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Review

The innate immune response to *Aspergillus fumigatus*

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Abstract

Despite the development of new treatments, the mortality due to invasive pulmonary aspergillosis remains above 50%, reaching 95% in certain situations. The battle against *Aspergillus fumigatus* involves several components of the pulmonary innate immune system: cells, mediators, and natural antifungal molecules involved in the recognition and elimination of the fungus, thereby preventing colonization of the respiratory system.

With the 10,000–15,000 l of air we inhale each day, the lungs are constantly exposed to a wide range of microorganisms, such as *A. fumigatus*. This fungus is ubiquitous in the environment and can release large numbers of spores able, due to their small size, to penetrate the respiratory tract. The spores of *A. fumigatus*, like any other pathogen, are then confronted with the innate immune system, a constitutive defense system that is permanently active and tightly regulated. The various elements of the pulmonary innate immune system—physical and cellular barriers and soluble mediators—are involved in the recognition and elimination of pathogens, thereby preventing colonization of the respiratory system. Consequently, the presence of spores in immunocompetent hosts is completely innocuous, because these spores are normally eliminated. However, changes in one of the components of the defense system may lead to the development of pulmonary infections. Thus, in immunocompromised individuals, the spores are able to develop and cause pulmonary mycoses. These mycoses, known as aspergillosis, are highly variable, with the range of presentations extending from an allergy-type illness, allergic bronchopulmonary aspergilloses, to a very serious generalized and frequently fatal infection: invasive pulmonary aspergillosis (IPA).

Keywords: *Aspergillus fumigatus*; Innate defense; Lung; Infection

Despite the development of new treatments, the mortality of IPA remains above 50%, reaching 95% in certain situations. Immunosuppression, whether due to drug treatment or secondary to an underlying disease, plays a key role in the occurrence of IPA. Patients suffering from hematological disorders and patients who have undergone transplantation are therefore at a particularly high risk of IPA.

1. The cells of the innate immune system

1.1. The epithelial cells

The epithelium of the upper airways and of the tracheal and bronchial tract consists of various types of cells, including ciliated cells and mucus-secreting cells. Inhaled spores are trapped in the mucus and then actively transported, by the beating of the cilia, towards the oropharyngeal junction, where they are either swallowed or expectorated. This physical barrier is strengthened by mechanical defenses, including the cough reflex and sneezing, which help to expel the inhaled particles. Most inhaled *Aspergillus fumigatus* spores are eliminated in this way. However, it has been shown *in vitro* that *A. fumigatus* can release factors capable of damaging epithelial cells and slowing the beating of cilia [1]. Furthermore, effective mucociliary elimination requires the production of large amounts of high-quality mucus. In cystic fibrosis patients, the mucus is dehydrated and hyperviscous, preventing the effective mucociliary elimination of microorganisms, thereby increasing the risk of chronic infections. *A. fumigatus* is one of the pathogens frequently isolated from the expectorations of these patients.

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The spores arriving in the alveoli are confronted with two other types of epithelial cell: type I and II pneumocytes. Type II pneumocytes secrete the pulmonary surfactant, the principal function of which is to decrease surface tension at the air–liquid interface, thereby facilitating the expansion of the alveoli during inspiration. This surfactant has also been implicated in the mechanisms of defense against microorganisms, through the antimicrobial activity of two of the proteins it contains: surfactant proteins A and D (SP-A, SP-D; see the section on collectins).

Diverse interactions between epithelial cells and pathogens have been described. Many intracellular pathogens, such as *Legionella pneumophila*, *Mycobacterium tuberculosis* and *Chlamydia pneumoniae* may enter epithelial cells and multiply within them. Although *A. fumigatus* has not been described as an intracellular pathogen, its conidia bind to respiratory epithelium cells and may subsequently be taken up by these cells into acid organelles [2–4]. They may survive in these organelles, and have in some cases been shown to germinate without causing lesions [5]. The discovery of this mechanism suggests that, like intracellular pathogens, *A. fumigatus* uses the respiratory epithelium cells as a refuge from the action of phagocytes and as a starting point for dissemination throughout the body.

1.2. Alveolar macrophages

The alveolar macrophages constitute the first line of phagocytic defense against infectious agents reaching the alveoli. Infectious agents are taken up into macrophages by phagocytosis and the spores of *A. fumigatus* are killed within the cells, preventing their germination. The elimination of spores by alveolar macrophages is highly effective, with 90% of spores killed within 30 h [6]. Macrophages are able to take up dormant or swollen spores (Table 1). Once within the phagolysosomal compartment, the dormant spores become active and swell. This swelling process is essential for elimination by macrophages, because these cells can only kill swollen spores [7].

1.3. Recruited polymorphonuclear neutrophils

Polynumorphoclear neutrophils constitute the largest population of intravascular phagocytes and are essential for host defense against microbial infections. The vascular network of the lung, including the capillaries in particular, is a major reservoir of neutrophils, containing 40% of all the body's neutrophils. During infection, these marginated neutrophils are rapidly recruited to the alveolar spaces to strengthen defenses. Following their recruitment, they may constitute more than 90% of the phagocytic cells present.

Neutrophils play an essential role in the elimination of *A. fumigatus*. Indeed, neutropenic subjects have the highest risk of developing IPA. It has been clearly demonstrated that recruited neutrophils can eliminate *A. fumigatus* filaments extracellularly, by releasing the contents of their granules into the extracellular medium, where they act directly on the fungus. However, in a model of alveolar macrophage depletion *in vivo*, we showed that the neutrophils recruited in the alveoli are also able to eliminate spores by phagocytosis [8] (Table 1). These findings are consistent with those of another study showing that neutrophils have fungicidal activity against spores *in vitro* [9].

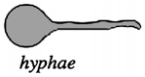
2. Microbicidal activity of respiratory cells

The cells of the upper and lower airways produce a broad range of antimicrobial molecules, helping ensure that the lungs remain sterile (Fig. 1). These molecules may act directly as endogenous antibiotics, or indirectly, by facilitating the elimination of infectious agents by phagocytes.

2.1. Oxidative mechanisms

The principal role of macrophages and neutrophils is the phagocytosis of microorganisms and the killing of these microorganisms in the phagocyte. Various microbicidal mechanisms come into play during phagocytosis, including oxidative mechanisms operating in the presence of oxygen and destroying

Table 1
Innate immune defense against the different phenotypes of *Aspergillus fumigatus*.

	 resting	 swollen	 germling	 hyphae
Recognition	DC-SIGN?	Dectin-1 TLR2 TLR4 DC-SIGN	Dectin-1 TLR2 TLR4	Dectin-1 TLR2 TLR4
Phagocytosis	AM PMN EC	AM PMN		
Intracellular killing		AM PMN EC?		
Extracellular killing			PMN	PMN

AM, alveolar macrophage; PMN, polymorphonuclear neutrophil; EC, epithelial cell; TLR, Toll-like receptor.

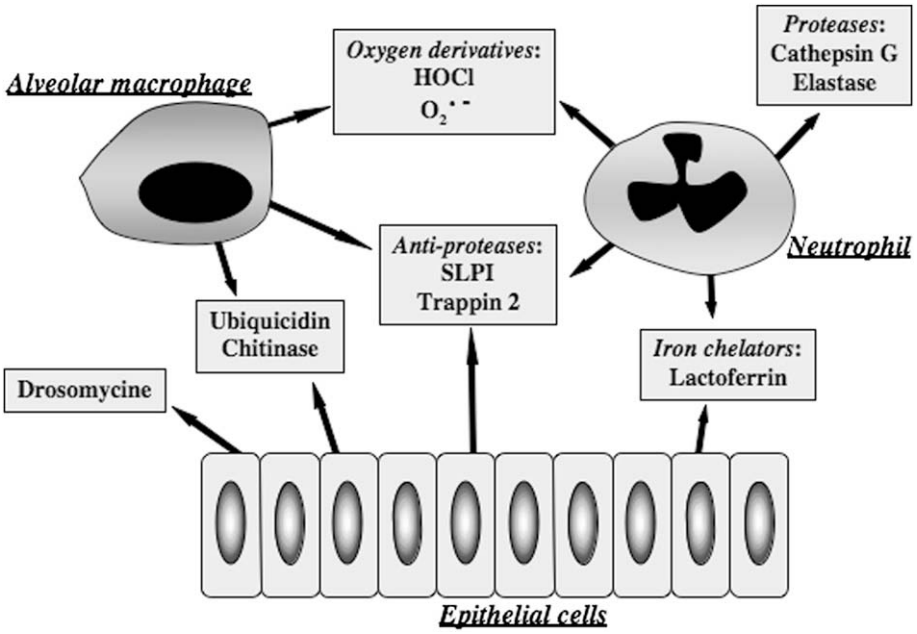


Fig. 1. Microbicidal activity against *Aspergillus fumigatus*. SLPI, secretory leukoprotease inhibitor; HOCl, hypochloric acid; $O_2^{\cdot -}$ superoxide anion.

microorganisms very efficiently. These mechanisms involve reactive oxygen species and reactive nitrogen species derived from nitric oxide (NO).

The enzymes involved in the production of reactive oxygen species during phagocytosis include myeloperoxidase (MPO) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, responsible for the production of hypochloric acid (HOCl) and superoxide anions, respectively. These enzymes are essential for the elimination of *A. fumigatus*. Indeed, the absence of MPO is associated with higher mortality rates in mice infected with *A. fumigatus* [10]. Work with macrophages from mice lacking NADPH oxidase (p47phox−/−) clearly demonstrated that the fungicidal activities of macrophages were associated with the production of reactive oxygen species [7]. These findings confirm those of a study *in vivo* demonstrating that mice lacking NADPH oxidase are more susceptible to infection with *A. fumigatus* (10). They explain why IPA is the leading cause of death in patients with chronic granulatomous disease (CGD), a disease characterized by the absence of NADPH oxidase from neutrophils and macrophages.

A. fumigatus does not seem to be able to inhibit these oxidative mechanisms; the antioxidant molecules produced by this fungus (melanin, catalase, mannitol, superoxide dismutase) have not been found to inhibit the oxidizing activity of phagocytes [11]. By contrast, the treatment of mice with corticosteroids inhibits reactive oxygen species production by macrophages, which continue to be able to take spores up by phagocytosis, but can no longer kill them [7].

Peroxynitrite is a powerful antifungal agent. However, the production and efficacy of reactive nitrogen species during *A. fumigatus* infection remain to be demonstrated. Studies of macrophages from mice lacking NO synthase (iNOS−/−) have shown that the fungicidal activity of macrophages is independent of the production of NO derivatives [7].

2.2. Antimicrobial molecules

Respiratory cells produce a large number of antimicrobial molecules, some of which have antifungal activity against *A. fumigatus*. This is the case for the lactoferrin produced by neutrophils and epithelial cells, which, by binding to iron, deprives the fungus of this element essential for its growth. Lactoferrin thus contributes to eliminating *A. fumigatus*, by inhibiting its growth [12]. Its antifungal effects against *A. fumigatus* were confirmed by a study *in vitro* that compared the activity of several antimicrobial peptides: this work suggested the possible use of peptides derived from lactoferrin or from ubiquicidin, another antimicrobial peptide produced by respiratory epithelial cells, as novel agents for the treatment of aspergillosis [13]. The secretory leukoprotease inhibitor (SLPI) and the pre-elafin trappin-2—serine protease inhibitors secreted by epithelial cells, macrophages and neutrophils—play an important role in maintaining the protease-antiprotease balance in the respiratory tract. They have also been reported to have antimicrobial properties, with activity against *A. fumigatus* in particular [14]. Elastase and cathepsin G, two serine proteases present in the azurophilic granules of neutrophils, have anti-*Aspergillus* activity and mice lacking one or other of these enzymes are more susceptible to *A. fumigatus* infection [15]. Chitinases, produced by epithelial cells and macrophages, are endo-β-1,4-*N*-acetyl glucosaminidases capable of degrading chitin, an essential component of the cell wall of *A. fumigatus* [16]. These enzymes play an important role in antifungal defenses. The human homolog of the drosomycin produced by *Drosophila* has been identified as an antimicrobial peptide. This peptide has antifungal activity specific for filamentous fungi, such as *A. fumigatus*, but seems to be mostly present in the skin [17].

3. Pathogen recognition systems

All the cells of the innate immune system need to be able to detect microorganisms, to activate the processes leading to pathogen elimination. This recognition is based on pathogen recognition receptors (PRRs), which recognize molecular motifs known as pathogen-associated molecular patterns (PAMPs) on the surface of pathogens. PRRs may be classified into three groups, defined on the basis of whether they are secreted, endocytic or induce cell signaling. The combined action of these different families of receptors leads to the recognition and effective elimination of pathogens.

3.1. Secreted PRRs

These receptors act as opsonins, facilitating the interaction of phagocytes with pathogens. The secreted PRRs potentially able to interact with *A. fumigatus* include:

3.1.1. Pentraxin 3

This molecule is produced and released by several types of cells, including macrophages, neutrophils, endothelial cells and dendritic cells, in response to inflammatory stimuli *in vitro* and *in vivo*. It fixes *A. fumigatus* spores. The susceptibility to *A. fumigatus* infection of mice lacking pentraxin 3 is due, in part, to defects in the recognition of spores by host macrophages [18].

3.1.2. Collectins

Collectins are members of the type C lectin superfamily. They recognize and bind to glycoconjugated structures in the cell walls of certain microorganisms, leading to the neutralization or opsonization of the microorganisms concerned. The collectins implicated in the recognition of *A. fumigatus* include surfactant proteins A and D (SP-A and SP-D), which are produced by type II epithelial cells and Clara cells. It has been shown *in vitro* that SP-A and SP-D bind to spores of *A. fumigatus* and increase the phagocytic capacity and fungicidal effects of alveolar macrophages and neutrophils. The effects of SP-D observed *in vitro* have been confirmed *in vivo*: in a mouse model of IPA, nasal administration of SP-D was shown to have a protective effect [19]. Mannose-binding protein (MBP), a serum collectin with several types of antimicrobial activity, also binds *A. fumigatus* spores *in vitro* [20]. However, its effects *in vivo* remain a matter of debate. Indeed, two *in vivo* studies with mouse models have generated conflicting results. The first demonstrated that the absence of MBP had no effect on the survival of immunocompetent mice infected with *A. fumigatus* [21]. By contrast, the second showed that MBP in mice immunocompromised by the administration of corticosteroids protected them against infection with *A. fumigatus* [22]. The difference in the results obtained with these two protocols probably results from the difference in the immune status of the animals studied, with immunosuppression in the second study revealing the role of MBP. These findings suggest that although MBP deficiency may not predispose immunocompetent subjects to IPA, it may have particularly serious consequences in the context of immunosuppression.

3.1.3. Complement factors

The complement system is a collection of more than 35 proteins involved in many functions, including inflammation, opsonization and pathogen destruction. Complement proteins are produced principally by the liver, but local pulmonary production of C2, C3, C4 and factor B by macrophages, of C1 by fibroblasts and of C3 and C5 by type II epithelial cells has been reported. A few relatively old studies addressed the interaction between *A. fumigatus* and complement.

One study reported that the absence of complement from the serum in the culture medium facilitated the destruction of spores by macrophages [23]. By contrast, Hector et al. demonstrated the importance of complement in anti-*Aspergillus* defenses, in an *in vivo* model of mice lacking C5; these mice were found to be more susceptible than controls to infection [24]. The C3 protein binds to *A. fumigatus,* causing dormant spores to generate hyphae, thereby inducing a transition in complement activation from the classical pathway to an alternative pathway leading to the formation of C3b and iC3b [25]. This binding to C3, converted into the C3b or iC3b form, facilitates phagocytosis by macrophages [26]. However, *A. fumigatus* produces an inhibitor capable of decreasing the binding of C3b to its surface. The inhibitory activity of this molecule is linked to *A. fumigatus* phospholipids [27]. This idea that *A. fumigatus* inhibits the activation of complement was taken up in a recent study suggesting that *A. fumigatus,* by binding to factors regulating complement, may inactivate the complement system, thereby escaping from its microbicidal effects [28].

3.2. Endocytic PRRs

Endocytic receptors are present on the surface of phagocytes and facilitate binding to the pathogen before its ingestion. The endocytic receptors implicated in the phagocytosis of *A. fumigatus* include DC-SIGN (dendritic cell-specific ICAM-3-grabbing non-integrin), the mannose receptor and dectin-1.

3.2.1. DC-SIGN

DC-SIGN is a lectin-type receptor thought to be expressed essentially by dendritic cells, although it has recently been detected on macrophages. It facilitates the binding of these cells to *A. fumigatus* spores and the internalization of the spores [29].

3.2.2. The mannose receptor

This lectin-type membrane receptor binds the carbohydrates rich in mannose typically produced by many microorganisms, facilitating their phagocytosis. It has been implicated in the recognition of spores by Langerhans cells [30], but has not been shown to be involved in fungal recognition by alveolar macrophages.

3.2.3. Dectin-1

This transmembrane receptor is expressed by macrophages, neutrophils and dendritic cells and recognizes β-1,3-glucans, major components of fungal cell walls, including those of *A. fumigatus*. The susceptibility of mice lacking dectin-1 to fungal infections demonstrates the importance of this receptor

for innate antifungal defense [31,32]. Dectin-1 is now recognized as a receptor essential for the phagocytosis of fungi by macrophages. Thus, the use of macrophages from mice lacking dectin-1 or the neutralization of dectin-1 by antibodies prevents the binding of zymosan particles to macrophages and their phagocytosis. Swollen or germinating *A. fumigatus* spores, which have abundant β-1,3-glucans on their surface, are recognized by dectin-1 [33]. This recognition is essential for the phagocytosis of swollen spores and germination tubes and for the production of oxygenated free radicals, which play an important role in the fungicidal activity of macrophages [34].

Dectin-1 also activates signaling pathways and interacts with signaling receptors, thereby amplifying the inflammatory response of these receptors. It may therefore also be considered to belong to the PRR family of signaling molecules.

3.3. Signaling PRRs

The binding of microbial motifs to signaling PRRs triggers an inflammatory response, strengthening the innate immune response and influencing the establishment of the adaptive immune response. The signaling PRRs involved in interactions with *A. fumigatus* include many Toll-like receptors (TLRs) and the dectin-1 receptor. However, a receptor of the nucleotide-binding domain leucine-rich repeat-containing protein family (NLR) was also recently implicated in these interactions.

3.3.1. TLRs

Respiratory epithelial cells, alveolar macrophages and neutrophils express all the TLRs, with the exception of TLR3, which is not expressed by neutrophils. TLRs 1, 2, 4, 5 and 6 are generally located at the cell membrane, whereas TLRs 3, 7, 8 and 9 are located in intracellular compartments, such as the endosomes. However, the distribution of TLRs may vary. For example, TLR4 is expressed intracellularly in respiratory epithelial cells.

Wang et al. [35] were the first to implicate TLRs in the recognition of *A. fumigatus*, with their suggestion that CD14 and TLR4 are involved in the recognition of *A. fumigatus* hyphae by human blood moncytes. Mambula et al. [36] carried out a broader study of the role of TLR4, TLR2, MyD88 and CD14 in the activation of murine peritoneal macrophages. They found that levels of TNF-α production were much lower in macrophages lacking TLR2 and MyD88 but not TLR4 than in other macrophages, despite similar levels of binding to the three forms of *A. fumigatus* (dormant spores, swollen spores and hyphae). Another study of peritoneal macrophages showed that TLR4 was involved in the recognition of spores, whereas TLR2 recognized both spores and hyphae. The authors of this study suggested that the expression of different phenotypes by *A. fumigatus* during its development might enable it to escape host defenses [37]. Meier et al. [38] in a study of a human cell line transfected with constructs encoding TLRs 1–10 confirmed the involvement of TLR2 and TLR4 and ruled out a role for other TLRs. They also confirmed the production of TLR2- and TLR4-dependent proinflammatory mediators. The discrepancies between these studies may be accounted for by the murine or human origin of the cells used and by the different tissues from which the macrophages originated. Indeed, one relatively old study [6] described differences in the interaction between *A. fumigatus* and macrophages according to whether the macrophages were of alveolar or peritoneal origin. The capacity of peritoneal macrophages to phagocytose and kill *A. fumigatus* spores was found to be much lower than that of alveolar macrophages. More recently, several studies have identified TLR9 as a PRR involved in the detection of *A. fumigatus*. These studies demonstrated the presence of hypomethylated DNA, the natural ligand of TLR9, in *A. fumigatus* [39] and the involvement of TLR9 in the production of proinflammatory cytokines [40]. While it was observed that the absence of different TLR or even of MyD88 did not render immunocompetent mice susceptible to the infection by *A. fumigatus* [41–43], studies using mouse models of IPA induced by immunosuppressed treatment showed a role of TLR2 [44], of TLR4 and MyD88 [41]. However, the absence of TLR9, unlike that of TLR2 and TLR4, does not seem to be deleterious in mouse models of IPA, instead tending to delay the death of animals infected with *A. fumigatus* [41].

There has been less work on the involvement of TLRs in cells other than macrophages and monocytes. *A. fumigatus* has been shown to interact with TLR2 and TLR4 in corneal epithelial cells, to induce the production of IL-8, but no such interactions have been demonstrated for respiratory epithelial cells. Indeed, a recent study showed that the interaction of respiratory epithelial cells with *A. fumigatus* induces the activation of two different signaling pathways: an MyD88-independent pathway leading to IL-8 synthesis and an MyD88-dependent pathway for which the mediators have yet to be identified [45].

The main message from all these studies is that TLR2 and TLR4 play a predominant role in the recognition of *A. fumigatus*. The findings for TLR4 are supported by the results of a clinical study of genetic polymorphism, which suggested the existence of an association between one particular haplotype of the TLR4 gene in donors and an increased risk of IPA in recipients of allogeneic hematopoietic cell transplants [46].

The PAMPs present on the surface of *A. fumigatus* and likely to activate TLR2 and TLR4 remain to be identified. Only one recent study has demonstrated the activation of macrophages by chitin, via TLR2 [47].

3.3.2. Dectin-1

In addition to its role as a phagocytic receptor, dectin-1 interacts with the TLRs to modulate the immune response. Dectin-1 cooperates with TLR2 in the production of inflammatory mediators by macrophages exposed to *A. fumigatus*. This cooperation acts at two levels: TLR2 does not affect the binding of spores to macrophages, but is essential for effective phagocytosis [48], and recognition by dectin-1 itself induces only a weak inflammatory response, but contributes to the inflammatory response induced by TLR2 [34]. The intracellular part of dectin-1 includes an ITAM motif (immunoreceptor tyrosine-based activation motif) responsible for triggering intracellular signaling and inducing recruitment of

the Syk kinase. In macrophages stimulated with zymosan, Syk is required to induce the oxidative response, but not for phagocytosis. Cooperation between TLR2 and dectin-1 may increase activation of the NF-κB transcription factor, but another transcription factor, AP-1, seems to be involved in the recognition of β-glucans via the dectin-1/Syk pathway.

3.3.3. NOD-2

The role of TLR2 and TLR4 in interactions between macrophages and *A. fumigatus* has been described in detail, but other activation pathways independent of TLRs also seem to be activated. MyD88-independent activation of MAP kinases has been demonstrated in a study *in vitro* [42]. A receptor from the NLR family, NOD2 has also recently been implicated. NOD-2 is a cytosolic receptor expressed essentially by leukocytes, dendritic cells and epithelial cells. The muramyl dipeptide (MDP) has been identified as the minimal peptidoglycan structure expressed by gram-positive and gram-negative bacteria recognized by NOD-2. Although NOD-2 has essentially been described as a receptor of bacterial molecules, it has recently been shown that *A. fumigatus* spores increase NOD-2 levels in alveolar epithelial cells and macrophages *in vitro* and in the lungs of a mouse model of IPA *in vivo* [49]. Thus, NOD-2 may be involved in the innate immune response.

4. Cytokines and chemokines in IPA

The cells of the innate immune system communicate with each other, via soluble mediators known as cytokines, to establish an organized and regulated host response.

The cytokine profile induced during the infection of mice with *A. fumigatus* depends on the immune status of the mice concerned (immunocompetent or immunocompromised) and the type of immunosuppression. Generally, in immunocompetent mice, the lungs contain many cytokines and chemokines, including TNF-α, IL-12, IFN-γ, IL-18, IL-6, IL-1β, IL-10, GM-CSF, MIP-1α, MCP-1, MIP-2 and KC [50,51]. Mice that have been rendered neutropenic or treated with corticosteroids show different profiles [50,51]. The administration or inhibition of some of these cytokines *in vivo* has provided evidence of their involvement in the occurrence or resolution of IPA.

4.1. TNF-α

TNF-α is a cytokine secreted principally by alveolar macrophages, but also by pulmonary epithelial cells. Several studies have demonstrated the importance of this cytokine in the host defense against *A. fumigatus*. TNF-α production is observed after intrapulmonary administration of *A. fumigatus* spores, in mouse models of IPA [51] and its neutralization leads to an increase in fungal load, a decrease in neutrophil recruitment and an increase in mortality [52]. The prior treatment of neutropenic mice with TNF-α increases the resistance of animals to infection with *A. fumigatus* [52]. The beneficial effects of TNF-α administration *in vivo* may be due to this cytokine increasing both the fungicidal effects of neutrophils against hyphae and the phagocytic activity of macrophages. However, despite its beneficial effects, TNF-α is not used in clinical practice due to its toxicity.

Work with e*x vivo* models has shown that wild-type murine alveolar macrophages stimulated with *A. fumigatus* produce TNF-α and that this production is inhibited by prior treatment of the macrophages with the corticosteroid, dexamethasone. This inhibition of TNF-α production is observed *in vivo* in mouse models of IPA based on immunosuppression with corticosteroids [51]. There is also anecdotal evidence from the report of a clinical case of aspergillosis in a patient with Crohn's disease treated with an inhibitor of TNF-α.

4.2. G-CSF and GM-CSF

G-CSF and GM-CSF are hematopoietic growth factors used to treat spontaneous neutropenia and neutropenia induced by chemotherapy for cancer. G-CSF is secreted by macrophages, endothelial cells and fibroblasts. It specifically increases neutrophil counts in the blood by increasing the multiplication of progenitor cells and decreasing the duration of the intramedullary period of maturation of these cells. G-CSF thus increases the capacity of these cells to migrate, to perform phagocytosis and to produce superoxide ions.

GM-CSF is secreted by macrophages, T lymphocytes, endothelial cells and fibroblasts. It favors the proliferation of myeloid cells and their differentiation into thrombocytes, neutrophils, macrophages, monocytes, eosinophils and basophils. GM-CSF enhances the antimicrobial functions of neutrophils, eosinophils and macrophages. It is used to accelerate the recovery of the myeloid system after bone marrow transplantation and in the treatment of leukopenia.

GM-CSF has been found in the lungs of mice infected with *A. fumigatus* and its neutralization by inhibitory antibodies has been shown to decrease neutrophil recruitment and to increase fungal load [53]. The capacity of these two growth factors to induce neutrophil recruitment has been studied in detail. A first study showed that treatment with human G-CSF increases the survival of neutropenic mice infected with *A. fumigatus*, possibly by increasing the number of neutrophils, although this treatment did not increase the survival of mice treated with corticosteroids [54]. However, other studies using mouse models of IPA induced with corticosteroids have shown that each G-CSF or IFN-γ restores the oxidative response of neutrophils and their ability to damage filaments [55]. Similarly, GM-CSF prevents the immunosuppressive effects of corticosteroids on macrophages. This inhibitory effect is also observed *in vivo*. These growth factors, administered alone or together with antifungal treatments, have been subjected to clinical trials and shown to have a beneficial effect as a preventive or curative treatment for fungal infections [56].

4.3. Interleukin-10

IL-10 is a pleiotropic cytokine produced essentially by Th2 lymphocytes, but also by macrophages, dendritic cells and B lymphocytes. It is considered to be an important regulator of the immune system.

IL-10 has an inhibitory effect on certain immune responses, including the oxidative and antifungal response of monocytes to filaments of *A. fumigatus*, but may also increase the capacity of these cells to carry out phagocytosis. IL-10 has been detected in the lungs and blood of model mice susceptible to infection with *A. fumigatus*. Mice with high serum concentrations of IL-10 are more susceptible to IPA, and are protected by the neutralization of this cytokine [57]. Similarly, mice lacking IL-10 are more resistant to IPA and have a lower fungal load in cases of systemic *A. fumigatus* infection [58]. IL-10 has been detected in the lungs of mice immunocompromised by the administration of corticosteroids [59,51]. In humans, higher serum IL-10 concentrations have been found in patients colonized with *A. fumigatus* than in uncolonized patients, and high IL-10 levels correlated with a fatal outcome [60]. Studies of genetic polymorphism of the IL-10 promoter have demonstrated differences in the level of IL-10 production between individuals: the IL-10-1082(AA) genotype, which is responsible for weak IL-10 production, is associated with resistance to the development of IPA [61]; by contrast, the IL-10-1082A allele, causing higher levels of IL-10 production, is associated with susceptibility to IPA; and the IL-10-1082GG genotype is associated with an increase in the risk of *A. fumigatus* colonization in patients with cystic fibrosis [62].

4.4. Interleukin-8

IL-8, also known as CXCL-8, is a chemokine produced by the cells of the pulmonary innate immune system, such as macrophages, epithelial cells and neutrophils, but also by T lymphocytes, fibroblasts and endothelial cells. In addition to its strong chemotactic influence on neutrophils, IL-8 has multiple effects on the degranulation of lysosomes and the liberation of their enzymes, the production of free radicals and the expression of adhesion molecules by neutrophils. The multiple stimuli inducing its production and its many effects make IL-8 a powerful inflammatory mediator.

Mice, unlike humans, have no IL-8 gene, but do express the now ineffective gene encoding the receptor for this cytokine (*Ilr8*). The mouse chemokines KC and MIP-2, from the CXC/ELR+ family, are considered to be the murine homologs most closely related to GRO-α and IL-8, respectively.

The role of KC and MIP-2 in the recruitment of neutrophils during experimental IPA has been extensively described. KC and MIP-2 are induced in response to the intratracheal administration of *A. fumigatus* spores in immunocompetent or neutropenic mice. The neutralization of the receptor shared by these two chemokines leads to a decrease in neutrophil recruitment in immunocompetent mice, resulting in fungal invasion and mortality rates similar to those for neutropenic mice. The role of KC has been clarified by a study of mice overproducing this chemokine. These mice, which are more resistant to *A. fumigatus* infection, present a lower fungal load and higher levels of neutrophil recruitment. In the lungs of these mice, larger amounts of IL-12 and IFN-g are recovered, suggesting that KC may regulate the expression of cytokines important for the host defenses [63].

The production of IL-8 by several types of human respiratory cell in response to stimulation with *A. fumigatus* has been demonstrated. Respiratory epithelial cells stimulated with proteases released by *A. fumigatus* or by fungal filaments [45] produce IL-8. Neutrophils stimulated with *A. fumigatus* antigens also produce IL-8, in a TLR2- and TLR4-dependent manner [64].

5. Conclusion

All of the experimental and clinical studies carried out to date, both *in vitro* and *in vivo*, demonstrate that the innate immune system provides the body with its most effective defense against IPA. Indeed, the battle against *A. fumigatus* involves several components of this immunity, in terms of cells (macrophages, epithelial cells and neutrophils), mediators (cytokines and chemokines), and the natural antifungal molecules formed (reactive oxygen species and proteases). These findings are supported by the implication of innate immune system receptors, such as TLRs and dectin-1, in these defenses. Adaptive immunity may also be triggered through CD4+T cells, but this process may not always be beneficial, as shown for bronchopulmonary aspergillosis. Innate immunity therefore plays a key role in the fight against IPA. Only when innate immunity is modified can *A. fumigatus* develop and invade the body.

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