$CD8^+$ T cells are vital for protection against viral pathogens and tumors; however, their relative contribution in host immunity against fungal infections is not as comprehensively understood as $CD4^+$ T cells. Multiple experimental models suggest that the former share redundant functions with the latter and confer protection in the setting of $CD4^+$ T-cell deficiency (Wuthrich et al. 2003, 2006; McAllister et al. 2004; Lindell et al. 2005; Marquis et al. 2006; Chiarella et al. 2007; Nanjappa et al. 2012b). In mice deficient in MHC class II, $CD8^+$ T cells suppress *H. capsulatum* infection by targeting macrophages laden with yeasts (Lin et al. 2005a). These cytotoxic $CD8^+$ T cells are primed by DCs through the process of cross-presentation (presentation of exogenous Ags on MHC class I). Furthermore, this lineage of T cells is critical for vaccine-induced immunity against *A. fumigatus*, *B. dermatitidis*, and *H. capsulatum* (Wuthrich et al. 2003, 2006; Hsieh et al. 2011; Carvalho et al. 2012; Nanjappa et al. 2012a). The most likely mechanisms through which memory $CD8^+$ T cells coordinate resolution of pathogen in these models is by the release of IFN-γ and IL-17, and cytotoxic effects on infected cells. Therefore, vaccines that elicit a robust $CD8^+$ T-cell response can potentially be used as an alternative strategy to prevent fatal mycoses in immunodeficient patients.

Humoral Immunity

Although recent advances have been made in our understanding of the contribution of humoral immunity to host defense against fungal infections, contrasting opinions on its involvement still exist. Much of the confusion stems from earlier studies that concluded antibodies were dispensable for resolution of fungal infections (Hurd and Drake 1953; Goren 1967; Diamond et al. 1974; Monga et al. 1979; Carrow et al. 1984). However, the advent of monoclonal antibody technology made it possible to identify the protective effects of immunoglobulins against fungi. Since then, the impact of immunoglobulins and B cells secreting them has been well scrutinized in *C. neoformans* (Aguirre and Johnson 1997; Rivera et al. 2005; Szymczak et al. 2013), *C. albicans* (Wahab et al. 1995; Matthews and Burnie 2001; Saville et al. 2008), *H. capsulatum* (Nosanchuk et al. 2003), and *Pneumocystis* sp. infections (Rapaka et al. 2010) (Fig. 4). The clinical importance of immunoglobulins in mycoses is evident from reports that patients with B-cell defects including X-linked hyperIgM (Winkelstein et al. 2003; de Gorgolas et al. 2005), hypogammaglobulinemia (Gupta et al. 1987; Wahab et al. 1995; Neto et al. 2000), and IgG2 deficiency (Marr et al. 2012) are susceptible to cryptococcosis. Additionally, pneumocystosis was reported in a patient with hypergammaglobulinemia who displayed inability to class switch from IgM to a more specific IgG subclass (Matheson and Green 1987).

Immunoglobulins elicit protective immune responses in the host by predominantly targeting Ags in the fungal cell wall, such as β-glucan (*A. fumigatus*, *C. albicans*, and *C. neoformans*), agglutinin like sequence 3 (*C. albicans*), glucuronoxylomannan (*C. neoformans*), and heat shock protein 60 (*H. capsulatum*) (Casadevall and Pirofski 2012). The mechanisms by which these antibodies mediate protection in the host are broadly classified into direct and indirect mechanisms. By definition, direct mechanisms

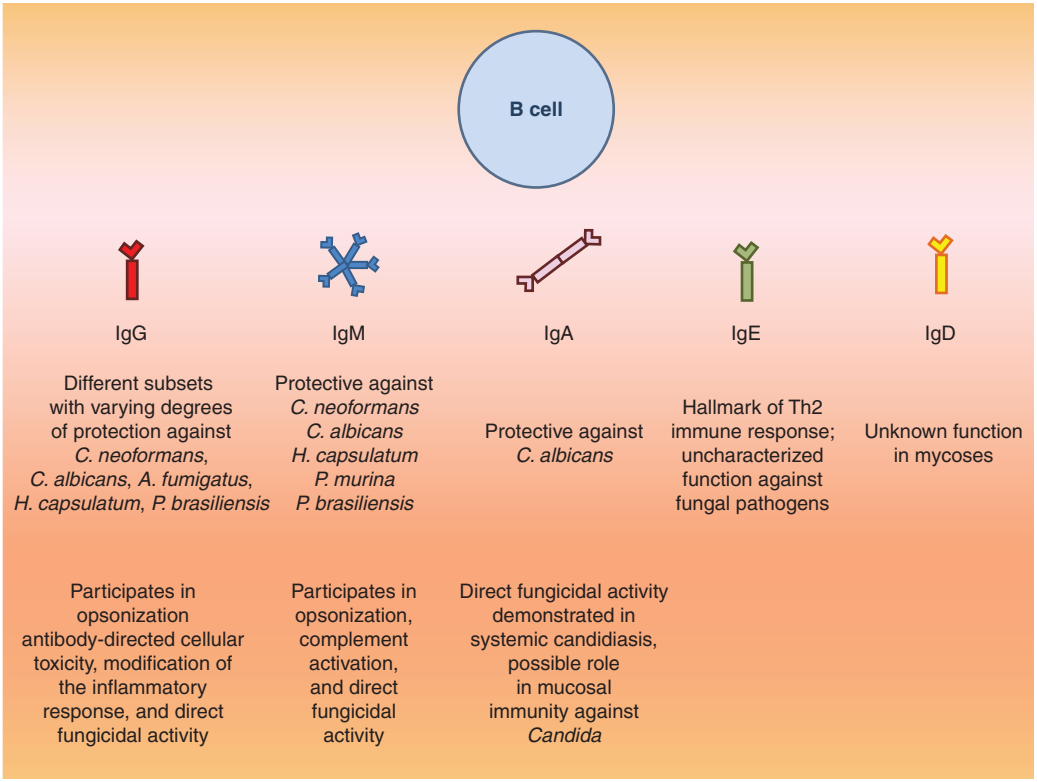

Figure 4. Functions of different subclasses of immunoglobulins in mycoses. On encountering fungal pathogens, B cells undergo clonal expansion and antibody class switching. Depicted below are the five major classes of immunoglobulins secreted by B cells, namely, IgG, IgM, IgA, IgE, and IgD. Whereas specific roles of the former three have been reported in fungal diseases, the impact of IgE in this setting remains unclear. IgD has the ability to bind to outer surfaces of certain bacterial pathogens in the respiratory mucosa (Chen et al. 2009); however, its importance in fungal infections has not been studied.

are those that result in inhibition of growth or microbicidal activity when immunoglobulins bind to the pathogen. The fungicidal activity of certain monoclonal antibodies is well illustrated against *C. albicans*, *C. neoformans*, and *A. fumigatus* (Torosantucci et al. 2005, 2009; Rachini et al. 2007). Additionally, antibody binding to the outer fungal surface prompts alteration in gene expression and fungal metabolism that ultimately suppress virulence of the pathogen (McClelland et al. 2010; Brena et al. 2011).

Indirect mechanisms comprise immunoglobulin-mediated resolution of infection by enhancing the microbicidal potential of effector cells. Opsonization, activation of complement pathway, and antibody-directed cell toxicity (ADCC) are associated with indirect effects of antibodies during infection and form integral components of host defense against fungal pathogens, such as *C. albicans*, *C. neoformans*, and *H. capsulatum* (Nabavi and Murphy 1986; Han et al. 2001; Shi et al. 2008). ADCC induces lysis of cells decorated with antibodies, whereas the former two actions trigger innate defense mechanisms including phagocytosis. In murine cryptococcosis, administration of an anticapsular IgG1 monoclonal antibody is associated with heightened production of IL-10 in the lungs and diminished levels of IFN-γ (Feldmesser et al. 2002). In this setting, dampening of the proinflammatory response is speculated to contribute to accelerated resolution of infection and improved survival in mice. Our knowledge of why the IgG1 monoclonal antibody elicits an

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anti-inflammatory response is incomplete, but a probable mechanism could be the suppression of the complement pathway facilitated by highly galactosylated antibodies (Karsten al. 2012). N-glycan galactosylation of IgG1 engages the inhibitory IgG receptor FcγRIIB and Dectin-1, which in turn suppress C5a receptor functions. In sharp contrast, administration of monoclonal antibody to *H. capsulatum* histone-like protein, H2B improves the outcome of infection by inducing Th1 cytokines and by enhancing the fungicidal activity of macrophages (Nosanchuk et al. 2003). Thus, antibodies shape the inflammatory response in fungal infections.

Fungal infections represent a serious threat in immunocompromised patients, such as those suffering from AIDS (Brown et al. 2012). The majority of these affected individuals have severe defects in cell-mediated immunity. Thus, there is an urgent requirement for immunotherapy that bypasses the need for $CD4^+$ T cells and combats fungal infections. One such strategy is DNA vaccination with *Pneumocystis* Ag, kexin linked to CD40 ligand (Zheng et al. 2005). This vaccination approach induces a robust antibody response in mice and confers protection during *Pneumocystis* pneumonia. Other fungal Ags that elicit a vigorous humoral response in experimental models have been reported (Devi 1996; Han et al. 1999; Fleuridor et al. 2001; Torosantucci et al. 2005; Sandini et al. 2011; Xin and Cutler 2011). Thus, immunoglobulin therapy represents a promising approach that could be used to treat mycoses in individuals with immunosuppression. However, an important consideration while administering these monoclonal antibodies to the host must be their dose. At a very high concentration, certain immunoglobulins show prozone-like effects and can be nonprotective or even deleterious during the course of the disease (Taborda and Casadevall 2001; Taborda et al. 2003).

CONCLUDING REMARKS

We have highlighted the major advances in knowledge concerning the development, maintenance, and function of the adaptive immune response to medically important fungi. We have tried to emphasize common pathways, mechanisms, and themes. The benefits of unearthing the pathways leading to adaptive immunity and the functions of this response are that (1) vaccines may be created that prevent or treat fungal diseases; (2) predictions may be made as to who will be at risk for serious fungal infections; and (3) biological agents may be developed that bolster immunity in the face of severe and life-threatening infections that are being diagnosed worldwide in increasingly large numbers of patients.

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