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Fungal immunology in clinical practice: Magical realism or practical reality?

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Abstract

Invasive fungal infections (IFIs) occur predominantly in immunocompromised individuals but can also be seen in previously well persons. The human innate immune system recognizes key components of the fungal cell wall as foreign resulting in a myriad of signaling cascades. This triggers release of antifungal molecules as well as adaptive immune responses, which kill or at least contain the invading fungi. However, these defences may fail in hosts with primary or secondary immunodeficiencies resulting in IFIs. Knowledge of a patient's immune status enables the clinician to predict the fungal infections most likely to occur. Moreover, the occurrence of an opportunistic mycosis in a patient without known immunocompromise usually should prompt a search for an occult immune defect. A rapidly expanding number of primary and secondary immunodeficiencies associated with mycoses has been identified. An investigative approach to determining the nature of these immunodeficiencies is suggested to help guide clinicians encountering patients with IFI. Finally, promising adjunctive immunotherapy measures are currently being investigated in IFI.

Key words: Fungal immunology, medical mycology, invasive fungal infection, inborn errors of immunity, biologic agents.

Introduction

Fungi are eukaryotic microorganisms that are ubiquitous in the environment and include yeasts, moulds, mushrooms, polypores, plant parasitic rusts, and smuts.[1] There are an estimated 100,000 described species, but the actual global fungal diversity is estimated at 0.8–5.1 M species.[1,2] Fungi play fundamental ecological roles as decomposers, pathogens of plants and animals, and they drive carbon cycling in soils and mediate mineral nutrition of plants.[2] We encounter them every day—in plant fruits and roots, in soil and air, on animals, and in fresh and salt water. In a recent study of soil collected from 365 sites globally analyzed by pyrosequencing, 963,458 (94.5%) sequences and 80,486 (85.4%) operational taxonomic units were classified as fungi.[2]

Despite our multiple encounters with fungi throughout our daily lives, through gardening or composting, food mould on the kitchen bench or refrigerator, or merely by inhaling, serious human fungal infections are quite rare in individuals with healthy immune systems. However, the increase in the numbers of immunosuppressed persons has led to the worldwide emergence of invasive mycoses as major causes of morbidity and mortality. There are an estimated 3 million (M) cases of chronic and 0.25 M cases of invasive pulmonary aspergillosis, 0.22 M cases of cryptococcal meningitis complicating human immunodeficiency virus (HIV)/AIDS, 0.7 M cases of invasive candidiasis, 0.5 M of *Pneumocystis jirovecii* pneumonia, 0.1 M cases of disseminated histoplasmosis, over 10 M cases of fungal asthma and 1 M cases of fungal keratitis, which occur annually worldwide.[3] The most significant opportunistic invasive mycoses include cryptococcosis,

candidiasis, pneumocystosis, aspergillosis, and mucormycosis, with a mortality rate in some settings approaching up to 75-95%, and endemic mycoses including histoplasmosis, coccidioidomycosis, penicilliosis (now talaromycosis), paracoccidioidomycosis, and blastomycosis.[4]

Here we discuss in brief the fungal cell wall, why and how the human host discerns fungal cell from host cell, and the adaptation measures with which fungi have countered. We discuss how modern medicine breaches natural human barriers to fungal infections contributing to invasive candidiasis and other rare IFIs. We summarize primary and secondary acquired immunodeficiencies, including new biologic agents and suggest an investigative approach for clinicians to explore for the presence of immunodeficiency. Finally, we highlight a few promising adjunctive immunotherapy measures currently being studied in IFI.

The clinical challenges of invasive fungal infection

IFI are myriad and their presentations so protean that one often must have a high clinical suspicion to make a diagnosis. Recognizing classic hosts such as those infected with HIV or with a hematological malignancy is relatively easy, but unfortunately, IFIs can surprise by occurring in previously well individuals! The diagnosis of IFIs is most perplexing and often further belated in patients with rare or unknown underlying risk factors.

An accurate diagnosis of an IFI may require advanced medical imaging, microbiological sampling, surrogate fungal blood markers, and a tissue biopsy, as per the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group definitions[5]—each of which can have logistic challenges. Invasive aspergillosis (IA) remains the most commonly diagnosed invasive mould infection in patients with hematological malignancies and solid organ transplant (SOT) recipients. However, clinicians need to have an awareness of the emergence of mucormycosis, as well as infections due to a myriad of rare moulds such as species of *Chrysosporium, Exophiala, Ochroconis, Rhizopus,* and *Scopulariopsis*. Moreover, while *Candida* and *Cryptococcus* are the most common yeasts to cause infections, rare yeasts, such as *Malassezia*, *Rhodotorula*, *Trichosporon*, *Geotrichum*, *Kodamaea,* and *Saccharomyces* also occur (reviewed in[6,7]). Furthermore, in patients from endemic regions, thermally dimorphic mycoses, including histoplasmosis, coccidioidomycosis, blastomycosis, talaromycosis, and emergomycosis must be entertained (reviewed in[8]).

Determining the infecting fungus beyond the more common *Candida, Aspergillus,* and *Cryptococcus* species requires morphological expertise supported in many cases by molecular identification. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) is commonly used in modern laboratories to rapidly identify bacteria and yeasts, but its performance with moulds is limited by the availability and size of reference libraries.[9,10] In a recent study comparing three libraries, the highest rate of species identification was seen with the Mass spectrometry identification online library where 72% of 221 moulds were identified. In comparison, the rate was a dismal 19.5% with the National Institutes of Health library and 13.6% with the Bruker mould library.[11] More importantly, fungi are sometimes difficult to culture—a prerequisite to the use of MALDI-TOF.

Molecular methods (e.g., DNA sequencing) are currently the gold standard for the identification of fungi to the species level, but these are expensive, require specialized equipment or expertise, and are not available in most clinical laboratories. As it is commonly a send-away test, turnaround time is slow, and accuracy may be limited by available primer sets and extraction techniques. Its potential to identify the presence of fungi directly from patient samples without the need for microbiological culture is attractive, although determining whether the organism found by molecular identification is pathogenic, commensal, or even a contaminant requires clinical nuance. *Aspergillus* polymerase chain reaction (PCR) is the most advanced fungal molecular tool and is verging on being incorporated in the upcoming revised IFI diagnostic definitions but still suffers from issues of standardization, turnaround time, and clinical interpretation (reviewed in[12]).

Beyond the difficult therapeutic decisions of selecting the most optimal antifungal therapy agent(s), clinicians are then faced with explaining why this specific patient presented with this specific fungal infection and at this specific time. The degree of immunosuppression in advanced HIV/AIDS is often inferred and largely explained by a low CD4+ T-cell count. The impact of chemotherapeutic agents may be largely due to neutropenia and other drug-specific immunosuppressant effects. However, assessing the immune state of the host beyond these two patient groups remains challenging, particularly outside of research settings. Understanding how the immunocompetent host recognizes and counteracts fungi is key to understanding situations where defences fail and mycoses result.

The fungal cell wall structure – simplified

Animals and fungi are kingdoms in the domain Eukaryota;[13] thus, from a cellular point of view, humans and fungi have more in common than is often appreciated. One major exception is nearly all fungi have a cell wall. It follows, then, that when encountering a fungal infection, innate immune systems of animals react predominantly to the foreign fungal cell wall components.

The fungal cell wall is predominantly composed of carbohydrate polymers interspersed with glycoproteins.[14] The three major components, found in all medically important fungi studied, are β-glucans (homopolymers of glucose with β-1,3-glucans

forming the scaffold and β-1,6 glucans forming the branches), chitin (a homopolymer of N-acetylglucosamine that provides structural stability), and mannans (mannose chains of varying lengths and configurations, often linked to proteins) (reviewed in[14,15]). Some fungi have other cell wall glycans such as chitosan (deacetylated chitin), α-glucan, and galactomannan. While these components intermingle in the cell wall, structurally, chitin and chitosan tend to be situated in the inner portion near the plasma membrane, glucans in the middle, and mannans on the outer portion.[15]

Breaching the natural barriers to fungal invasion with modern medicine

The human host is usually prepared to defend against fungal infections from multiple routes of natural exposure, most commonly via sinopulmonary, gastrointestinal, and dermatological routes. Each site has unique anatomical and cellular host defences, such as alveolar macrophages in the lungs and epithelial barriers in the skin and vagina. Breaching these natural anatomical barriers are particularly common means by which IFIs develop. The increasing use of invasive devices, especially intravascular central lines but also cardiac pacemakers, ventricular assist devices, urological stents, and joint prostheses has resulted in a contaminant increase in catheter-related bloodstream infection and other nosocomial fungal infections.

Candida spp. are the most common fungal pathogens causing serious hospital-acquired infections.[16] Certain *Candida* spp., especially *Candida albicans*, are part of the human microbial flora; hence, most candidal infections are endogenous in origin. In recent data pooled from the national healthcare safety network encompassing more than 17,000 healthcare facilities in the United States, *Candida* spp. were ranked fourth for all hospital-acquired infections, third for central line-associated bloodstream infections, second for catheter-associated urinary tract infections and tenth for surgical site infections.[17]

Most nosocomial outbreaks of invasive candidiasis and invasive infections with other yeasts have been associated with intravascular catheters.[18] A central venous catheter is present in at least 70% of non-neutropenic patients with candidemia at the time that the diagnostic blood culture is obtained (reviewed in[16]). In neonates, known risk factors for candidemia include very low birthweight, prematurity, central venous catheter use, necrotizing enterocolitis, total parenteral nutrition, and prior or prolonged broad-spectrum antibacterial drug use (reviewed in[19]).

Non-*albicans*, nosocomial *Candida* outbreaks have included *C. parapsilosis*[20,21] and recently *C. krusei* in South Africa.[19] In a study of 290 neonates or preterm infants and 17 paediatric patients with complicated gastrointestinal surgery based in Southern Italy, six blood stream infections were caused by *Malassezia furfur* and four by *Candida* spp. (1.4%; two *C. parapsilosis*, one *C. albicans*, and one *C. glabrata*).[22] Rarer nosocomial fungal infection outbreaks include a multistate outbreak of *Exserohilum rostratum* infections, caused by epidural, paraspinal, and peripheral joint injections of fungus-contaminated vials of methylprednisolone;[23,24] *Sarocladium kiliense* bloodstream infections in 67 patients at eight hospitals in Santiago, Chile, found to be associated with intravenous ondansetron,[25] and *Saprochaete clavata* hypothesized to be associated with apheresis platelet concentrates[26] (reviewed in[27,28]). These cases emphasize that fungi that normally have very low virulence can cause serious infections if allowed access to sterile parts of the body by disruption of natural anatomic barriers.

Candida auris, a multiresistant organism first reported in patients with ear infections in 2009,[29,30] followed by blood stream infections in 2011,[31] has been associated with multiple nosocomial outbreaks globally.[32–34] It is often misidentified by standard microbiological techniques. *C. auris* can be difficult to treat as isolates are usually resistant to fluconazole and have variable susceptibility to other azoles, amphotericin B and echinocandins.[34]

Recognition by the innate immune system

Our immune system has evolved very sophisticated adaptive and innate systems to counteract and defend against fungi. Innate effector cells, mainly macrophages and neutrophils, are the first line of defense against inhaled fungal spores. Innate fungal recognition is accomplished via sensing of cell wall and intracellular pathogen-associated molecular patterns (PAMPs) by soluble, membrane-bound, and intracellular pattern recognition receptors (PRRs) of myeloid and epithelial cells.[35] Innate recognition of fungi leads to signal transduction events, which in turn leads to functional responses such as phagocytosis, cytokine and chemokine release, antimicrobial activity, and involvement and modulation of the adaptive immune system.[35]

Innate immune system PRRs consist of four major classes: C-type lectin receptors (CLRs), Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, and retinoic acid inducible gene I (RIG-I)-like receptors.[14] Of these, CLRs appear to be most important for antifungal defenses—they bind glycans using conserved carbohydrate-recognition domains and play major roles in recognizing fungal cell wall components (β-glucans and mannans). CLRs important in fungal recognition include Dectin-1 and -2, which recognize β-1,3 glucan and α-mannans, respectively, and mannose receptors, which recognize exposed mannose residues (reviewed in[36,37]). A newly described CLR, MelLec, senses melanin on fungal conidia.[38] Soluble CLRs may be found circulating in the serum and other body fluids or exist as transmembrane receptors on immune cells such as monocytes, dendritic cells, and neutrophils.[36]

Receptors recognizing cell wall exposed mannans include two CLR, the mannose receptor (CD206, also known as the macrophage mannose receptor) and Dendritic Cell-Specific

Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN or CD209).[14] Other mannose receptors include langerin and Dectin-2. Receptors on dendritic cells that recognize mannose facilitate uptake of mannosylated antigens by dendritic cells for presentation to T cells.

One of the most studied CLRs is Dectin-1.[39] This transmembrane receptor is highly expressed on myeloid cells and has specificity for β-1,3-glucans. Upon receptor engagement, various signalling cascades are triggered, including the Caspase recruitment domain-containing protein (CARD)-9 dependent cascade, resulting in phagocytosis, the respiratory burst, and cytokine/chemokine gene induction (reviewed in[14]). In addition to Dectin-1, CARD9 serves as a critical adapter protein for Dectin-2 and mincle.[40] Hence, patients with CARD9 mutations have a more severe phenotype than those with Dectin-1 mutations. Other receptors that recognize β-glucans include complement receptor (CR) 3, and the scavenger receptors, CD5, CD36, and scavenger receptor class F member 1 (SCARF1) (reviewed in[14,41]). Recently, two additional β-glucan receptors were described; natural killer cell activating receptor NKp30[42] and ephrin type-A receptor 2 (EphA2), an oral epithelial cell PRR.[43]

Surface components on fungi also stimulate TLR2 and TLR4. TLRs initiate intracellular signaling pathways using adapter proteins, which ultimately activate the transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and interferon-regulatory factors.[44] Important TLR-dependent cellular responses that promote antifungal immunity include type I interferons (IFNs) and the proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-12 (reviewed in[37]). Defects in IL-1 receptor associated kinase-4 (IRAK4) and myeloid differentiation factor 88 (MyD88) lead to susceptibility to infections with bacteria such as pneumococcus, TLR3 signaling defects are specific for susceptibility to herpes simplex virus type 1 encephalitis, while mutations in nuclear factor-B essential modulator (NEMO) and other downstream mediators generally induce broader susceptibility to bacteria, viruses, and fungi (reviewed in[44,45]). Patients with NEMO mutations have presented with PJP infection.[46,47] An increased risk for IA in allogeneic stem cell transplantation was suggested with a specific donor TLR4 haplotype.[48] Single nucleotide polymorphisms (SNPs) in Dectin-1 and DC-SIGN were combined with other established clinical risk factors and *Aspergillus* PCR screening results as a predictive model for IA, in a retrospective cohort of high-risk haematology patients.[49] While testing donors for specific polymorphisms predisposing to mycoses has not been adopted in routine clinical care, in the future, this type of immunogenomic study could be used to inform decisions regarding antifungal prophylaxis.

Fungi are potent activators of the complement system. Complement activation fragment C5a, which is formed in response to *Candida* infection, induces the cellular release of the inflammatory cytokines IL-6 and IL-1β, thereby augmenting the host proinflammatory response.[50] Further, serum antibodies to fungal cell wall components, particularly mannans and β-glucans, can initiate classical pathway activation upon binding and promote recognition by phagocytic Fc receptors (FcRs). Activation of the lectin pathway occurs when recognition of exposed mannans by mannose-binding lectin (MBL) triggers MBL-associated serine proteases.[14] SNPs of the gene coding for MBL are present in nearly 40% of the general population, resulting in reduced cross-linking and secretion of nonfunctional MBL. Heterozygotes and compound heterozygotes for this gene record a serum MBL one third and one-sixth that of normal homozygotes. Patients with IA had significantly lower serum MBL levels compared to febrile immunocompromised controls, and a larger proportion met the definition of MBL deficiency.[51]

From the above discussion, it is apparent that multiple innate receptors are stimulated by fungi and an immune cell may recognize multiple ligands on a fungus. For example, immune sensing of *C. albicans* is mediated by multiple recognition systems including Dectin-1-3, Mincle, mannose receptor, DC-SIGN, CR3, FcRs, MBL, and Langerin (reviewed in[36]). Similarly, several CLRs are involved in the recognition of *Aspergillus* spp. This includes Dectin-1 and -2, mannose receptor, DC-SIGN, MBL and the lung surfactant proteins (SP) SP-A and SP-D (reviewed in[36]). Each ligand-receptor system activates specific signaling pathways, with some being proinflammatory, while others are anti-inflammatory. In addition, receptor crosstalk occurs. Thus, the overall host response to fungal infection depends on multiple factors, including the host immune status, site of infection, fungal morphotype (e.g., yeast vs hyphae), cell wall complexity, and fungal virulence traits. The response occurs in a coordinated way involving innate, adaptive and trained immunity.[52] A comprehensive review of fungal PRRs and associated fungal PAMPs can be found in Lionakis and Levitz[35] and in Goyal et al.[36] A simplified illustration of cryptococcal cell wall components acting as PRRs and their respective PAMPs is provided as Figure 1.

Fungal avoidance of innate receptors

While the extent varies depending on the fungal species, mannans sitting on the outer surface shield β-glucans, chitin and chitosan, thereby impairing recognition of these ligands. Some fungi further "mask" ligands to reduce the stimulation of innate immunity. For example, *Cryptococcus* spp. have a capsule composed of glucuronoxylomannan and galactoxylomannan, which serves as its major virulence factor (reviewed in[53]). The capsule completely encompasses the cell wall and in the absence of opsonization, encapsulated *C. neoformans* are poorly phagocytosed.

Importantly, fungal cell walls are dynamic, constantly remodeling structures. By varying the distribution of their components

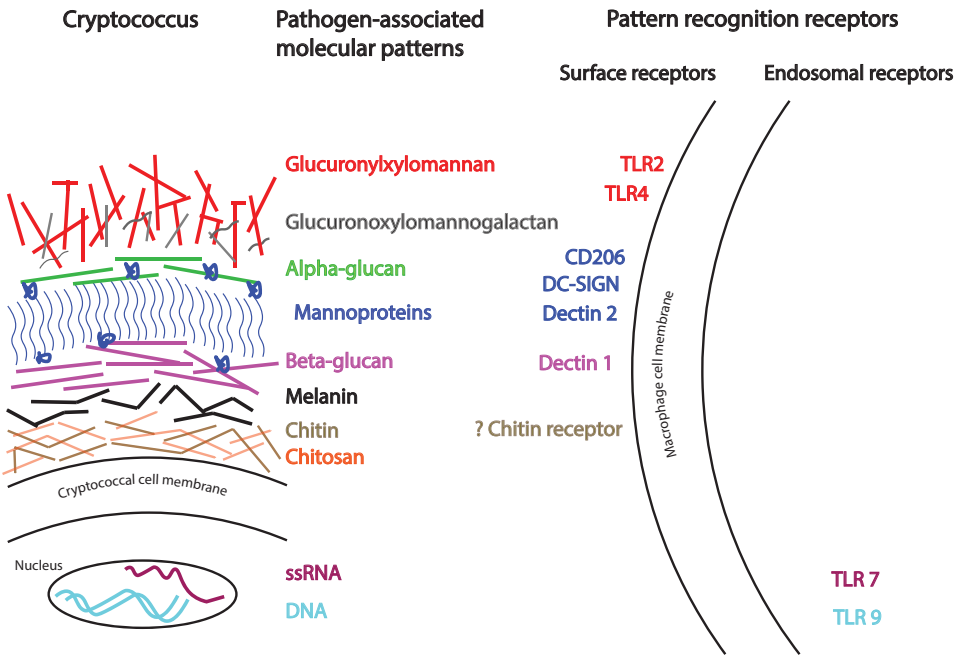

Figure 1. The architecture of the cryptococcal cell wall and capsule, highlighting selected pathogen-associated molecular patterns and their respective pattern recognition receptors. Engagement of receptors leads to activation of signaling pathways that result in cytokine release and other cellular events. CD206 (also known as mannose receptor); DC-SIGN, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (also known as CD209); TLR, Toll-like receptor.

and their cross-linking, they enable morphologic changes to allow for external stress, cellular division, and mating.[53] For example, the more virulent *Histoplasma* chemotype II strain uses α-glucans to mask cell wall β-glucans and secretes *Eng1*, an endoglucanase which enzymatically prunes exposed β-glucans. Together, this results in reduced Dectin-1 recognition of yeasts and reduced cytokine production by phagocytes.[54] The ability to change morphotype by alteration of its surface glycans results in reduced host recognition, reduction in inflammatory response, and increased virulence. Thus, despite the fundamental constructs of fungal cell walls, each fungus presents unique challenges to the host. The host must contend with myriad inter- and intra-species fungal cell wall variations.

Primary immunodeficiencies/ inborn errors of immunity

Primary immunodeficiency diseases are a group of disorders caused by inborn genetic defects in the cells or tissues of the immune system. While severe primary immunodeficiency states usually manifest in childhood, they may occasionally be diagnosed in seemingly immunocompetent adult patients presenting with a severe IFI. Classical primary immunodeficiencies have tended to be of Mendelian inheritance, where single genes confer susceptibility to multitude of infections, in a completely penetrant manner and with a predictable effect on immune cells. Since then, there has been a growing number of monogenic single-gene inborn errors of immunity recognized, with selective susceptibility only to specific infective pathogens.[55–57] Routine assessment of these patients with simple investigations looking for immune defects is often unrevealing. More sophisticated analysis of individual patients may identify inborn errors of immunity such as mutations in the genes encoding for CARD9,[40,58–64] Dectin-1,[65,66] signal transducer and activator of transcription (STAT)-1, 3, or 4,[67–70] and IL-12 receptor beta-1.[71] As discussed below, other patients have been found to have anti-cytokine antibodies such as auto-antibodies to IFNγ.[72–74] GM-CSF,[75,76] IL-17,[70,77–80] and IL-22[78,79] (reviewed in[35]).

The International Union of Immunological Societies (IUIS) Inborn Errors of Immunity Committee not only publishes and updates a comprehensive classification of the inborn errors of immunity[81] but also has a clinician-targeted publication with helpful phenotypic descriptions.[82] In its most recent iteration published in 2018, it describes 320 inborn errors, classified into nine categories, with helpful flowcharts, gene names, and phenotypic descriptions. These categories are: combined immunodeficiencies (CID) (cellular and humoral), CID with syndromic features, predominantly antibody deficiencies, diseases with immune dysregulation such as hemophagocytosis, phagocytosis related congenital disorders, defects in intrinsic and innate immunity, autoinflammatory disorders, complement deficiencies, and phenocopies of primary immunodeficiencies. The phenotypic descriptions include some predisposition to bacterial, viral, mycobacterial, and fungal infection.

A detailed knowledge of each signaling cascade is beyond the scope of this review. An update on the immunological and clinical features of inborn errors of immunity that result in mucosal and systemic fungal infection susceptibility has been reviewed by Lionakis and Levitz[35]. However, illustrative examples of CARD9 deficiency and IFNγ/IL-12 pathway impairment are discussed.

CARD9 loss of function is associated with fungal susceptibility but not viral or bacterial susceptibility. It also may exhibit surprising tissue tropism as evidenced by the particularly severe central nervous system manifestations that CARD9-deficient patients often develop. CARD9 deficiency has been reported in families where multiple individuals have been diagnosed with chronic mucocutaneous candidiasis (CMC), deep dermatophytosis,[59] *Candida* encephalitis[60,62,64] and the rarely invasive *Exophiala spinifera* infection.[61] While there are many homozygous mutations reported, mostly in consanguineous families, none of the heterozygous individuals was symptomatic, consistent with an autosomal recessive inheritance (reviewed in[60]). Detailed studies of patients with CARD9 mutations with extrapulmonary aspergillosis and cerebral candidiasis show impaired proinflammatory cytokines and an inability to accumulate neutrophils in the affected tissue.[63,83] Administration of granulocyte-macrophage colony stimulating factor (GM-CSF) has been attempted to improve the outcome of a few CARD9 deficient patients with CNS candidiasis[58,84,85] but may lead to differential response dependent on the specific affected signaling pathway.

The IL-12/ IFNγ signalling cascade is particularly important in clearing intracellular fungal and bacterial pathogens such as *Cryptococcus, Histoplasma,* and mycobacteria. Activated macrophages secrete IL-12, which stimulates NK and T cells to produce IFNγ. This cytokine then stimulates macrophage IFNγ receptors, resulting in nuclear translocation of STAT1 and the transcription of IFNγ-related genes. Defects include IFNγ receptor 1 or 2 gene (*IFNγR1* or *IFNγR2*), IL-12 receptor beta-1 gene (*IL12RB1*), *IL12B* gene, and *STAT1* gene and have variously been associated with CMC, histoplasmosis, cryptococcosis, coccidioidomycosis, and nontuberculous mycobacterial infections (reviewed in[35]).

IFN-gamma autoantibodies

Apart from genetic defects in the IFNγ/IL-12 axis, acquired autoantibodies against IFNγ have been found in patients presenting with non-tuberculous mycobacteria (NTM) infections[86–88] and fungal infections including cryptococcosis,[73] talaromycosis,[72,74] and histoplasmosis.[89] Anti-IFNγ autoantibodies has been associated with HLA-DRB1*16:02 and HLA-DQB1*05:02[86] and has a predominance in East Asians.[86,88] Rituximab, a chimeric monoclonal antibody against human CD20 primarily found on B cells, has been tried in four patients with high anti-IFNγ antibodies and NTM with promising clinical and immunological responses.[90]

Idiopathic CD4 lymphopenia

Idiopathic CD4 lymphopenia (ICL) is a rare, heterogeneous syndrome, diagnosed after the exclusion of agents known to depress CD4 T cell counts including HIV.[91] It is usually identified when previously well patients present with an opportunistic infection. Current studies use a CD4 cutoff of less than 300 cells/ml or less than 20% of T cells.[92–94] In a study of 39 patients with ICL, many had concurrent CD8 lymphopenia, low B cell or low NK cell numbers, and 5 (12.8%) recorded low cell numbers in all four subtypes.[94] Cryptococcosis and NTM infections were the most frequent presenting opportunistic infection.[94] In a separate study of 25 ICL patients presenting with infection, B cell (CD19+), CD8+, NK cell subsets were also deficient in 19 (76%), 13 (52%), and 7 (28%), respectively.[92] Up to 20% of patients with ICL were identified incidentally when lymphopenia is noted on routine blood work.[92] An open-label Phase I/IIa dose-escalation study of recombinant human IL-7 in nine patients with ICL found this therapy to be safe.[93] There was a resultant increase in peripheral CD4 T-cells at week 3, which peaked at week 5 and was sustained to week 12.[93]

Secondary immunodeficiencies

Having survived past childhood, adults who develop serious IFIs usually have secondary immunodeficiencies. Secondary immunodeficiency disease states occur when the immune system is compromised due to an environmental factor such as HIV/AIDS or as a consequence of immunosuppressive therapy in association with organ transplantation, cancer, or autoimmune illnesses.

Those with HIV/AIDS with advanced CD4-lymphopenia are classically at risk for cryptococcosis, pneumocystis, oesophageal candidiasis, and the endemic mycoses. Apart from CD4 depletion, HIV leads to B, NK, and dendritic cell (DC) dysfunction, which all contribute to susceptibility to microbial pathogens, including fungi (reviewed in[95]). Another complication seen in HIV-infected persons coinfected with IFI is immune reconstitution inflammatory syndrome (IRIS) where infective symptoms may paradoxically recur or newly occur after the commencement of antiretroviral therapy. The diagnosis of IRIS is made clinically in the patient who presents with an exaggerated and/or atypical inflammatory response which is temporally associated with the restoration of immune function and in whom an alternative diagnosis has been excluded.[96] IRIS has been described as a complication of many IFI, including cryptococcosis, histoplasmosis, pneumocystis, and talaromycosis (reviewed in[96]).

Hematological stem cell transplant (HSCT) recipients are particularly at risk for pulmonary IA though rarer organisms have been reported. Data from the Transplant-associated infection

surveillance network (TRANSNET) database of IFIs in HSCT recipients from 2001 to 2006, showed IA was the most common IFI (43%), followed by invasive candidiasis (28%), zygomycosis (8%), fusariosis (3%), pneumocystosis (2%), and cryptococcosis (0.6%) and a multitude of rare moulds and yeasts.[97] *A. fumigatus* caused 44% of the 425 cases of aspergillosis, and concerningly *C. glabrata* was the most common organism causing candidiasis (33%).[97] In a more recent but smaller study of four HSCT centers in the United States, *Candida* spp. accounted for 34% of the 54 IFIs, *Aspergillus* spp. for 32%, followed by Mucorales (13%), Pneumocystis (6%), *Exophiala* (4%), and *Alternaria* (2%).[98] The wide range of fungal pathogens seen in the severely immunosuppressed patients underlines the importance of good mycology diagnostics. This is especially critical as many of these rarer fungi are resistant to commonly used empiric antifungal agents. Dismissing rare fungi as contaminants in the immunosuppressed host is perilous.

Adding to the complexity of treatment-induced immunodeficiency in HSCT and SOT patients, a number of studies highlights the importance of genetic polymorphisms in modulating IFI susceptibility, reviewed in Herrero-Sanchez et al.[99] For example, pentraxin-3 is a soluble PRR produced by phagocytes that binds to and opsonizes *Aspergillus* conidia. Donor and recipient SNPs in the pentraxin-3 gene increase IA susceptibility in allogeneic HSCT[100,101] and SOT, respectively.[102] This suggests that well-defined immunodeficiency states may be compounded by subtle primary immunological defects.

New biologic agents

There has been a proliferation of new biologic agents in clinical practice many of which have been associated with susceptibility to specific and in some cases unusual fungal infections (reviewed in[35,103–106]). Keeping pace with these novel agents, particularly their cellular and humoral impact, is challenging. There are two European Society of Clinical Microbiology and Infectious Diseases (ESCMID) publications which summarize the infection risks of these agents; Reinwald et al.[105] review drugs impacting intracellular signalling pathways and Redelman-Siti et al.[104] review immune checkpoint inhibitors, cell adhesion inhibitors, sphingosine-1-phosphate receptor modulators,and proteasome inhibitors. Examples of these agents and their respective fungal susceptibility are reviewed in Table 1.

The utility of giving antifungal prophylaxis when using many of these agents is only starting to be defined. However, antipneumocystis prophylaxis is recommended for patients receiving idelalisib throughout the entire course of therapy and for 2 to 6 months after discontinuation and those on Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), programmed cell death 1 (PD-1)/PD-ligand 1 (PDL1) blockade who are

Table 1. New biologic agents and risk of invasive fungal infections (reviewed in[35,103–106]).

Inhibitor mechanism	Example(s)	Fungal susceptibility
TNF-α inhibitors	infliximab, etanercept, adalimumab, certolizumab	histoplasmosis, PJP (reviewed in[107]), cryptococcosis, coccidioidomycosis, and blastomycosis
IL-6R	tocilizumab	cryptococcosis[108], PJP (reviewed in[107])
IL-17A	secukinumab ixekizumab brodalumab	candidiasis (reviewed in[109])
IL-23/IL-17 blockers	ustekinumab	mucosal candidiasis (reviewed in[109])
Janus kinase inhibitors - JAK1/2 and 1/3 inhibitors	tofacitinib ruxolitinib baricitinib	cryptococcosis[110–112], talaromycosis[113], candidiasis[114]
Bruton's tyrosine kinase (BTK) inhibitor	ibrutinib (reviewed in[115])	cryptococcosis[116,117], PJP[118,119], aspergillosis, mucormycosis
Vascular endothelial growth factor (VEGF)	bevacizumab	aspergillosis[120], fusariosis[121]
α-4/β-7 integrin	natalizumab vedolizumab	cryptococcosis[122,123], candidiasis[124]
Anti-programmed cell death 1 (PD-1) / PDL-1	nivolumab pembrolizumab	fusariosis[125]
Sphingosine-1- phosphate receptor	fingolimod	cryptococcosis[126–128], histoplasmosis[129]
Cytotoxic T-Lymphocyte associated protein 4 (CTLA-4)	ipilimumab	PJP[130]
Ubiquitin-proteosome	bortezomib	PJP[131]
Ras/PI3K/Akt/ mTOR	idelalisib, buparlisib, rigosertib, duvelisib	PJP[132]
BCR-ABL, c-Kyt, other off-target kinases	imatinib, dasatinib, sorafenib	candidiasis[133], PJP[134], rhodoturulosis[135]
B cell lymphoma (BCL)-2	venetoclax	aspergillosis[136], candidiasis[136], PJP[137]

expected to receive 20 mg of prednisone (or equivalent) for at least 4 weeks.[104,105]

A suggested simple clinical algorithm to assess primary and secondary immunodeficiency in the setting of IFI

Many individuals presenting with a new IFI will have a known secondary immunodeficiency, such as HIV/AIDS or receipt of immunosuppressive agents. Those with candidemia may have a predisposing central venous catheter. Diagnosing the presence of a secondary immunodeficiency condition such as an undiagnosed haematological malignancy, HIV/AIDS, or solid organ neoplasia is important in all persons presenting with a newly diagnosed IFI. A thorough clinical history and examination is critical in guiding targeted investigation including medical imaging. Simple blood investigations include a complete blood count (CBC) and blood smear, HIV testing, CD4/CD8/ natural killer (NK) cell subsets by flow cytometry, immunoglobulin levels, complement testing, and supportive ancillary tests such as nonspecific inflammatory markers and albumin levels (Fig. 2). This list is not comprehensive and a referral to a clinical immunologist will assist in guiding these investigations.

In particular, a thorough clinical assessment of all previous infections should be obtained. For example, multiple episodes of bacterial, viral, and fungal infections in a child or young adult might suggest a combined immunodeficiency. While the types of infections will inform the specific testing, an evaluation of humoral immunity by immunoglobulin, complement and B cell numbers, and adaptive immunity by T and NK cell subset and function are important. Advanced flow cytometry is often required in the diagnostic algorithm. Details of the various diagnostic tools required are reviewed elsewhere.[138–141]

Patients with unexplained IFI and/ or atypical mycobacterial disease may have an IL-12/ IFNγ pathway defect or IFNγ autoantibodies or other rarer inborn errors of immunity. Patients with IFI without other bacterial or viral or parasitic should be further divided into those limited to chronic mucocutaneous candidiasis (CMC), those with mould infections, or both. Those with just CMC most often have an IL-17 pathway defect. Patients with unusual CNS IFI (e.g., CNS candidiasis in the absence of gastrointestinal involvement) and infections predominantly limited to fungi, should be considered for CARD9 mutations. These inborn errors of immunity are best assessed by whole genome sequencing (WGS), whole exome sequencing (WES), or targeted gene sequencing.[140,142–144] A two-tiered investigative approach has been suggested: first focusing on the common, actionable, and severe primary immunodeficiency diseases, followed by a genome-wide approach using either WES or WGS with a large, frequently updated gene candidate list.[142] An approach to a patient presenting with an IFI is illustrated in Figure 2.

There are no cost-effectiveness studies to guide or justify these tests, and it is often difficult to know how far to investigate for immunodeficiencies. Access to some of these investigations is also currently limited to research laboratories; thus, a personalized and pragmatic approach is necessary. We advocate that individuals without a known risk factor for IFI, particularly when presenting with a severe and or recurrent IFI, be evaluated for an undiagnosed immunodeficiency, preferably in consultation with an expert clinical immunologist. A diagnosis of primary immunodeficiency may lead to increased awareness of infections, potential prophylaxis strategies and occasionally direct therapeutics. Further, familial linkages may assist early diagnosis in other family members.

Immunotherapeutics for invasive fungal infections

The challenges and promise of immunotherapeutics in IFI have been recently reviewed in Armstrong-James et al.[145] In HIV-infected patients with cryptococcal meningitis, adjuvant exogenous IFNγ was shown to be safe and improved clearance rates of cryptococci from the cerebrospinal fluid, although there was no impact on mortality rates compared with standard therapy.[146,147] While the use of adjuvant exogenous IFNγ in cryptococcal meningitis has not been adopted as part of routine care, it may have utility in selected difficult to treat patients.

In a prospective open-label study, eight patients with aspergillosis and invasive candidiasis received recombinant IFNγ thrice a week for 2 weeks in addition to standard antifungal therapy. This led to clinical improvement and an increase in HLA-DR positive monocyte levels.[148] This strategy was also used in a patient with ICL and progressive cryptococcal meningitis who had a good clinical and immunological response.[149] Interestingly an open-label study published more than 10 years ago, explored IFNγ use in 32 HSCT recipients with IFIs and found this to be relatively safe. Only one episode of lymphopenia was reported and this reversed with the cessation of IFNγ.[150] No worsening of graft-versus-host-disease (GVHD) occurred, and indeed, about a third of those with acute and chronic GVHD recorded improvement.[150] Two successful case reports of adjuvant IFNγ in the treatment of refractory IFI in pediatric patients with acute lymphoblastic leukemia have been recently reported: a 3 year-old with disseminated *Candida dublinensis*[151] and an 8 year-old with disseminated *Aspergillus terreus*.[152] These studies, while promising, must be interpreted cautiously due to the lack of randomized control groups.

An elegant example of translational science is the successful use of topical imiquimod in the treatment of four patients with chromoblastomycosis, a difficult to treat, chronic progressive subcutaneous mycoses most often caused by Fonsecaea pedrosoi.[153] *F. pedrosoi* was shown to be recognized by CLRs but not TLRs,[154] resulting in defective inflammatory response and chronicity. Imiquimod is a synthetic compound that stimulates

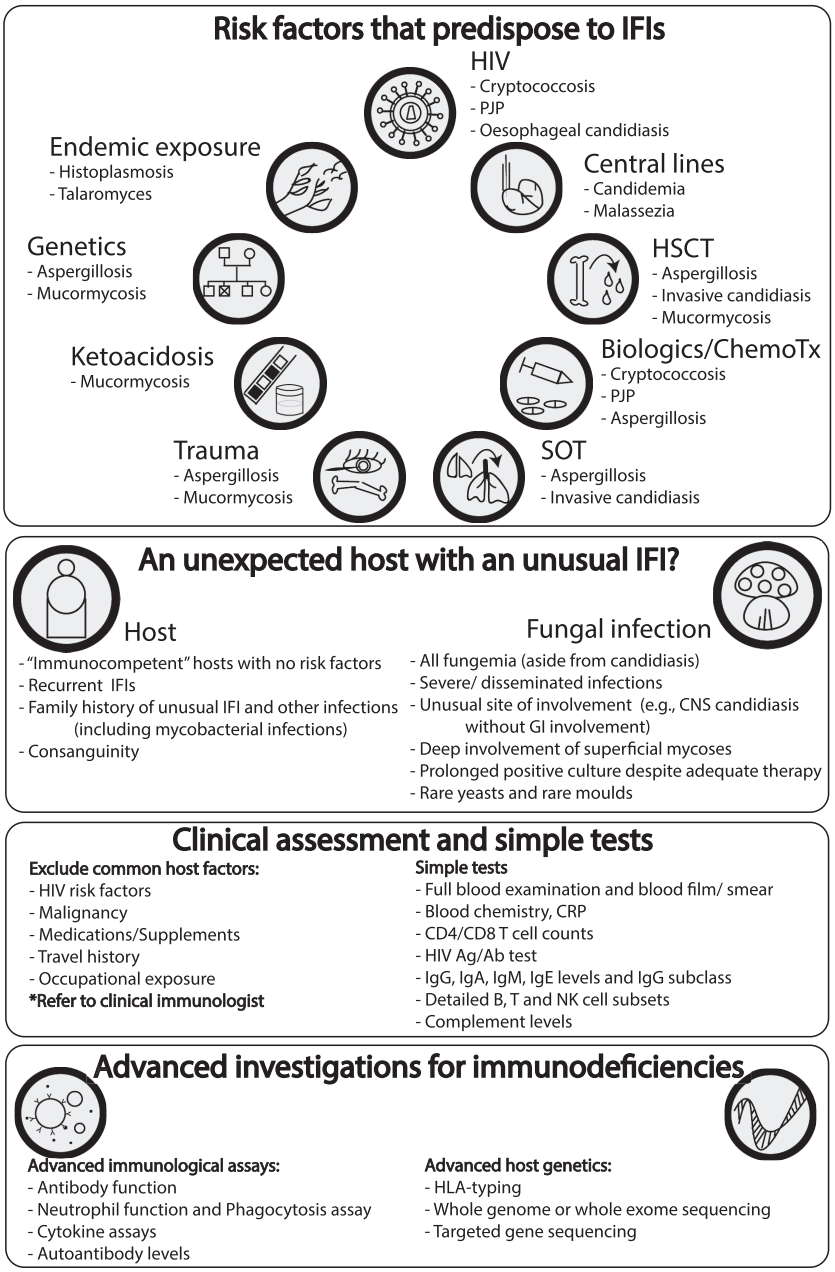

Figure 2. A suggested simple clinical algorithm to assess for primary and secondary immunodeficiency in the setting of IFI. ChemoTx, chemotherapeutics; CNS, central nervous system; CRP, C-reactive protein; GI, gastrointestinal; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; HSCT, hematological stem cell transplant; IFI, invasive fungal infections; Ig, immunoglobulin; PJP, *Pneumocystis jirovecii* pneumonia; SOT, solid organ transplant.

both the innate and acquired immune pathways through activation of TLR7.[153] Its topical administration led to complete cure in one patient, and partial improvement in three patients, though concerningly these showed morphological transformation from a yeast form to a filamentous form.[153]

Immune checkpoint inhibitors restore T-cell responses and thus have been recently proposed to be of potential utility in refractory fungal infections, which are controlled by T-cell effector function. Recently, IFNγ dosed thrice weekly and a single dose of 250 mg nivolumab, an anti-PD1 inhibitor, was used to treat severe mucormycosis. This resulted in a rise in absolute lymphocyte count, monocyte HLA-DR expression, and CD8 T-cells, a decrease in T-cell PD-1 expression and clinical recovery.[155]

The need to keep pace with new therapeutic agents which exploit and expose the host immune system is difficult but important. The gap between research settings and the reality of clinical practice remain wide, particularly in the realm of immunogenomics. The data and literature are growing exponentially. Discoveries are occurring so rapidly that clinicians need a basic framework of fungal immunology assisted by some helpful

resources as reference. An openness to embrace scientific collaborators should further enhance clinical care.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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