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Immune Responses in Fungi Infections

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Summary

Fungi are cosmopolitan and the infections occur in more than a quarter of the world's population with a very low inherent virulence. The increased incidence of these infections and the diversity of fungi causing them, has paralleled the emergence of growing population of immunosuppressed patients. **Surveillance and elimination of fungal pathogens relies on the sentinel behavior of several cells of the innate immune system and the recruitment of effector cells. Defects in antifungal effector functions of these cells result in susceptibility to many fungal pathogens.** The decision of how to respond will still be primarily determined by interactions between pathogens and cells of the innate immune system, but the actions of T cells will feed back into this dynamic equilibrium.

Keywords: Immune Responses, Fungi, Infections

Introduction

1.1 Background

Fungi are saprophytic and parasitic nucleated microorganisms that has no chlorophyll and exist in dimorphic forms; yeasts and molds (Tobias *et al.*, 2006). Yeasts (e.g. *Cryptococcus neoformans*) grow as unicellular round- or oval-shaped organisms, whereas molds (e.g. *Aspergillus fumigatus*) form long tubular structures termed hyphae that extend into a branch-like network known as a mycelium. Dimorphic fungi (e.g. *Candida albicans*) assume both yeast and hyphal states of growth based on environmental conditions, including interactions with the mammalian immune system (Tobias *et*

al., 2006). Fungi are cosmopolitan and the infections occur in more than a quarter of the world's population with a very low inherent virulence (Armstrong-James *et al.*, 2017). The increased incidence of these infections and the diversity of fungi causing them, has paralleled the emergence of growing population of immunosuppressed or immunocompromised patients whose mechanisms of host defense have been impaired by primary disease states (e.g., AIDS, cancer), a mobile and aging population with an increased prevalence of chronic medical conditions, and the use of new and aggressive medical and surgical therapeutic strategies, including broad-spectrum antibiotics, cytotoxic chemotherapies, and organ transplantation (Armstrong-James *et al.*, 2017; Tobias *et al.*, 2006).

Healthcare associated mycoses are almost always opportunistic and the community acquired fungal infections comprise of opportunistic and endemic mycoses which vulnerability depend on exposure to a pathogenic fungus in the natural environment and predisposing risk factors (Lockhart *et al.*, 2009).

The first protective mechanism that appears in fungi infection is innate and constitute physical barriers supplemented by circulating and tissue-associated cells, and humoral factors (Quintin, 2018). It separates the organism from the environment: i.e. skin and the mucous membranes of the respiratory, gastrointestinal and genito-urinary tracts. The skin and mucous membranes are physical barriers, and they have

antimicrobial substances on their surface, some of them synthesized by the epithelial and endothelial cells. Also, they have a commensal microflora of saprophytic microorganisms that impede colonization by pathogenic microorganisms (Romani, 2004). Once the fungi have passed the physical barriers, they are met with a series of innate mechanisms of defense, including cellular membranes, cellular receptors and several humoral factors. In the tissues, phagocytes, consisting of neutrophils, mononuclear leukocytes (monocytes and macrophages) and dendritic cells, have an essential role, and natural killer cells, gdT-cells and non-haematopoietic cells, such as epithelial and endothelial cells are involved in the host defence. The host defence mechanism can adapt to different kinds of fungal infection; For example, macrophages are the primary cells involved in fungal killing during infection by *Cryptococcus* and *Pneumocystis*, whereas neutrophils are the primary effector cells in preventing infection by *Candida albicans* and *Aspergillus fumigatus* (Blanco and Garcia, 2008).

Fungi are recognized by cells of the innate immune system (neutrophils, monocytes, macrophages, dendritic cells and Natural Killer cells) (Quintin, 2018; Khanna *et al.*, 2016), which bind components of fungal cell walls using pattern recognition receptors (PRRs) on their surface. C-type lectin receptors (CLRs, e.g. Dectin-1) are particularly important PRRs in anti-fungal immunity, although several other PRRs are also involved including the mannose receptors and Toll-like receptors (TLRs, e.g. TLR2). A dendritic cell decorated with anti-fungal PRRs. Upon recognition of fungi through these receptors, signaling results in generation of Th1/17 adaptive immune responses and activation of innate effector cells (Quintin, 2018).

2.0 Innate immune responses to fungal infection

Innate immune responses restrict fungal growth and invasion in mammalian hosts. The first step in this process involves fungal recognition by germline-encoded receptors in innate immune effector cells (Mayer, 2013). Fungal polymers

that trigger inflammatory responses have been identified; these include β -1, 3/ β -1, 6 glucans, glucuronoxylomannan, phospholipomannan and galactomannan. The role of cognate mammalian receptors in antifungal defense is discussed with emphasis on receptors with known signaling properties and in vivo studies that approximate the natural route of pathogen acquisition. Ex vivo assays that measure fungal cell phagocytosis suggest that a wide range of recognition receptors on different cell types mediate fungal cell uptake (Tobias *et al.*, 2006).

2.1 Soluble Receptors

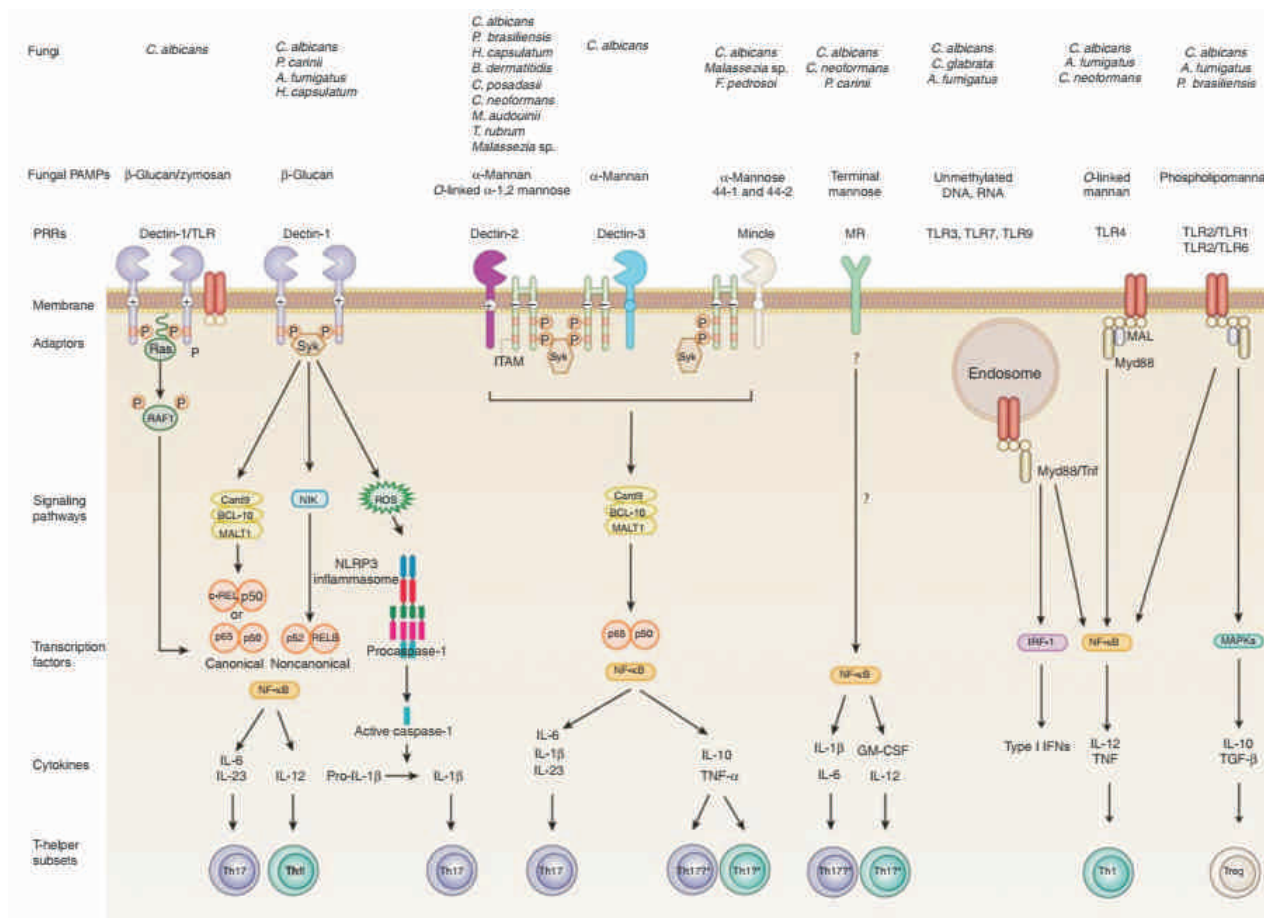
Mice deficient in soluble pentraxin-3 (PTX3)—an opsonic receptor that binds to conidia, but not hyphae, by way of a galactomannan-sensitive interaction — are highly vulnerable to pulmonary *A. fumigatus* infection. PTX3 alveolar macrophages display a major deficit in conidial internalization and killing. PTX3 binds to yeast cells of *Paracoccidioides brasiliensis*, but not to *C. albicans*, which suggests that PTX3 recognizes specific fungal species and morphologic forms. Mice deficient in mannose-binding lectin-A—a serum opsonin for mannan-containing organisms — display normal resistance to *C. albicans* infection. In *A. fumigatus* induced asthma, however, mannose-binding lectin-A appears to enhance pro-asthmatic T helper (Th)2 responses, which suggests that this opsonin enhances fungus-specific T cell responses (Tobias *et al.*, 2006).

2.2 PRRs in Fungal Recognition

The presence of fungal infection is detected by germline-encoded innate receptors that recognize conserved structures present on the surface of fungi (Rivera, 2014). Sensing of fungal-associated molecular patterns such as cell wall constituents (i.e., carbohydrate polymers and glycoproteins) and intracellular components (i.e., DNA) relies on the expression of a plethora of PRRs in immune and nonimmune cells. These innate receptors or pattern-recognition receptors are expressed by innate cells and mediate the activation of multiple signaling cascades that facilitate the production of factors directly involved in fungal containment by innate cells as well as influence the development of adaptive immunity (Rivera, 2014).

After recognition, PRRs can mediate various cellular functions, from fungal uptake and killing to the production of cytokines and chemokines, which could influence the resultant immune responses. Two main families of innate receptors have been clearly shown to be involved in fungal recognition, toll-like receptors (TLR) and C-type lectins receptor (CLR). TLRs 1–4, 6, 7 and 9 have been found to recognize a variety of fungal pathogens. The discovery of Toll-like receptors (TLR) as key receptors in immunity, followed by

the identification of dectin-1 as a β -glucan receptor, paved the way to the discovery of new receptors involved in fungal recognition and their subsequent cellular responses (Salazar and Brown, 2018). Other members of PRRs several families classified based on their structure and function, include nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs), and retinoic acid inducible gene (RIG)-like receptors (RLRs) (Salazar and Brown, 2018).



(Verma *et al.*, 2014)

Figure 1. PRR and signaling pathways that lead to differentiation of antifungal T-helper cells.

2.3 Toll-like receptors and the Myeloid Differentiation 88 (MyD88) adaptor protein

During fungal infection the generation of inflammatory responses, fungal killing and host survival depend on multiple Toll-like receptor (TLR-) and non-TLR mediated pathways. Purified fungal cell wall constituents can activate more than one TLR-dependent signaling pathway; for example, in vitro macrophage tumor necrosis factor (TNF) production in response to *C. albicans* phospholipomannan is

TLR2-, TLR4- and TLR6-dependent. Macrophage TNF and interferon (IFN)- γ secretion triggered by *C. albicans* yeast cells depends on TLR2 and TLR4, and hyphal cells trigger only TLR2-dependent responses. In systemic candidiasis, in vivo study of TLR2 in mice were reported sensitive in primary infection than control mice, resistant and indifference, regardless if animals were inoculated with yeast or hyphal forms. In *C. neoformans*, the major capsular component

glucuronoxylomannan activates TLR4-dependent NF κ B translocation in vitro, but TLR2-deficient animals die more rapidly than control mice after in vivo intranasal infection. TLR2 and TLR4 are activated by *C. neoformans*, *C. albicans* and *A. fumigatus* ligands in vitro, it is likely that deficiency of one of these receptors can be partially or fully compensated in animal models of infection owing to the redundancy in signaling mechanisms induced by live fungal cells (Hohl *et al.*, 2006).

MyD88 is an intracellular adaptor protein that transmits signals from TLRs (Hohl *et al.*, 2006), and MyD88-deficient animals are more susceptible to infection with *C. neoformans* and *C. albicans* as well as show delayed clearance of *A. fumigatus* spores at early times after infection (Bretz *et al.*, 2008; Biondo *et al.*, 2005). Fungal infections are not reported to be a significant problem for patients with defects in MyD88, thus suggesting that TLR/MyD88 are not essential for antifungal defence in paediatric patients. The enhanced susceptibility of patients with TLR4 polymorphisms to invasive aspergillosis during stem cell transplantation and to candidemia in individuals with TLR1 polymorphism suggests that although not essential, TLR-dependent signals do contribute to effective protection against fungal infection (Rivera, 2014).

2.4 C-Type Lectin Receptors (CLRs)

CLRs comprise a superfamily of proteins that can recognize diverse self and non-self-molecules, and thus CLRs can regulate a wide range of physiological and pathological processes. They are constituted of soluble and membrane-bound proteins characterized by the presence of at least 1 C-type lectin domain (CTLD), some of which act as carbohydrate recognition domains (CRD). Conserved residues in the CRD allow recognition of diverse sugar structures, including the EPN (Glu-Pro-Asn) and QPD (Gln-Pro-Asp) motifs which confer specificity for mannose and galactose, respectively. However, other CTLDs lack these components and can recognize a broader range of ligands, including protein and lipids. Structurally, most membrane-bound CLRs consist of a cytoplasmic tail, a transmembrane region, and at least 1 CTLD in the extracellular

domain. Others have multiple CTLDs, such as the mannose receptor (MR), which has 8 extracellular CTLDs (Salazar and Brown, 2018).

2.4.1 Dectin-1

Fungal cell walls are rich in β -glucans and mannans. Dectin-1 (the best-characterized receptor involved in antifungal immunity) is expressed on nearly all phagocyte populations, human B cells, human mast cells, keratinocytes, epithelial cells, and subsets of T cells also express this receptor (Salazar and Brown, 2018) and mediates uptake of fungi with exposed β -glucans. However, for some fungi, the β -glucans are mostly in the inner cell wall, masked by mannans, α -glucans or capsule, and may not be available to interact with dectin-2. The cytoplasmic tail of dectin-1 contains an immunoreceptor tyrosine activation motif which is necessary for its signaling properties (Brown, 2006).

Dectin-1, a natural killer-like C-type lectin receptor, is highly expressed on myeloid cell subsets at the alveolar/airway interface and in the gastrointestinal tract — two portals of fungal invasion. Dectin-1 binds and internalizes fungal cells and signals through cytosolic immunoreceptor tyrosine-based activation motif-like domain, to induce the production of reactive oxygen species, and inflammatory cytokines and chemokines (Hohl *et al.*, 2006).

Recently, the CLR dectin-1, has been demonstrated to mediate specific recognition of β -glucans found in the cell walls of *Candida* and *Aspergillus* spp. Dectin-1 and TLR2 may collaborate to augment proinflammatory cytokine production. Phagocytosis of (ballistoconidia) can also be mediated by either dectin-1 or TLR2. Though dectin-1-deficient mice have shown increased susceptibility to disseminated candidemia. In vitro, the inert β -glucan-rich particle zymosan derived from *Saccharomyces cerevisiae* activates at least two distinct dectin-1-dependent signaling cascades in dendritic cells. This results in the production of interleukin (IL)-2, IL-10 and reactive oxygen species in a spleen tyrosine kinase (Syk)-dependent manner, and in the generation of TNF and IL-12 in a Syk-independent fashion. *C.*

albicans triggers dectin-1-dependent signals that induce cyclooxygenase-2 expression and arachidonic acid release for eicosanoid production (Tobias *et al.*, 2006). Yeasts and molds only express β -glucan on their surface when they are in specific morphologic states (Hohl *et al.*, 2006).

2.4.2 The Dectin-2 Cluster

The genes encoding these receptors are located in a cluster, near dectin-1, on chromosome 6 in mice and chromosome 12 in humans, and members of this family include clec6a (dectin-2), clec4d (MCL), clec4e (macrophage-inducible C-type lectin or Mincle) (Lionakis *et al.*, 2017), DCIR, DCAR, and BDCA-2. Apart from DCIR, all of the other receptors have short cytoplasmic tails that lack signaling motifs and associate with the FcR-g chain, an adaptor containing an ITAM motif (Graham and Brown, 2009). Dectin-2 expression appears to be primarily restricted to myeloid cells, including monocytes, macrophages, neutrophils, and DCs (Lionakis *et al.*, 2017). Dectin-2 – the typical member of this family – contains 1 CRD in the extracellular domain that can recognize α -mannans and O-linked mannoproteins from several fungi, including *Candida*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*, in a Ca²⁺-dependent manner. This interaction can modulate cytokine and ROS production, as well as fungal killing (Becker *et al.*, 2015). Dectin-2 recognizes *C. albicans*, *S. cerevisiae*, *Microsporium audouinii*, *Trichophyton rubrum*, *A. fumigatus*, *H. capsulatum*, *P. brasiliensis*, *Malassezia* sp., *B. dermatitidis*, and *C. posadasii*. It recognizes *C. albicans* and *Malassezia* via N- and O-linked α -mannan, respectively, on their surface. 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 (Verma *et al.*, 2014). Dectin-2-deficient mice are more susceptible to primary *C. albicans* infection (Saijo *et al.*, 2010; Robinson *et al.*, 2009).

In vivo, dectin-2 has been shown to exert a protective role against *C. albicans*, *C. glabrata*, and *T. rubrum* infections and, together with MCL, they have been suggested to promote Th17

responses to *Fonsecaea pedrosoi* and *Blastomyces dermatitidis*, respectively, while Mincle can antagonize these responses. Mice deficient in MCL show impaired vaccine resistance against *B. dermatitidis* infections, and this lack of resistance was associated with a reduced recruitment of Th17 cells to the lung upon recall following experimental challenge (Salazar and Brown, 2018).

Mincle is another FcR-g-coupled activating receptor that recognizes pathogenic fungi and Mycobacteria. Mincle binds glycolipids, such as trehalose-6, 6-dimycolate (TDM) from Mycobacterium tuberculosis, and novel glyceroglycolipids from *Malassezia*. Mincle-deficient mice are more susceptible to primary infection with *C. albicans* and *Malassezia* (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-g-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. 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 (Salazar and Brown, 2018).

2.5 Mannose receptor and DC-SIGN

Mannans or mannoproteins are surface exposed on most fungal cell walls, and for some fungi are secreted. Mannosylation of proteins can occur via either N-linkages or O-linkages. Exposed mannose groups are recognized by mannose receptors present on a wide variety of cell types, particularly dendritic cells (DC) and macrophages. As with dectin-1, mannose

receptors are competent to mediate both phagocytosis and signaling responses. The major mannose receptors are the macrophage mannose receptor and DC-SIGN (Harrison and Levitz, 2009).

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- κ B activation and the production of IL-12, GM-CSF, IL-8, IL-1b, and IL-6 (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). Mice that lack the mannose receptor mount normal immune responses against *C. albicans* and are not more susceptible to systemic infection than wild-type mice. However, MR has been reported to induce Th17 cell differentiation of human T cells in response to *C. albicans*, memory and not naive T cells were the major source of the IL-17 produced. 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 naive cells into Th17 cells, whereas the MR might play a more prominent role in triggering IL-17 production by memory cells (Verma *et al.*, 2014).

Human dendritic cells and alveolar macrophages express dendritic cell specific intercellular adhesion molecule grabbing nonintegrin (DC-SIGN) — a receptor that mediates binding and internalization of *A. fumigatus* conidia by way of galactomannan as well as of *C. albicans* yeast cells. Antibody-mediated ligation of DC-SIGN activates intracellular signaling cascades; however, it remains unclear whether fungal ligands activate DC-SIGN-driven signaling cascades and thereby influence DC maturation and elaboration of inflammatory mediators (Tobias *et al.* 2006).

2.6 Cell-mediated killing of fungi

Tissue macrophages and dendritic cells are some of the first cells to encounter fungi and play pivotal roles in restriction of fungal growth and

dissemination. In the case of *A. fumigatus* and *P. carinii* from sp. *muris*, alveolar macrophages ingest conidia or cysts into phagolysosomes and kill fungal cells through phagocyte oxidase-dependent, inducible nitric oxide synthase-independent, killing mechanisms. MyD88 is required for the killing of *C. albicans* yeast cells, but not of *A. fumigatus* conidia, whereas dectin-1 is essential for the killing of *P. Carinii* from sp. *Muris* cysts. Neutrophils are rapidly recruited to sites of infection; these cells express TLRs and dectin-1, and inactivate fungal spores and hyphae. It was demonstrated that differences in the ability of rat and murine alveolar macrophages to kill *C. neoformans* accounts for the relative sensitivity of mice and resistance of rats to cryptococcosis. Depletion of rat alveolar macrophages enhanced fungal growth in vivo, whereas depletion of murine alveolar macrophages, remarkably, enhanced resistance to infection. Thus, the inferior killing by murine alveolar macrophages resulted in fungal opsonization persistence, a process that could be reversed by depleting these cells from mice. In contrast to other fungal species, phagocytic uptake of *C. neoformans* in humans and mice requires antibody (Hohl *et al.*, 2006).

1.0 Adaptive Immune Responses to Fungal Infections

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 antigen to naive 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 antigen presentation via MHCI. For antigens that enter through the exogenous pathway, engagement of CD8⁺ proceeds through a mechanism termed cross-presentation in which antigens are shuttled into the class I MHC pathway. In contrast to the requirement of antigen processing for activation of T cells, B cells directly react to fungal antigens and secrete immunoglobulins that may influence the outcome of infection (Verma *et al.*, 2014).

The adaptive immune responses to fungal

infection involve the cell mediated and humoral responses. The cell mediated responses include effector cells and the humoral effector molecules include antibodies which play a role in combating fungal infections (Venkateswaran, 2011).

3.1 Antibodies (Effector Molecules)

B cells mediate humoral responses to fungal infections by producing fungus-specific antibodies. Antibodies are produced in response to fungal infections. These antibodies are chiefly directed towards a variety of fungal polysaccharide and protein antigens (heat shock protein 90 (Hsp90) and mannoprotein 65 (MP65) in *C. albicans*). The role of antibodies in host defence against fungi has been extensively studied in *C. neoformans* and *C. albicans*, and protective antibodies have been detected in the serum of experimentally infected animals. They eliminate and inhibit adherence of fungi (direct microbicidal or microbistatic action of antibodies on attachment to microbial surfaces), reduce biofilm formation, and promote fungal ingestion and killing by phagocytes (Venkateswaran, 2011).

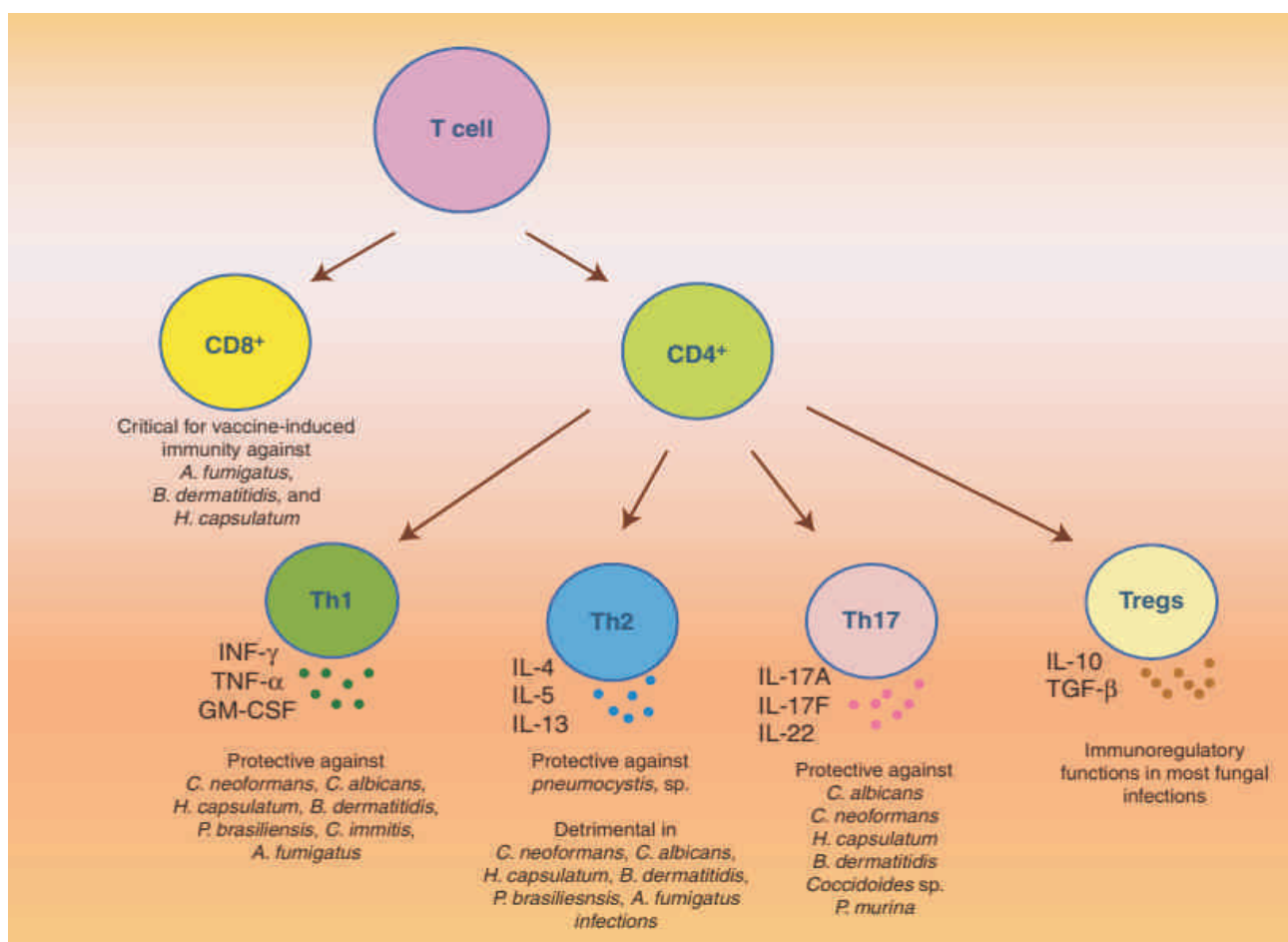
Antibodies are involved in activation of cellular responses; transforming cytokine expression via Fc receptor activation. The existence of protective and harmful antibodies to fungal pathogens suggests that the efficacy of the protective antibody response depends on the composition of the antibodies with respect to their isotype and epitope specificity and titre. Venkateswaran (2011) found that during *C. neoformans* infection in mice, polysaccharide-binding antibodies co-operate with cellular

immune mechanisms in the lung to produce enhanced granulomatous inflammation which helps in containment of infection. TH1 derived antibodies are usually of immunoglobulin IgG2 isotype, they act as excellent opsonins and promote fungal engulfment and killing. TH2 derived antibodies are of IgG1 isotype, these antibodies are associated with the induction of a non-protective immune response that causes suppression of the protective TH1 mediated immunity.

3.2 Cell Mediated Immunity

The clinical circumstances in which fungal infections occur are associated with impaired cell-mediated immunity. AIDS and severe haematological malignancies are examples of acquired defects in T-cell function that predispose individuals to fungal infections. T cells are the main effector cells which mediate cellular immune responses.

In case of fungal infections T cell activation is crucial for the development of optimal protective immunity. Both Cd4+ and CD8+ T cells are required for clearance of fungal pathogens. CD4+ T cells produce essential lymphokines that activate and recruit phagocytic cells to sites of fungal infection. CD8+ T cells lyse infected cells and limit tissue damage by reducing inflammatory responses. But, in case of several fungal pathogens the presence of CD4+ T cells are essential for host survival, during the primary stages of the disease, and CD8+ T cells are necessary to restrict infection. Therefore, host resistance to fungi depends on the induction of T cell mediated cellular immunity, cytokines and effector phagocytes (Venkateswaran, 2011).



(Verma *et al.*, 2014)

Figure 2. Schematic illustration of different subsets of T lymphocytes and their roles in combating different fungal pathogens.

3.3 Th1 Cells

CD4+ T cells (T helper cells) are classified as TH1 (producing IL 2, TNF, IFN gamma) these recruit and activate phagocytic cells for antimicrobial action. 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. A dominant TH1 response is necessary for the elimination of fungal pathogens. The absence of the TH1 signature cytokines like IFN and TNF leads to overwhelming occurrence of disease. Following exposure, APCs produce IL-12 that is critical for Th1 lineage commitment (Venkateswaran, 2011). 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. Patients receiving adjunctive IFN-g

immunotherapy display augmented protection against aspergillosis, cryptococcosis, and coccidioidomycosis. However, increased susceptibility to histoplasmosis occurred in mice lacking IFN-g and in a patient with a genetic deletion mutation in IFN-greceptor-1 gene (Verma *et al.*, 2014). Th1 cells orchestrate antifungal immune responses through the release of proinflammatory cytokines IFN-g, TNF-a, and GM-CSF. The signature Th1 cytokine, IFN-g, 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*. 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).

3.4 Th2 Cells

Th2 responses are comprised of CD4⁺ T-cell-derived cytokines IL-4, IL-5, and IL-13, and B-cell-secreted IgE. They trigger the differentiation of B cells and antibody production. The mechanism by which antigen-presenting cells prime CD4⁺ T cells and generate either TH1 or TH2-biased responses in response to fungal challenge remains unknown, but it is probably related to the induction of an inflammatory response upon initial exposure. Increased production of TH2 cytokines leads to the development of the disease. For the vast majority of fungal infections, Th2 immunity manifests a detrimental influence on the host. 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, infection with *H. capsulatum* or *C. neoformans* induces a dominant IL-4 response that is associated with impaired host resistance (Szymczak and Deepe, 2009). In pulmonary aspergillosis and cryptococcosis, undesired activation of Th2 response leads to non-protective allergic inflammation in mice (Muller *et al.*, 2013). These animals show signs of eosinophilia, goblet-cell hyperplasia, and heightened susceptibility to the pathogen. The mechanisms by which Th2 cytokines dampen host immunity are multifactorial. 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 (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 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 contrast, Th2 cytokines are protective in pneumocystis but not other mycotic diseases are incomplete. Nelson *et al.* (2011), reported that alternatively activated macrophages driven by IL-13 show increased fungicidal capacity in *Pneumocystis murina* infection, as opposed to classically activated cells (Venkateswaran, 2011).

3.5 Th17 Cells

TH17 cells have a role to play in immune responses to fungi and induce through TLR- and non-TLR-dependent signaling. TH17 cells are a subset of CD4⁺ T helper cells and are distinct from TH1 and TH2 cells. CD4⁺ T cells in presence of TGF- β and IL-6 express the transcription factor ROR γ c (retinoid-related orphan receptor gamma c) and become TH17 cells. TH17 cells on activation secrete IL-17 and synthesize a plasma membrane receptor- IL-23R. The binding of IL-23 produced by DCs to the IL-23R leads to the proliferation of the TH17 cells (Zuniga *et al.*, 2013). The importance of IL-17 responses in antifungal immunity is best studied in diverse models of experimental candidiasis. Conti *et al.* (2009) reported that mice deficient in IL-17 receptor fail to generate functional Th17 cells and are vulnerable to systemic and oral candidiasis. 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). Likewise, in vulvovaginal *C. albicans* infection, impairing Th17 cell differentiation leads to heightened fungal burden at the site of challenge (Verma *et al.*, 2014). The antifungal effector activities of neutrophils are impaired due to the action of IL-23 and IL-17 even in the presence of IFN- γ . IL-12 and IL-23 cytokines cross-regulate each other and therefore, the relative levels of these cytokines may determine the outcome of infection. These cytokines lead to the activation of inflammatory program of neutrophils by opposing the IFN- γ -dependent activation of indoleamine 2, 3-dioxygenase (IDO) (Cheng *et al.*, 2010) and also induce the release of metalloproteinases (MPs) and oxidants, causing tissue damage (inflammatory pathology) associated with TH17 cell activation. The role of IDO and kynurenines is to limit the inflammatory status of neutrophils

against fungi by maintaining a balance, because though some degree of inflammation is required for protection, mostly in mucosal tissues, excessive inflammation worsens the disease and prevents pathogen clearance (Venkateswaran, 2011; Romani, 2008).

TH17 cells, activated by several fungal components were found to play an inflammatory role previously attributed to uncontrolled TH1 responses. TH17 cell activation, and not failure to activate these cells, was associated with defective clearance of fungal in experimental settings of aspergillosis and mucosal candidiasis. Experimental observation state that IL-17 neutralization increased fungal clearance, prevented inflammatory pathology and restored protective TH1 antifungal resistance. 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. The role of IL-17-producing T cells in protection versus pathology in fungal infections is still controversial. Studies have shown that TH17 are also involved in control of fungal infections, it is known that IL-17A and IL-17F are involved in neutrophil recruitment and granulopoiesis. It was observed that defective neutrophil recruitment was associated with the susceptibility to disseminated candidiasis in IL-17R^{-/-} mice. Therefore, TH17 cells were considered to be protective against candidiasis. Also, IL-23 which helps in differentiation of TH17 cells is known to be essential for IL-22 production. IL-22 which is expressed by TH17 cells is involved in the control of fungal growth at mucosal and non-mucosal sites in both candidiasis and aspergillosis (Verma *et al.*, 2014).

3.6 T regulatory cells (Tregs)

T regulatory cells (sometimes known as suppressor T cells) are a specialized subpopulation of T cells; they suppress activation of the immune system and maintain immune system homeostasis. CD4⁺ T cells activated in the presence of TGF- β and IL-2 upregulate expression of the transcription factor Foxp3 (forkhead box P3) and develop into inducible Tregs, natural Tregs exist in the thymus. Tregs suppress the immune responses. A reciprocal relationship exists between the development of Tregs and TH17 cells, so that naive T cell activation in the presence of innate stimuli divert iTreg generation to TH17 generation. They are thought to have a role in minimizing damage caused by immune activation, by functional antagonism of TH17. In mice with candidiasis, CD4⁺CD25⁺ Treg cells, producing IL-10 and TGF, prevent complete elimination of the fungus from the gastrointestinal tract; allow fungal persistence and development of memory immunity (Venkateswaran, 2011; Romani, 2008).

Conclusion

Fungal diseases cause life-threatening infections in the context of primary and acquired immunodeficiencies all over the world. Innate immunity is essential for both the development and modulation of adaptive immunity. Pathogens are recognized by innate immune-associated receptors, namely TLRs, NLRs, RIG-like receptors and CLRs. The initial recognition of pathogens by these receptors induces inflammatory reactions at the infected site, and also triggers adaptive immunity against the pathogens. The innate and adaptive arms of the immune system respond differently to specific morphologic states of the fungal pathogen, by distinct inflammatory and CD4⁺T cell responses to fungi infections. Differences in innate and adaptive immune responses to different morphologic states reflect, in part, differences in fungal cell wall composition, and might represent a host adaptation tailored to the severity of an invasive threat and to the risk of excessive tissue damage caused by inflammation.

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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