Agostinho Carvalho *Editor*

Immuno-genetics of Fungal Diseases

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Preface

Fungal infections represent a worldwide healthcare problem. They are estimated to occur in over a billion people each year, and recent evidence suggests the rate is increasing. Vaccines are not available and, particularly in immunocompromised hosts and critically ill patients, the management of invasive fungal diseases (IFDs) is a challenging endeavor associated with unacceptable mortality and morbidity rates. Critical risk factors for IFD include hematologic malignancies, stem cell or solid organ transplantation, primary or acquired immunodeficiencies, and long-term intensive care treatment and immunosuppressive therapy. Despite the availability of a broad spectrum of antifungal drugs, successful treatment of IFD is often hampered by limitations in diagnostic approaches that do not allow a rapid and reliable identification of infection, and in the assessment of host-derived biomarkers of susceptibility. Of note, the risk of IFD and its clinical outcome vary remarkably even among patients with similar predisposing clinical conditions and microbiological exposure. Since there is no evidence for geographical or genomic factors influencing fungal virulence, susceptibility to IA is thought to depend mainly on genetic predisposition and degree of pathogen exposure, with interactions between the two likely contributing substantially to the risk of infection.

The inter-individual variability in the development and progression of IFD raises fundamental questions about their actual pathogenesis. Clinical and epidemiological studies have reported an increasing number of both monogenic defects and common polymorphisms associated with susceptibility to fungal disease. The study of genetic variation regulating the immune response provides important insights into the human immunobiology by pinpointing directly relevant immune molecules and pathways. Genetic studies of susceptibility to infection have typically focused on defects of antibody production, lack of T cells, phagocytes, natural killer cells, or complement, each of which can cause a classic immunodeficiency syndrome. More recently, genetic defects that impair pathogen recognition by the innate immune system and increase susceptibility to selected fungi have also been reported.

This book responds to a pressing demand for timely and authoritative information offering a comprehensive overview of the current state of the art of immunogenetics of fungal diseases. Worldwide leading experts in the field address ongoing developments in the elucidation of the genetic bases regulating the molecular and cellular processes that contribute to human susceptibility to fungal disease in both patients with primary and acquired immunodeficiencies. Moreover, genetics of

susceptibility to fungal disease is discussed within possible strategies aimed at decoding the host-fungus dialogue and at its exploitation towards personalized medical interventions. The discovery of accurate and reliable genetic markers of susceptibility may be a turning point towards innovative clinical tools to predict risk and severity of disease, efficacy of antifungal prophylaxis and therapy, and eventually contribute to the successful design of antifungal vaccines and patient-tailored immunotherapy.

Braga, Portugal Agostinho Carvalho

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Fungal Infections in Primary and Acquired Immunodeficiencies

1

Sarah P. Georgiadou and Dimitrios P. Kontoyiannis

Abstract

IFIs are important causes of morbidity and mortality in patients with either primary or acquired immunodeficiency. A wide spectrum of invasive mold and yeast infections are variably implicated depending on the type of immune deficit. A high index of suspicion is needed as prompt diagnosis of IFIs remains a challenge. Establishment of diagnosis is based on host factors, clinical evidence, and microbiological examination. Advancement in molecular diagnostic methods (e.g. serum biomarkers such as 1,3-β-D-glucan or galactomannan) and high-resolution radiological imaging has improved our diagnostic evaluation. The antifungal armamentarium has expanded rapidly in the past few decades.

Abbreviations

CGD	Chronic granulomatous disease
CNS	Central nervous system
HSCT	Hematopoietic stem cell transplantation
IA	Invasive aspergillosis

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IC	Invasive candidiasis
IDSA	Infectious Diseases Society of America
IFI	Invasive fungal infection
IPA	Invasive pulmonary aspergillosis
SOT	Solid organ transplantation

1.1 Introduction

IFIs important causes of morbidity and mortality in patients with either primary or acquired immunodeficiency. A wide spectrum of invasive mold and yeast infections are variably implicated depending on the type of immune deficit. A high index of suspicion is needed as prompt diagnosis of IFIs remains a challenge. Establishment of diagnosis is based on host factors, clinical evidence, and microbiological examination. Advancement in molecular diagnostic methods (e.g., serum biomarkers such as 1,3-β-D-glucan or galactomannan) and high-resolution radiological imaging has improved our diagnostic evaluation. The antifungal armamentarium has expanded rapidly in the past few decades. Initiation of antifungal treatment requires careful consideration of several factors such as risk stratification, local fungal epidemiologic patterns, concomitant comorbidities, drug interactions, prior history of antifungal use, and the pharmacologic profile of the antifungal agents. In this chapter, we discuss the most common IFIs in patients with either primary or acquired immunodeficiencies.

1.2 Primary Immunodeficiencies

The term primary immunodeficiency disease represents a group of heterogeneous disorders resulting from inherited defects of the immune system. Depending on which component of the immune system is affected, a number of isolated and pleiotropic immune defects have been described, including humoral immune deficiencies, immunodeficiencies of lymphocytes and natural killer (NK) cells, and disorders resulting from phagocytic and complement defects. Primary immunodeficiencies are usually diagnosed during early life (with more than 80% of cases diagnosed before the third decade of life) and may present with recurrent, protracted, or life-threatening infections caused by common pathogens or with infections caused by opportunistic agents, including fungi. As an effective host immune response against fungal organisms depends on the coordinated contribution of both innate and adaptive immunity [1], IFIs are more common and severe in patients with profound innate defects of the macrophage/monocyte axis with or without defects in T-cell function (Table 1.1).

Table 1.1 Summary of primary immunodeficiencies and associated fungal infections

Disease	Fungal infections
Phagocytic disorders	
Chronic granulomatous disease	*Candida*, *Aspergillus*, *Rhizopus*, *Scedosporium*, *Trichosporon*, *Paecilomyces*, *Acremonium*, *Exophiala*, *Penicillium*, *Absidia*, *Fusarium*, *Microascus*, *Inonotus*, *Chrysosporium*, *Cladophialophora*, *Neosartorya*, *Alternaria*
Myeloperoxidase deficiency	*Candida*
Leukocyte adhesion deficiency	*Candida*, *Aspergillus*, *Fusarium*
Congenital neutropenias	*Candida*, *Aspergillus*, *Mucor*
Defects in the IFN-γ/IL-12 axis	Endemic fungi (*Histoplasma*, *Paracoccidioides*)
Chédiak-Higashi syndrome	NS
Cellular and combined immunodeficiencies	
Severe combined immunodeficiency	*Candida*, *Aspergillus*, *Cryptococcus*, *Acremonium*
DiGeorge syndrome	*Aspergillus*
X-linked hyper-IgM syndrome	*Candida*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*
Wiskott-Aldrich syndrome	*Candida*, *Aspergillus*
Humoral immunodeficiencies	
Common variable immunodeficiency (CVID)	*Candida*, *Aspergillus*, *Histoplasma*, *Penicillium*, *Trichophyton*
X-linked/autosomal recessive agammaglobulinemia	NS
IgA deficiency	NS
IgG subclass deficiency	NS
Complement	
Classic, late or alternative complement defects	NS
Mannose-binding lectin pathway defects	*Candida*, *Aspergillus*
Other primary immunodeficiencies	
Hyper-IgE syndrome	*Candida*, *Aspergillus,* Endemic fungi (*Histoplasma*, *Coccidioides*), *Cryptococcus*, *Trichosporon, Penicillium*, *Scedosporium*
Chronic mucocutaneous candidiasis	*Candida*, *Cryptococcus*, *Histoplasma*
GATA2 deficiency	*Candida*, *Aspergillus*, *Histoplasma*
CARD9 deficiency	*Candida*, *Trichophyton*, *Phialophora*, *Exophiala*

NS not susceptible

1.2.1 Phagocytic Disorders

1.2.1.1 Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is a genetically heterogeneous condition characterized by recurrent, severe bacterial and fungal infections and excessive inflammatory reactions leading to granuloma formation [2]. CGD is caused by defects (absence or malfunction) in the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. This enzymatic defect results in the inability of phagocytes to destroy certain catalase-producing bacteria (such as *Staphylococcus aureus*, *Nocardia* spp., and Gram-negative bacteria, such as *Serratia marcescens*, *Burkholderia cepacia*, and *Salmonella* spp.) as well as fungi [3–6]. The diagnosis is made by neutrophil function testing, and the exact defect is determined by genotyping [7]. The frequency of CGD has been estimated to range between 1/200,000 and 1/250,000 live births [8]. The disease primarily affects males as most mutations are X-linked; less patients carry an autosomal recessive form [8, 9]. CGD is the primary immunodeficiency with the highest incidence of fungal infections. Most IFIs among CGD patients are caused by *Aspergillus* spp. and, to a lesser extent, by *Candida* spp. and other fungi [10–13]. The frequency of, and mortality from, IFIs has been markedly reduced since the advent of itraconazole prophylaxis and the use of voriconazole and posaconazole for treatment of filamentous fungal infections (e.g., *Aspergillus*) [14]. The prolonged prophylactic use of interferon (IFN)-γ in patients with CGD appears to be safe although significant reduction in the frequency of serious infections and mortality remains controversial [15, 16]. However, IFIs continue to occur, even in the era of routine prophylaxis with triazoles, and remain the leading cause of infectious death in CGD [5, 17].

Aspergillus infections in CGD patients: *Aspergillus* spp. constitute a major pathogen, being responsible for one-third of all deaths. *Aspergillus fumigatus* and *A. nidulans* are the most commonly isolated species [18]. Importantly, *A. nidulans* infections are seldom reported in other immunocompromised patients, indicating a unique interaction between this fungus and the CGD host and leading to more severe implications than that of *A. fumigatus* [19, 20]. The lungs, the liver, and bones are the most commonly affected organs [18, 21]; however, chronic lymphatic and skin involvement have also been described [5]. Most cases of IPA are suggested on routine chest imaging. Of note, the serum biomarkers such as galactomannan and 1,3-β-D-glucan are of limited value in CGD. Radiographic findings of pulmonary disease include segmental and multilobar consolidation, perihilar infiltrates, multiple small nodules, peripheral nodular masses, and pleural effusions. In contrast to neutropenic patients, the incidence of "classic" radiological signs of lung infection such as the halo, air crescent, and other signs of cavitation appears low [22]. Routine use of itraconazole for prophylaxis has been implemented for almost 15 years, although posaconazole may be an alternative. Management of IA typically requires prolonged courses of antifungal therapy. Surgery may be required for complete resolution in selected cases, especially in the setting of osteomyelitis or infections due to *A. nidulans* [21, 23, 24]. In the case of refractory or life-threatening IA, other interventions may be considered as adjunctive therapy. Transfusion of granulocytes from healthy donors may

partially restore the patient's impaired phagocytic activity and potentially improve outcome [25]. IFN-γ has also been used as adjunctive therapy of IA in CGD patients in a number of case reports though its effectiveness is controversial [16, 26, 27]. HSCT is the only curative strategy for CGD and may be appropriate in selected patients with refractory IA [28].

Candida infections in CGD patients: *Candida* is considered an uncommon pathogen in the CGD population. In a recent review of 68 cases of non-*Aspergillus* IFIs in 65 CGD patients, only six cases (8.8%) were due to *Candida* infections and the main sites of involvement were the liver, the spleen, skin, and soft tissue [29]. In the US registry of 368 CGD patients, *Candida* spp. were isolated from 20% of meningitis cases (the most common cause), 11% of fungemia cases, 7% of suppurative adenitis cases, 4% of subcutaneous abscesses cases, 2% of liver abscesses, and 2% of pneumonia cases [8]. In the largest European cohort to date of 429 patients with CGD, *Candida* spp. were isolated from 3% of patients with septicemia, 2% of those with pneumonia, <1% of liver abscesses, and 0.5% with lymphadenitis [28]. There are no data on the relative frequency of different *Candida* spp. isolated from CGD patients. A number of antifungal agents, including azoles, echinocandins, and amphotericin B, have been used in the treatment of *Candida* infections in CGD patients. Immunotherapy with IFN-γ has also been proposed for the treatment of IC in CGD patients, either for prevention or adjunctive therapy [29].

Other fungal infections in CGD patients: Data concerning non-*Aspergillus* IFIs in CGD patients are limited in the description of individual cases or small case series. Nevertheless, such infections are far less common than IA in this patient population. Among other IFIs in these patients, *Paecilomyces* spp. have been frequently reported, being the third most common cause of osteomyelitis in the US registry of 368 CGD patients (8% of cases) and the cause in 1% of pneumonia cases [8]. Nonetheless, in a recent review of 68 cases of non-*Aspergillus* IFIs in 65 CGD patients, the most prevalent fungal infections were associated with *Mucorales* spp. and *Trichosporon* spp. found in nine cases each (13.2%), followed by *Scedosporium* spp. in eight cases (11.7%) and *Paecilomyces* spp. in six cases (8.8%). The most commonly affected organs were the lungs, skin and soft tissue, gastrointestinal tract, liver, and central nervous system [29].

A number of other fungal species have also been reported sporadically to cause IFIs in CGD patients, including *Acremonium*, *Exophiala*, *Penicillium*, *Absidia*, *Fusarium*, *Chrysosporium*, *Cladophialophora*, *Neosartorya*, and *Alternaria* [10, 11, 29]. As the literature on non-*Aspergillus* IFIs in patients with CGD is scattered, it is difficult to define the best treatment for each of these rare infections.

1.2.1.2 Myeloperoxidase Deficiency

Myeloperoxidase (MPO) deficiency is an autosomal recessive inherited disorder, with a variable clinical phenotype. It is the most common primary phagocyte disorder as 1 in 4000 individuals has complete MPO deficiency, whereas 1 in 2000 has a partial defect [30, 31]. Myeloperoxidase is the most abundant enzyme in the azurophilic granules and plays a critical role in bacterial killing by neutrophils and monocytes [32].

More than 95% of MPO-deficient patients are entirely asymptomatic. Of the small percentage of MPO-deficient patients with clinical findings, infections due to different *Candida* strains are the most frequently reported [30]. Mucocutaneous, meningeal, and bone infections, as well as sepsis, have been described [33–35]. The susceptibility to invasive *Candida* infections appears to be increased in the presence of other comorbidities, especially diabetes mellitus [36]. Antifungal prophylaxis is not routinely recommended.

1.2.1.3 Leukocyte Adhesion Deficiency

Leukocyte migration to sites of inflammation is a dynamic process, involving multiple steps in an adhesion cascade. Various adhesion molecules are expressed on both resting and stimulated endothelial cells and leukocytes. Defects in a number of these adhesion molecules result in rare, inherited leukocyte adhesion deficiency (LAD) syndromes and are typically diagnosed very early in the neonatal period: LAD I, in which the β-2-integrin family is deficient or defective; LAD II, in which the fucosylated carbohydrate ligands for selectins are absent; LAD III, in which activation of all β-integrins is defective; and LAD IV, with Rac2 (Ras-related C3 botulinum toxin substrate 2) deficiency, which is involved in the regulation of NADPH oxidase and the actin cytoskeleton. Although the infection burden of these newborns might be severe, only a few case reports have shown increased susceptibility of these patients to IFIs caused by *Candida* spp., *Aspergillus* spp., and *Fusarium* spp. [37–39].

1.2.1.4 Congenital Neutropenias

The term "congenital neutropenia" is used to indicate neutropenia starting at or around birth, due to a primary bone marrow failure syndrome. It refers primarily to the following three conditions: severe congenital neutropenia (Kostmann syndrome), cyclic neutropenia, and Shwachman-Diamond syndrome. Interestingly, despite the protracted neutropenia, IFIs are infrequent in these patients whose other immune functions are intact. Nevertheless, a few cases with IFIs due to *Candida* spp., *Aspergillus* spp., and mucormycosis have been published [40–43]. Treatment with recombinant human granulocyte colony-stimulating factor (G-CSF) increases neutrophil count and decreases the incidence of severe infections; in the absence of response to G-CSF, HSCT is indicated [44, 45].

1.2.1.5 Defects in the IFN-γ/IL-12 Axis

This heterogeneous group of immune disorders is caused by defects in components of the IL-12, the IL-12 receptor, or the IFN-γ receptor. IL-12 is the main stimulus for production of IFN-γ by Th1 T cells and NK cells; IFN-γ is a critical cytokine in the development of innate and adaptive immune responses to a variety of infectious agents [46]. Patients with these defects have been reported to develop disseminated infections caused by endemic mycoses (*H. capsulatum* and *Paracoccidioides brasiliensis*) [47, 48].

1.2.1.6 Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome (CHS) is a rare autosomal recessive disorder with severe congenital neutropenia that is characterized by recurrent pyogenic infections, hypopigmentation, progressive neurologic dysfunction, and mild coagulation defects. Eighty percent of these patients develop eventually the "accelerated phase" of the disease characterized by massive lymphohistiocytic infiltration of virtually all organ systems which is frequently lethal. Optimal treatment is HSCT [49]. However, these patients do not exhibit increased susceptibility to IFIs.

1.2.2 Cellular and Combined Immunodeficiencies

1.2.2.1 Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is a syndrome caused by mutations in different genes whose products are essential for the development and function of both T and B cells. In some cases, the molecular defect results in isolated T-cell dysfunction or T-cell lymphopenia, while B-cell numbers are normal. However, since B cells depend on signals from T cells to produce antibodies, serious T-cell dysfunction typically affects humoral immunity. NK cells are present in approximately 50% of patients with SCID and may partially provide protection against bacterial and viral infections in these patients. The incidence of SCID is estimated to be 1:50,000–1:500,000 live births. More than half of SCID cases are X-linked [50, 51]. The classic symptoms of SCID are chronic diarrhea, failure to thrive, and severe recurrent infections that are almost always fatal in the first year of life without treatment [52, 53]. Intracellular pathogens are usually implicated (especially *Pneumocystis jiroveci*), viruses, bacteria, and fungi [54–56].

Regarding IFIs, persistent mucocutaneous *Candida* spp. infection is frequent [57]. Moreover, cases of *C. albicans* meningitis and IPA in patients with SCID have been described [55, 58–60]. Sporadic cases of other rare fungal infections have also been reported: a severe disseminated cryptococcal infection in a 23-month-old boy [61] and an invasive gastrointestinal infection due to *Acremonium falciforme* in an 11-month-old girl with SCID who had received a haploidentical T-cell-depleted bone marrow transplantation [62]. The most common curative strategy for all forms of SCID is HSCT or, alternatively, gene therapy [63].

1.2.2.2 DiGeorge Syndrome (22q11.2 Deletion)

DiGeorge syndrome (DGS) is characterized by signs and symptoms associated with defective development of the pharyngeal pouch system. The classic triad of features of DGS on presentation is congenital cardiovascular abnormalities, hypoplastic thymus, and hypocalcemia (resulting from parathyroid hypoplasia) [64]. Thymic hypoplasia in DGS results in a range of T-cell deficits. Most patients with DGS have mild defects in T-cell numbers and are not overtly immunodeficient. Nonetheless,

approximately 1% have a complete absence of thymic tissue and severe immunodeficiency. This form of DGS, called complete DGS, is considered to be a type of SCID with analogous clinical manifestations. A few cases of opportunistic IFIs such as pulmonary and disseminated aspergillosis have been described [65, 66].

1.2.2.3 X-Linked Hyper-IgM Syndrome

The hyperimmunoglobulin M (hyper-IgM or HIGM) syndromes include a heterogeneous group of conditions characterized by normal or increased levels of serum IgM associated with deficiency of IgG, IgA, and IgE and poor antibody function [67]. CD40 ligand (CD40L) deficiency is the most common form of hyper-IgM syndrome. It is inherited as an X-linked condition. The estimated minimal incidence is approximately 1 in 1,000,000 live births. This disease affects the interaction between activated CD4+ T cells and cell types expressing CD40 (B cells, dendritic cells, monocyte/macrophages, platelets, activated endothelial and epithelial cells) and leads to a combined cellular and humoral immunodeficiency [68]. The clinical phenotype of CD40L deficiency is marked not only by recurrent sinopulmonary infections but also by opportunistic infections and liver disease [67]. *Pneumocystis jiroveci* pneumonia is a common clinical feature [69]. Data from the HIGM syndrome registry of the Latin American Society for Immunodeficiencies (LASID) in 58 patients demonstrated several IFIs including *Candida* (n = 6), *Aspergillus* (n = 2), *Paracoccidioides brasiliensis* (n = 1), *Histoplasma capsulatum* (n = 1), and *Cryptococcus neoformans* (n = 1) [70]. In a previous review of 79 patients from the national registry of the USA, the most common fungal pathogens implicated were *Candida*, *Cryptococcus*, and *Histoplasma* [71]. Similarly, in a recent cohort of 11 HIGM patients, nine patients (82%) had IFIs such as *P. jiroveci* and *Candida albicans* and *Paracoccidioides brasiliensis* [72]. Cases of cryptococcal meningoencephalitis or disseminated disease have also been reported [73–76]. Treatment of hyper-IgM syndrome requires regular administration of intravenous immune globulins. However, the only definitive cure for CD40L deficiency is HSCT [77, 78].

1.2.2.4 Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome (WAS) is an X-linked disorder caused by mutations in the gene that encodes the Wiskott-Aldrich syndrome protein (WASp). WASp encodes a 502–amino acid protein that is expressed in hematopoietic stem cell lineages and is associated with cell signaling and cytoskeleton reorganization. The main clinical features of WAS include susceptibility to infections related with adaptive and innate immune deficiency, thrombocytopenia, eczema, and increased risk for autoimmunity and malignancy [79]. WAS is a rare disorder with an estimated incidence of approximately 1 in 100,000 live births. Susceptibility to infections in patients with WAS depends largely upon the effect of the type of mutation on the WASp expression and function. Patients with severe WASp deficiency may have recurrent infections during early infancy, but in the majority of cases, the frequency of infections increases with age [80]. IFIs were reported in 12 out of 50 patients