Immune System and Pathogenesis

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Abbreviation

AIDS	Acquired immunodeficiency syndrome
CLRs	C-type lectin receptors
DCs	Dendritic cells
HIV	Human immunodeficiency virus
IFIs	Invasive fungal infections
Ig	Immunoglobulin
IL	Interleukin
ILCs	Innate lymphoid cells
NK cells	Natural killer cells
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
PTX-3	Pentraxin-3
T_H cells	T helper cells
TLRs	Toll-like receptors
T_{reg} cells	Regulatory T cells

2.1 Introduction

Humans are constantly exposed to fungi, but only a limited number of fungi can cause infection, and clinical disease is rare in non-immunocompromised or noncritically ill patients [1, 2]. The development and severity of invasive fungal infections (IFIs) are closely related to the dysfunction of the patient's immune system. As the world population is changing and with the development of new treatments for patients with haematological malignancy and cancer, haematopoietic stem cell or solid organ transplantation and acquired immunodeficiency syndrome (AIDS), the number of immunocompromised patients has increased over the past two decades and will further increase in the future. As a consequence, the incidence of IFIs will also continue to rise [3].

The host defence mechanisms against fungi are numerous and range from protective mechanisms that were present early in the evolution of multicellular organisms ("innate immunity") to sophisticated adaptive mechanisms, which are specifically induced during infection and disease ("adaptive immunity") [1, 4].

2.2 Pathogenesis

Fungal infections can originate from exogenous source by inhalation of fungal conidia (such as *Aspergillus* sp., *Fusarium* sp., *Mucorales* or pathogens of endemic mycoses) or inhalation of aerosolized basidiospores (*Cryptococcus neoformans*), from endogen source mainly the gastrointestinal tract for commensals (*Candida* sp.) or reactivation of a latent infection. The pathogenicity of the clinically important fungi is mediated

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by a number of virulence factors that facilitate adherence to mucosa and promote formation of biofilms [2], the ability to acquire iron from intracellular host sources [5], the ability to evade host defences (escape from phagocytosis), the production of tissue-damaging hydrolytic enzymes (such as secreted proteases, hydrolyses, elastases, phospholipases and haemolysins) [2], the ability to grow at 37 °C (thermotolerance) [1], the ability to adapt to oxygen-limited microenvironments [6] and the ability to exist in different forms (yeast and hyphal growth forms) and to reversibly switch from one to the other during infection [1]. In particular, dimorphic fungi transform from saprobic filamentous moulds to unicellular yeasts in the host [1]. Some species of *Candida* can grow in different forms (yeasts, blastospores, pseudohyphae and hyphae) depending on infections sites [7]. Also filamentous fungi such as *Aspergillus* sp., inhaled as unicellular conidia, can germinate and transform into branching hyphae (the invasive form of filamentous fungi), in the lungs of an immunosuppressed patient [1, 2].

2.3 Host Immune System Response to Fungal Infection

Figure 2.1 summarizes the host innate and adaptive immune responses that cooperate with one another to eliminate the fungal pathogens. Cell-mediated immunity is the main mechanism of defence, but certain types of antibody response are also protective against fungal infection [4].

2.3.1 Innate Immune System Response to Fungal Infection

The physical barrier of body surfaces and the mucosal epithelial surfaces of the respiratory, gastrointestinal and genitourinary tract are the first line of defence [1, 2]. The skin und mucous membranes have antimicrobial substances on their surface, some of them synthesized by the epithelial and endothelial cells [4]. Furthermore, they have a commensal microflora of saprophytic microorganisms that impede colonization by pathogenic fungi [1]. Most of the inhaled fungal conidia, as well as most of the inspired particles, are eliminated by the action of the cilia of the epithelium of the upper part of the tracheobronchial tree (mucociliary clearance). But fungi, such as *Aspergillus fumigatus*, synthesize toxins that are able to inhibit this ciliary movement [4].

Once fungi invade the mucosa, the host response is mediated by innate immune cells with phagocytes consisting of polymorphonuclear neutrophils, mononuclear leukocytes (monocytes and macrophages), dendritic cells (DCs) and natural killer (NK) cells and by soluble mediators such as complement or different peptides [2]. The response to fungi is activated by soluble and innate cell-associated pattern recognition receptors (PRRs) which are able to recognize conserved structures of microorganisms called pathogen-associated molecular patterns (PAMPs) [2]. Well-known fungal PAMPs include proteins and polysaccharides such as mannan, galactomannan, α- and β-glucan and chitin [2, 8]. Recognition of fungi by the many PRRs is a highly complex and dynamic process. The most important soluble PRRs in the immune response against *Candida* sp. are C-type lectin receptors (CLRs), and against *Aspergillus* infection, opsonization with pentraxin-3 (PTX-3) is also critical [2]. The most important cell-associated PRRs against *Candida* and *Aspergillus* are CLRs, Toll-like receptors (TLRs) and NOD-like receptors [8, 9]. Among the PRRs, the (transmembrane and soluble) CLR receptors mainly recognize β-glucan and mannan. Dectin-1 is the most important CLR. Dectin-1 signalling is crucial for triggering phagocytosis and antifungal activity [10] and plays a key role in balancing T helper type 1/T helper type 17 response [11]. Polymorphisms in dectin-1 are associated with colonization of the genitourinary tract by *Candida* species, recurrent vulvovaginal candidiasis and aspergillosis [9, 12].

PTX-3, secreted by macrophages and epithelial cells during *Aspergillus* infection, binds galactomannan and coated conidia. This step is important because neutrophils take up PTX-3-coated spores much more efficiently than uncoated spores [13]. TLRs are a protein family of cellular receptors that mediate recognition of fungal pathogens

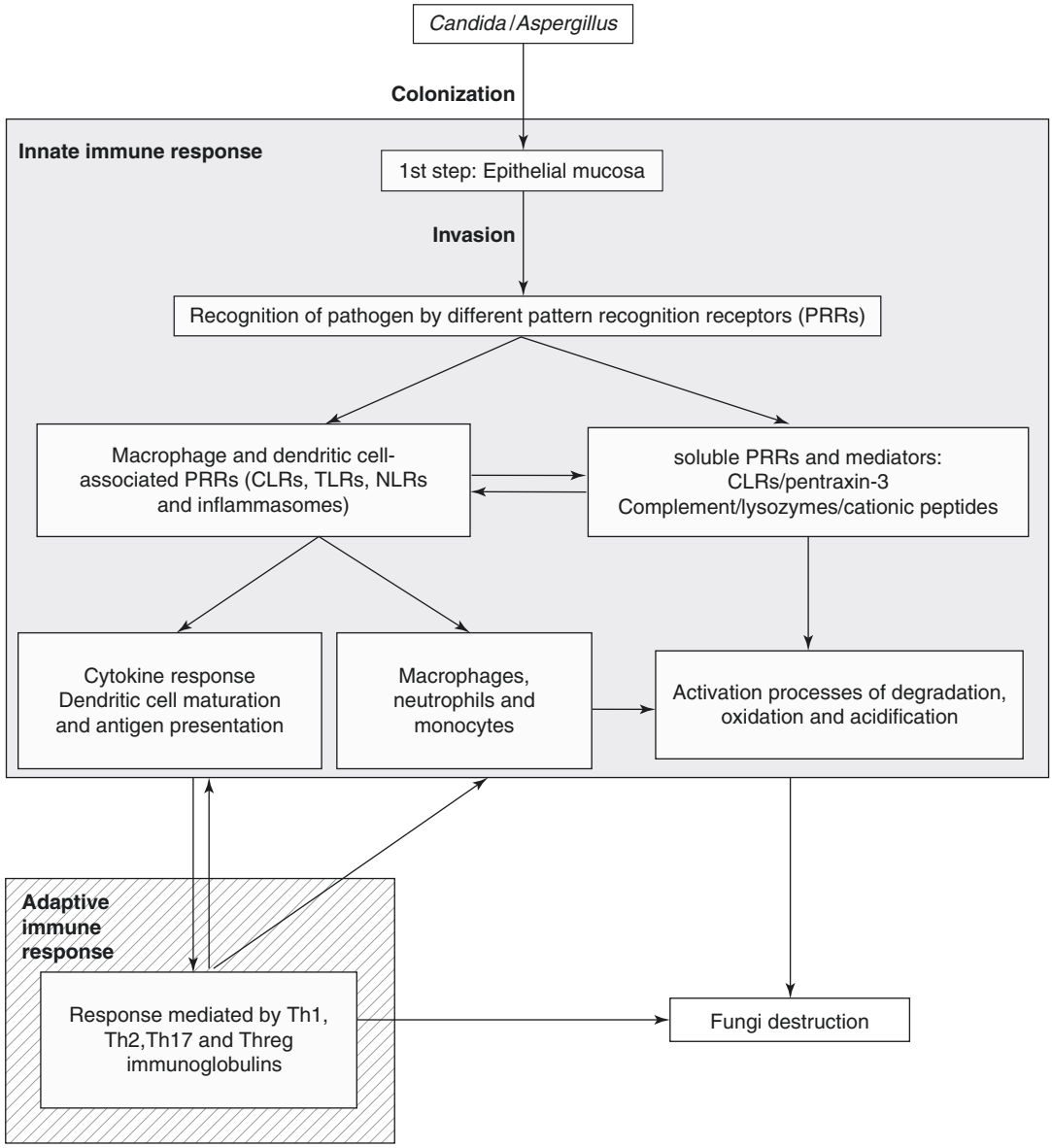

Fig. 2.1 Outline of the host immune response to fungal infections. *CLRs* C-type lectin receptors, *NLRs* NOD-like receptors, *TLRs* Toll-like receptors [2]

and subsequent inflammatory response. Some genetic polymorphisms such as polymorphisms with TLR3, TLR4 or TLR9 have been related to a higher risk of invasive aspergillosis [14, 15]. TLR1 polymorphisms have been also associated with an increased susceptibility to candidemia [16].

A critical point in the defence is the production of chemotactic factors at the site of fungal infection for effective recruitment of leukocytes at that site. These chemotactic factors include chemokines, produced by a variety of cells (including leukocytes, epithelial cells, endothelial cells, fibroblasts and smooth muscle cells), peptides derived from activation of complement pathway, leukotrienes and products synthesized by the fungi [4].

2.3.2 Innate Immune Cells and the Link with Adaptive Response

Polymorphonuclear neutrophils, mononuclear leukocytes and DCs have a different capacity of killing fungal cells depending on the species [1, 4, 17]. For example, macrophages are the primary cells involved in fungal killing during infection with *Cryptococcus* and *Pneumocystis*, whereas neutrophils are the primary effector cells in preventing infection by *Candida albicans* and *Aspergillus fumigatus*. Polymorphonuclear neutrophils are responsible for the destruction of hyphae of *Aspergillus fumigatus*, and they are able to kill the conidia that escape destruction of macrophages [1]. In neutropenic patients, NK cells play an important role in host defence against invasive aspergillosis by secreting IFN-γ, and IFN-γ is crucial for controlling *Aspergillus* infection. In an antigen-independent manner, NK cells induce cell death of the infected cells [8].

Furthermore, DCs can phagocytose both growth forms of *Aspergillus* sp., conidia and hyphae, via two different phagocytic mechanisms [4]. DCs also show greater fungicidal activity compared to macrophages or neutrophils in respect of *Histoplasma capsulatum* [4, 17].

But most important, the antigen-presenting DCs play a vital role in linking innate and acquired immunity against fungi [1, 2]. DCs sample fungi at the site of infection, transport them to the draining lymph nodes and initiate a T-cell response [1] via the secretion of cytokines by promoting the differentiation of naive $CD4^+$ T cells into effector T helper (T_H) cell subtypes: T_H1, T_H2, T_H17 or regulatory T (T_{reg}) cells [2]. The cytokines that drive the differentiation of each particular T_H phenotype are inhibitory to the development of the others, thereby maximizing the potential that only one type of T_H response is initiated at any one time [18].

T_H1 response leads to the production of protective pro-inflammatory cytokines such as IFN-gamma, interleukin (IL)-6 and IL-12, which are essential for clearance of a fungal infection by promoting cell-mediated immunity and phagocyte activation. The T_H2 response is associated with the production of IL-3, IL-4, IL-5 and IL-10 [2] and usually results in susceptibility to invasive fungal infection or allergic responses [4]. The T_H1/T_H2 response is a dynamic process that primarily has an antifungal effect but also plays a key part in balancing pro-inflammatory and anti-inflammatory signals.

IL-17 secreting T lymphocytes (T_H17 cells) are another subset of $CD4^+$ T_H cells. T_H17 cellular response is found early during an immune response and is associated with production of IL-17A, IL-17F and IL-22 [2, 18]. Differentiation of naive $CD4^+$ T cells to the T_H17 phenotype is driven initially by IL-1β, while maturation and terminal differentiation are dependent upon IL-23 signalling. IL-17A and IL-17F are involved in neutrophil recruitment and granulopoiesis. IL-22 is involved in the control of fungal growth at mucosal and non-mucosal sites [19].

T_{reg} cells, also known as suppressor T cells, suppress activation of immune system and maintain immune system homeostasis [19].

To a smaller extent, $CD8^+$ (cytotoxic) T cells and also B lymphocytes, which are cells that secrete antibodies—immunoglobulins (Ig)—principally of IgG type, are also involved in the immunological response to fungal pathogens [17]. In a CD4-deficient host, $CD8^+$ cells may come into play. DCs also activate $CD8^+$ cells by antigen presentation. In contrast, B cells directly react to fungal antigens. Although the role of acquired humoral-mediated immunity against IFIs was uncertain in the past, it has been shown that humoral immunity can protect against fungal infections if certain types of protective antibodies are available in sufficient quantity [4]. The main recognized functions of such antibodies include prevention of adherence, toxin neutralization, antibody opsonization and antibody-dependent cellular cytotoxicity. However, it appears that humoral factors by themselves are unable to prevent fungal development and they are not important in the first stage of infection [4].

Recently, a novel population of innate lymphocytes called innate lymphoid cells (ILCs) has been identified. ILCs differ from T cells because they lack a T-cell receptor. IL-17-producing ILCs have been described as being important in the defence against and the control of fungal pathogens at the mucosal barrier [8].

2.4 Link Between Immunopathogenesis and Clinical Risk Factors for Most Common Invasive Fungal Infections

Patients with high risk for invasive candidiasis include critically ill and severely immunocompromised patients. Factors associated with higher risk of candidiasis in patients admitted to an intensive care unit are mainly associated with mucosa disruption caused by surgery or catheters and *Candida* overgrowth due to antibiotic pressure. In contrast, in severely compromised patients, factors predisposing for candidiasis are similar to those that predispose to aspergillosis [2].

Predisposition to invasive aspergillosis includes cytopenia of all cells of innate immune response with prolonged neutropenia being the most important. Other risk factors for aspergillosis are defective neutrophil function (such as that seen in patients with chronic granulomatous disease), presence of graft-versus-host disease, receipt of corticosteroid therapy or immunosuppressive agents, cytomegalovirus disease and iron overload [2, 20].

The risk factors for mucormycosis are also similar to those of aspergillosis, but diabetes mellitus with poor metabolic control and the use of deferoxamine and voriconazole prophylaxis should also be taken into account [20].

Low CD4 levels, particularly found in HIV-infected/AIDS patients, are the main risk factors for developing fungal diseases with pathogens such as *Pneumocystis jirovecii* or *Cryptococcus neoformans* [20, 21].

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