

Aspergillus fumigatus and Related Species

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The genus *Aspergillus* contains etiologic agents of aspergillosis. The clinical manifestations of the disease range from allergic reaction to invasive pulmonary infection. Among the pathogenic aspergilli, *Aspergillus fumigatus* is most ubiquitous in the environment and is the major cause of the disease, followed by *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus nidulans*, and several species in the section *Fumigati* that morphologically resemble *A. fumigatus*. Patients that are at risk for acquiring aspergillosis are those with an altered immune system. Early diagnosis, species identification, and adequate antifungal therapy are key elements for treatment of the disease, especially in cases of pulmonary invasive aspergillosis that often advance very rapidly. Incorporating knowledge of the basic biology of *Aspergillus* species to that of the diseases that they cause is fundamental for further progress in the field.

Invasive aspergillosis (IA) is one of the most serious clinical invasive fungal infections, resulting in a high case-fatality rate among immunocompromised patients (Lin et al. 2001). The disease is caused by saprophytic molds in the genus *Aspergillus*. Established by Micheli in 1729, the genus *Aspergillus* was created to accommodate an asexual fungus that produces spore chains or columns radiating from central structures. As a priest, Micheli recognized the resemblance of the spore-forming structures with that of the aspergillum (holy water sprinkler), and therefore applied the name *Aspergil-*

lus to the fungus. More than 100 years later, *Aspergillus fumigatus*, the most important pathogenic species of the genus, was described by Fresenius (1863), and the role of aspergilli in human disease was first recognized by Virchow in 1856 (Virchow 1856). Virchow noticed the similarity between the aspergilli described from cases of animal infections and those he had observed in human disease. IA was later first recognized by Rankin, when he reported disseminated aspergillosis in a patient with aplastic anemia (Rankin 1953). For the subsequent 60 years, the number of cases of IA increased



Editors: Arturo Casadevall, Aaron P. Mitchell, Judith Berman, Kyung J. Kwon-Chung, John R. Perfect, and Joseph Heitman
Additional Perspectives on Human Fungal Pathogens available at www.perspectivesinmedicine.org

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substantially because of an increase in the number of immunocompromised patients, such as those with malignancies, AIDS, solid organ transplantation (SOT), and those patients receiving immunosuppressive treatments for countless underlying conditions (Denning 1998; Kontoyiannis and Bodey 2002; Marr et al. 2002a).

MYCOLOGY

Taxonomy and Nomenclature of the Pathogenic *Aspergillus* Species

The genus *Aspergillus* is characterized by the formation of flask-shaped or cylindrical phialides either in a single or double series on the surface of a vesicle at the apex of a conidiophore (Raper and Fennell 1965). Conidia are deciduous and globose, oblong to elliptical in shape, and present various colors. In 1926, Thom and Church assembled all available information on *Aspergillus* in a monograph (Thom and Church 1926). In 1965, Raper and Fennell expanded this work by adding many new species and provided descriptions of the species classified into 18 informal groups (Raper and Fennell 1965). In 1985, Gams and coinvestigators revised the groups and assigned them to 18 sections as a formal taxonomic status (Gams et al. 1985). Currently, there are approximately 250 species assigned to 17 sections in the family Aspergillaceae (Houbraken and Samson 2011) and this number will continue to grow as new species are described. The major species known to cause disease in humans are found in five *Aspergillus* sections: *Fumigati*, *Flavi*, *Nigri*, *Terrei*, and *Nidulante*. The most common species causing invasive disease is *A. fumigatus* in the section *Fumigati*. *Aspergillus flavus* (section *Flavi*), *Aspergillus niger* (section *Nigri*), and *Aspergillus terreus* (section *Terrei*) are secondary to *A. fumigatus* as etiologic agents in SOT and hematopoietic stem cell transplantation (HSCT) recipients as well as patients with leukemia (Perfect et al. 2001; Marr et al. 2002a; Pagano et al. 2010; Pappas et al. 2010). *Aspergillus nidulans* (section *Nidulante*) rarely causes IA in immunocompromised patients, but is a common etiology of IA

in patients with chronic granulomatous disease (CGD) (Cohen et al. 1981; Henriot et al. 2013). *A. fumigatus*, *A. flavus*, and *A. nidulans* reproduce asexually and sexually, and their sexual states have been described in the genus *Neosartorya* (O’Gorman et al. 2009), *Petromyces* (Horn et al. 2009), and *Emericella* (Kwon-Chung and Bennett 1992), respectively. Because the description of a sexual state merely completes the characterization of the fungus that is usually already known (Kwon-Chung and Bennett 1992), these names have been abandoned in 2013 as the fungal nomenclatural rule was changed to use only one name for one fungal species (Hawksworth et al. 2011). Sexual reproduction in heterothallic aspergilli, such as *A. fumigatus* and *A. flavus*, requires two strains of compatible opposite mating types. Sexual reproduction in homothallic species, such as *A. nidulans*, is accomplished by a single strain harboring the two compatible mating-type loci required for mating. In heterothallic or homothallic species, mating produces ascospores, sexual spores formed by meiosis, which are enclosed inside sexual fruiting bodies called cleistothecia. Most ascospores have a furrow or slit (Houbraken and Samson 2011).

A. fumigatus and Other Pathogenic Members of Section *Fumigati*

The section *Fumigati* is of special interest to the field of infectious diseases because this section includes not only *A. fumigatus* but also 11 other species as the etiologic agents of IA. Section *Fumigati* is characterized by the formation of a conidiophore with columnar conidial heads consisting of flask-shaped vesicles, uniseriate phialides, and long chains of conidia. Conidia are bluish-green to pale green, generally hydrophobic, and have a size of 2.5–3.5 μm . It has been estimated that *A. fumigatus* is the etiologic agent of various forms of aspergillosis in more than 200,000 patients a year (Brown et al. 2012). It is unclear whether this estimate represents only those caused by *A. fumigatus* or includes its morphologically indistinguishable sister species. Molecular diagnostic approaches have revealed that there are at least 11 species that could



easily be confused with *A. fumigatus* based on their conidial morphology. Besides *A. fumigatus*, the sister species *Aspergillus udagawae* (*Neosartorya udagawae*), *Aspergillus pseudofischeri* (*Neosartorya pseudofischeri*), *Aspergillus lentulus*, *Aspergillus felis*, *Aspergillus hiratsukae* (*Neosartorya hiratsukae*), *Aspergillus fischeranus* (*Neosartorya fischeri*), *Aspergillus viridinutans*, *Aspergillus fumisynnematus*, *Aspergillus fumigatiaffinis*, *Aspergillus novofumigatus*, and *Aspergillus laciniosa* (*Neosartorya laciniosa*) have been reported sporadically in cases of aspergillosis. Of these, *A. udagawae*, *A. lentulus*, and *A. pseudofischeri* have been reported more frequently (Padhye et al. 1994; Alcazar-Fuoli et al. 2008; Alhambra et al. 2008; Vinh et al. 2009b; Gyotoku et al. 2012; Zbinden et al. 2012) than the other eight species (Lonial et al. 1997; Guarro et al. 2002; Yaguchi et al. 2007; Alcazar-Fuoli et al. 2008; Shivaprakash et al. 2009; Vinh et al. 2009a; Barrs et al. 2013; Malejczyk et al. 2013; Pelaez et al. 2013; Singh et al. 2013). A sexual state (*Neosartorya*) was described in nine of the 12 pathogenic species in the section *Fumigati* (Samson et al. 2007; O’Gorman et al. 2009; Swilaiman et al. 2013). It could be that the three asexual species, *A. fumisynnematus*, *A. fumigatiaffinis*, and *A. novofumigatus*, require nonconventional growth conditions to undergo the sexual cycle, as was the case with *A. fumigatus* (O’Gorman et al. 2009).

ECOLOGY

Aspergillus species from section *Fumigati* as well as the other four major aspergilli known to cause disease in humans (*A. nidulans*, *A. flavus*, *A. terreus*, and *A. niger*) are found in a wide variety of substrata, including soil, compost piles, fruits, organic debris, animals, and humans (Thom and Church 1918; Samson et al. 2007; Barrs et al. 2013). Although most of these species are ubiquitous in the environment (Raper and Fennell 1965), *A. fumigatus* remains the major cause of IA. One reason for the prevalence of *A. fumigatus* as the main clinical cause of aspergillosis is the exceedingly high abundance of the species, with the average human inhaling hundreds of airborne conidia daily

(Latgé 1999). *A. fumigatus* is more ubiquitous than other aspergilli owing to their superior ability to survive and grow in a wider range of environmental conditions compared to other species. In the saprophytic cycle, conidia released from conidiophores are readily dispersed in the environment. Conidia remain dormant until encountering the environmental conditions that allow activation of their metabolism. Once metabolically active, conidia swell and germinate into hyphal filaments that grow into mycelium and produce conidia bearing fruiting structures (conidiophores) (Fig. 1).

Although *A. fumigatus* grows optimally at 37°C, it can be isolated wherever soil and decaying vegetation reach temperatures ranging between 12°C and 65°C, as well as pH between 2.1 and 8.8 (Jensen 1931). Owing to its pronounced ability to tolerate high temperature, self-heating compost piles are major environmental sources (Latgé 1999; Kwon-Chung and Sugui 2013). *A. fumigatus* also contains numerous genes encoding glycosylhydrolases and extracellular proteinases in its genome, whose products support the ability to grow successfully by degrading polysaccharides from plant cells and acquiring nitrogen by degradation of proteinaceous substrates (Tekaiia and Latgé 2005; Abad et al. 2010). Not only can *A. fumigatus* grow on a wide variety of substrates, but it can also tolerate stresses imposed by freezing or dehydration for prolonged periods (Kozakiewicz and Smith 1994). Furthermore, conidia of *A. fumigatus* disperse more readily than most other aspergilli because of the notorious hydrophobicity of *A. fumigatus* conidia (Bayry et al. 2012). The ubiquitous nature of *A. fumigatus* is also partly because of its ability to produce potent secondary metabolites and abundant efflux pumps that serve as defense systems (Kwon-Chung and Sugui 2013).

LABORATORY IDENTIFICATION AND BIOLOGY

Identification of the *Aspergillus* species is based on a combination of biotype and genotype. A biotype involves cultural and morphological characteristics, whereas a genotype is defined

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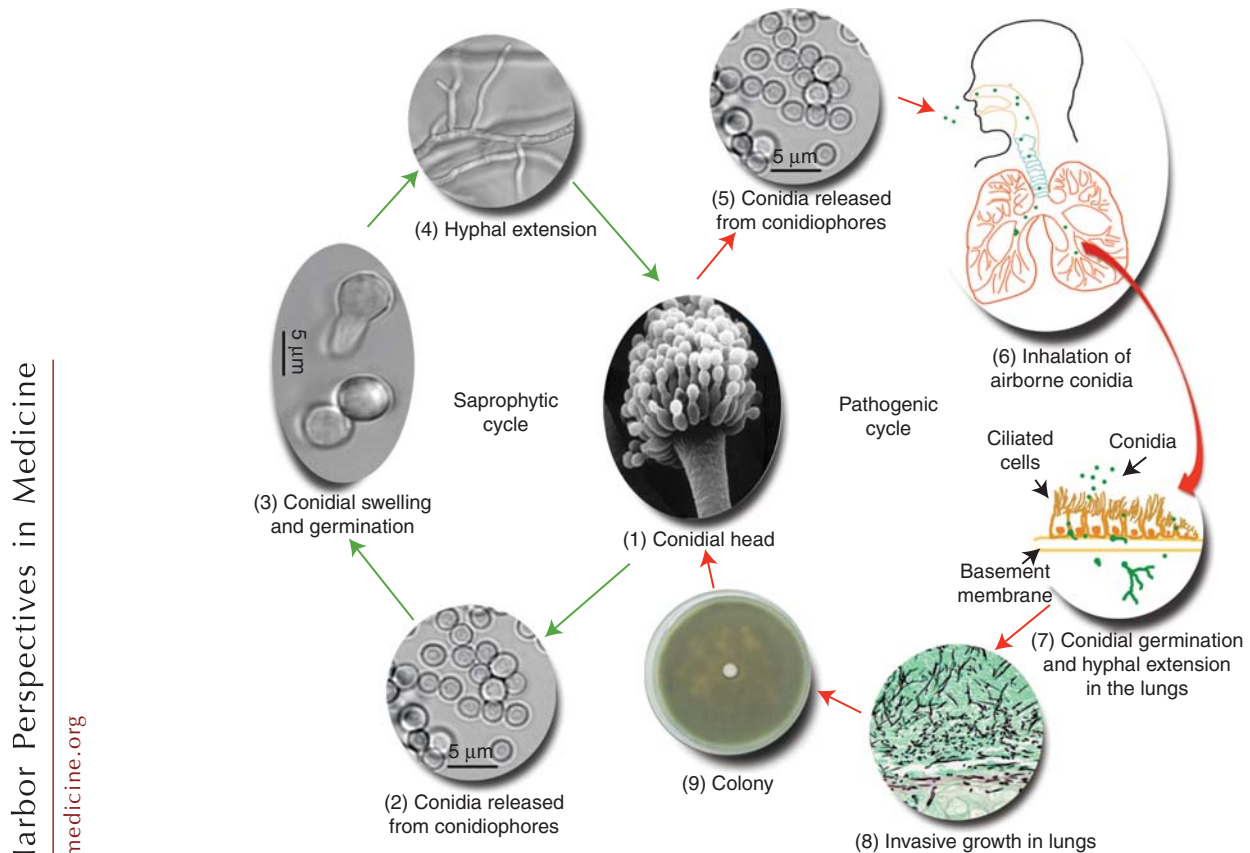


Figure 1. Saprophytic and pathogenic cycles of *A. fumigatus*. Saprophytic cycle: (1) *A. fumigatus* conidial head bearing conidia (spores). (2) Mature conidia are highly hydrophobic and readily dispersed. (3) On germination, conidia swell and germinate into hyphal filaments. (4) Extension of hyphal filaments forms an interwoven net called mycelia. Pathogenic cycle: (5) and (6) Airborne conidia are constantly inhaled by humans. (7) In severely immunosuppressed patients, conidia may escape from host defenses and grow invasively. (8) Extensive hyphal growth in the lungs of an immunocompromised patient. (9) Colony growth.



by the sequences of certain genes. Etiologic agents of aspergillosis are typically very distinct in their appearance; thus the biotype (shape, size, and color of the conidial head, thermotolerance, etc.) is a good indicator of their identity. For example, the bluish-green color of *A. fumigatus* conidial heads is clearly distinct from the yellowish-green, tan, dark green, or black conidial heads of *A. flavus*, *A. terreus*, *A. nidulans*, and *A. niger*, respectively (Fig. 2). In instances in which differences in the color of conidial heads are not clear, other characteristics can be helpful in species identification. For example, *A. nidulans* possesses deep green conidial heads, which

might be mistaken for the bluish-green color from *A. fumigatus* (Fig. 2). Unlike *A. fumigatus*, however, *A. nidulans* conidia are produced by biserial phialides. The presence of Hülle cells enclosing the cleistothecia produced by *A. nidulans* can also serve as a differential marker. Hülle cells are thick-walled, variously shaped, cells that often encase cleistothecia (Raper and Fennell 1965; Kwon-Chung and Bennett 1992). Conidial size can also aid in the identification of some species. *A. fumigatus*, *A. terreus*, and *A. nidulans* all have conidia ranging from 2 to 3.5 μm , whereas conidia from *A. flavus* and *A. niger* are larger (3–5 μm) (Raper and Fennell

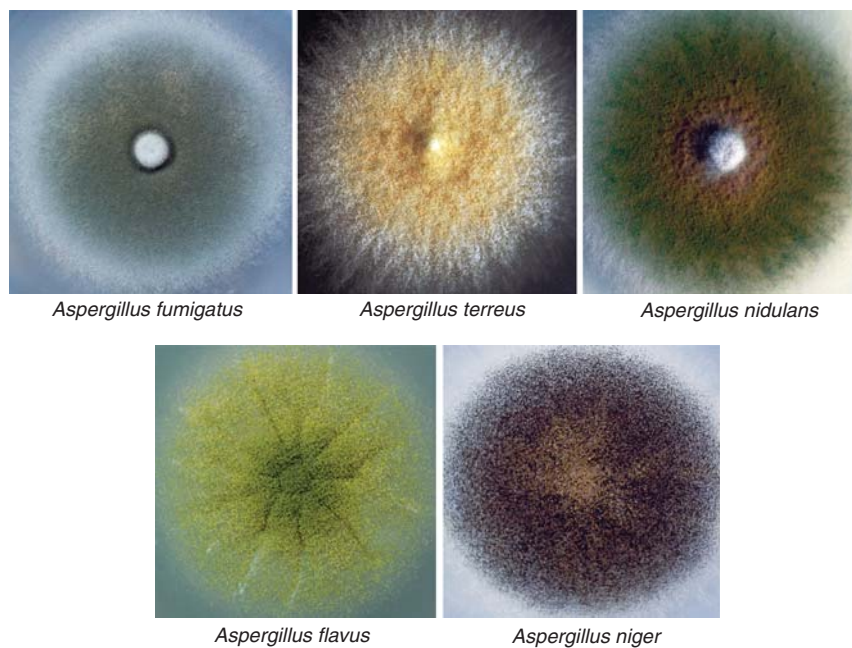


Figure 2. Major pathogenic species of *Aspergillus*. *A. fumigatus*, *A. terreus*, *A. nidulans*, *A. flavus*, and *A. niger* were inoculated on Czapek-Dox agar and grown at 37°C.

1965). Although differences in biotype can often be sufficient to distinguish *A. fumigatus* from other aspergilli, identification should be confirmed by their genotype. Internal transcribed spacer (ITS) sequences, along with others such as fungal sequences from the genes *MCM7*, *TSR1*, *RPB1*, and *RPB2*, are useful for species identification (White et al. 1990; Peterson 2008; Schmitt et al. 2009).

Although differentiation between *A. fumigatus* and the pathogenic species from other sections of *Aspergillus* is relatively straightforward because of their distinct morphology, differentiation between *A. fumigatus* and its sister species becomes difficult because of their morphological resemblance. In fact, relying solely on morphological characteristics has led to misidentification of several of the sister species. One of the first clues suggesting misidentification of the sister species was the extensive aerial hyphal growth with delayed and sparser conidiation on agar compared to the fast and abundant production of conidia in *A. fumigatus*. Thermotolerance is another key biotype that

can aid in the differentiation of *A. fumigatus* from its sister species. Whereas *A. fumigatus* is able to grow at 50°C but not at 10°C, the sister species are generally able to grow at 10°C but not at 50°C (Samson et al. 2007; Sugui et al. 2010; Posteraro et al. 2011; Barrs et al. 2013). Compared to biotype analysis, which requires a few days to weeks before the characteristic fruiting structures are produced, genotype analysis can be performed as soon as the species grows efficiently to allow DNA isolation. The ITS sequence is reliable in determining the *Aspergillus* sections, but lacks variability in species identification within the section *Fumigati*. Alternatively, the sequences from the genes encoding β -tubulin, rodlet A, and calmodulin are more suitable for the distinction between *A. fumigatus* and its sister species (Samson et al. 2007). A minimum of 99% sequence identity is the typical threshold adopted for species determination in *Aspergillus*.

Aspergillus species differentiation can be clinically important for antifungal treatment selection. Two studies analyzing the minimal in-

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hibitory concentration (MIC) distribution of more than 5000 isolates, including *A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*, showed that some species are significantly more susceptible to amphotericin B, itraconazole, or voriconazole (Espinel-Ingroff et al. 2010, 2011). In the case of *A. pseudofischeri* and *A. udagawae* in the section *Fumigati*, susceptibility to amphotericin B and itraconazole is somewhat controversial. For voriconazole, on the other hand, there is a general consensus that these species are less susceptible than *A. fumigatus* (Balajee et al. 2006; Vinh et al. 2009b; Posteraro et al. 2011). The variable susceptibility to antifungals may have contributed to the different outcomes observed in patients; although treatment with antifungals was effective in several cases with *A. pseudofischeri* and *A. udagawae*, fungal growth was uncontrolled in other cases despite antifungal treatment (Coriglione et al. 1990; Summerbell et al. 1992; Jarv et al. 2004; Ghebremedhin et al. 2009; Vinh et al. 2009b; Posteraro et al. 2011; Gyotoku et al. 2012; Toskova et al. 2013). *A. lentulus* is overall less susceptible than *A. fumigatus* to amphotericin B, itraconazole, and voriconazole based on its high MICs (Balajee et al. 2006; Alcazar-Fuoli et al. 2008; Symoens et al. 2010). These in vitro susceptibilities correlate, to some extent, with the findings in clinical settings. Death of a significant number of patients infected by *A. lentulus*, despite treatment with antifungals, substantiates that *A. lentulus* is more refractory to antifungal agents than *A. fumigatus* (Alhambra et al. 2008; Montenegro et al. 2009; Zbinden et al. 2012).

PATHOGENESIS

One of the most relevant questions in understanding the pathobiology of *A. fumigatus* is identifying virulence determinants. This is complicated because of the redundancy of genes with the same functions, pleiotropic effects of several genes, and complex enzymatic systems encoded by gene clusters. Moreover, as a successful saprophyte, *A. fumigatus* does not possess any particular set of virulence factors. According to the classical definition of “virulence factors,” which

includes genes that affect virulence but are dispensable for normal growth, only a few genes/molecules fulfill these requirements. Two of these factors are conidial melanin and gliotoxin. *A. fumigatus* does not require either of these two factors for growth; however, loss of conidial melanin rendered *A. fumigatus* nearly avirulent when inoculated intravenously in BALB/c mice (Tsai et al. 1998) and, in the absence of gliotoxin synthesis, virulence was reduced in a host immune-status dependent manner. Reduced virulence was observed in non-neutropenic mice immunosuppressed with hydrocortisone (Sugui et al. 2007; Spikes et al. 2008) but not in neutropenic mice immunosuppressed with cyclophosphamide and cortisone (Cramer et al. 2006; Kupfahl et al. 2006). Melanin and gliotoxin are part of the fungal defense system to survive in an extreme environment, such as in soil in which thousands of microorganisms cohabit. To a certain degree, such a defense system may also protect aspergilli in the host environment (Kwon-Chung and Sugui 2013).

As an opportunistic pathogen, nearly all published *A. fumigatus* mutant strains defective in genes responsible for its normal growth through proper hyphal extension exhibit a virulence defect in murine models. However, it is clear that not every *A. fumigatus* mutant strain with an in vitro growth defect displays a virulence defect. Similar to in vitro growth, lung fungal burden has been repeatedly directly correlated to virulence in the murine model of IA. Several of these genes can be considered as virulence factors because they are crucial for fungal establishment within nutrient-limited host tissue. For instance, iron and zinc are of prime importance for *A. fumigatus* growth. Consequently, genes encoding proteins involved in acquisition of these two metals are indispensable for *A. fumigatus* to cause disease (Moreno et al. 2007; Haas 2012; Moore 2013). The calcineurin signaling pathway is another example of a system that is required for normal growth and that deeply impacts virulence of *A. fumigatus* (Steinbach et al. 2007). Deletion of the *cnaA* gene encoding the catalytic subunit calcineurin A affects the extension process of hyphal filaments. Without the ability to extend, hyphal

invasion of the host tissue and virulence is compromised; thus, even if the fungus is able to withstand host defenses it will not cause IA. In fact, virulence of a *cnaA* deletion mutant in a murine model was greatly reduced compared to the wild-type strain (Steinbach et al. 2006; da Silva Ferreira et al. 2007). In general, the array of genes and molecules associated with *A. fumigatus* virulence can be classified based on their involvement in specific biological processes. Thermotolerance, cell-wall composition and maintenance, defense against host immune response, toxin production, and nutrient uptake during host invasion are just some examples of the processes involved (Fig. 3). The importance and the role of genes involved in these processes in *A. fumigatus* pathogenesis have been extensively reviewed (Hohl and Feldmesser 2007; Askew 2008; Abad et al. 2010; Sales-Campos et al. 2013).

The epithelium is the first point of contact for fungal conidia within the lung and represents an important surface of interaction. Despite this, the interactions between epithelial cells and *A. fumigatus* remain poorly understood (Svirshchevskaya et al. 2012). It has been reported that epithelial cell lines, such as A549, are able to internalize conidia readily, whereas primary cell lines are not. A major innate immune response to inhaled conidia is the synthesis of proinflammatory cytokines, such as IL-8, Granulocyte macrophage colony-stimulating factor, and the proapoptotic factor tumor necrosis factor- α . Inhibition of apoptosis, which has been regarded as a mechanism by which a pathogen overcomes host defense, has been shown to be specifically induced by conidia. In addition, conidia are in contact with many different fungicidal factors present in the upper layer of the mucous epithelium. Among them,

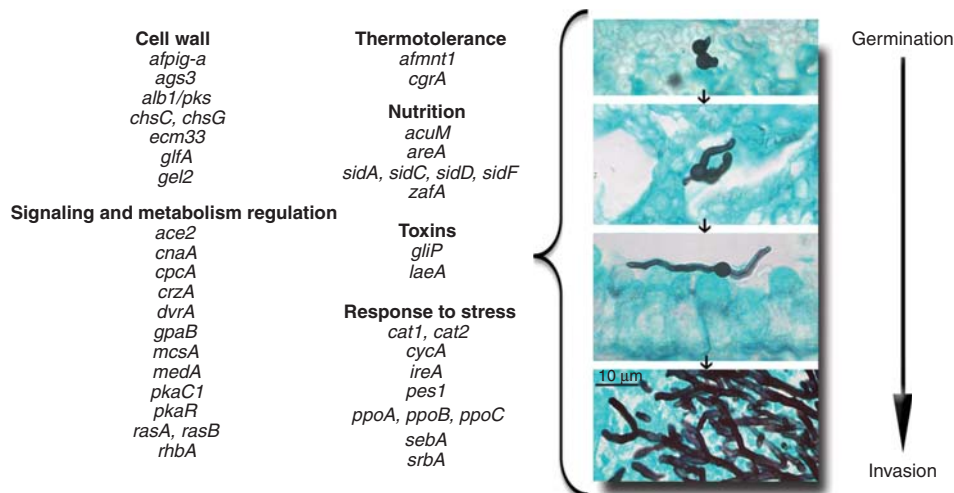


Figure 3. Select genes associated with *A. fumigatus* virulence. The listed genes interfere with various aspects of *A. fumigatus* survival, growth, and response to host defenses. Growth stages of *A. fumigatus* inside of lungs of mice immunosuppressed with hydrocortisone are represented. Fungal cells were stained with Grocott's methenamine silver stain. This list includes: genes controlling the cell wall (Mellado et al. 1996; Langfelder et al. 1998; Tsai et al. 1998; Mouyna et al. 2005; Maubon et al. 2006; Romano et al. 2006; Li et al. 2007; Schmalhorst et al. 2008), signaling and metabolism regulation (Panepinto et al. 2003; Krappmann et al. 2004; Liebmann et al. 2004; Fortwendel et al. 2005, 2012; Steinbach et al. 2006; Zhao et al. 2006; da Silva Ferreira et al. 2007; Cramer et al. 2008; Ibrahim-Granet et al. 2008; Soriani et al. 2008; Ejzykowicz et al. 2009, 2010; Gravelat et al. 2010), thermotolerance (Bhabhra et al. 2004; Wagener et al. 2008), nutrition (Hensel et al. 1998; Schrettl et al. 2004, 2007; Hissen et al. 2005; Moreno et al. 2007; Liu et al. 2010), toxin genes (Bok et al. 2005; Sugui et al. 2007; Spikes et al. 2008), and response to stress (Paris et al. 2003; Tsitsigiannis et al. 2005; Reeves et al. 2006; Willger et al. 2008; Feng et al. 2011; Dinamarco et al. 2012; Grahl et al. 2012).

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the most active factors are the surfactant proteins SPA and SPD, the pentraxin-3-ficolin complexes, and the β -defensins (Okamoto et al. 2004; Alekseeva et al. 2009; Madan et al. 2010; Ma et al. 2013).

After *A. fumigatus* conidia bypass mucociliary clearance and epithelial defenses, they reach the lower airways (Cohen et al. 2011). Conidia that reach the alveoli are unable to withstand the immune assault mounted by normal hosts and are effectively removed by innate immune cells (Tekaiia and Latgé 2005). When they reach the alveoli, conidia are phagocytosed by alveolar macrophages. Although these conidia present several antigens and allergens, it remained an enigma as to why airborne conidia do not continuously activate host innate immune cells and induce detrimental inflammatory responses following their inhalation. This mystery was solved when it was shown that the hydrophobin layer organized in a rodlet configuration on the surface of the dormant conidia imparts immunological inertness. This was shown using conidia that were lacking the rodlet protein (RodAp) after it had been removed biologically (germ tube), chemically (hydrofluoric acid treatment, HF), or genetically (*rodA* mutant) (Aimanianda et al. 2009). For example, in contrast to wild-type conidia, and despite being dormant, conidia of the $\Delta rodA$ mutant and HF-treated conidia that lack RodAp but have exposed immunogenic determinants induced significant up-regulation of cytokines as well as costimulatory and antigen presenting molecules on human dendritic cells. The immunosilencing role of the surface hydrophobins is not only seen in *A. fumigatus* but is a common feature of all aerial airborne molds. Considering the fact that dormant conidia are ubiquitous in the atmosphere, the inert nature of the hydrophobin rodlet layer makes teleological sense, as it prevents undue and exacerbated host response by innate immune cells, and hence avoids inflammation and host damage (Aimanianda et al. 2009). Once internalized by macrophages, microbes are usually killed by reactive oxygen species (ROS). In the case of *A. fumigatus*, resting conidia are extremely resistant to ROS. Although this resistance is also caused by the presence of

hydrophobins, the most important defense mechanisms against ROS are the underlying layer of melanin (Heinekamp et al. 2013) that scavenges host ROS in a more efficient way than the classical catalases and superoxide dismutases, as well as the many antioxidant proteins controlled by the transcriptional regulator Yap1 (Lessing et al. 2007).

After phagocytosis, conidia germinate inside the phagolysosome (Ibrahim-Granet et al. 2003) and shed their rodlet layer that is then degraded in the phagolysosome. The release of the rodlets results in the exposure of polysaccharides and immunogenic proteins that initiate a proinflammatory response. In addition, the melanin layer ruptures because of the internal osmotic pressure resulting from conidial swelling. Both events facilitate contact of host cells with the fungal PAMPs and increase cell-wall permeability to phagocyte toxic molecules. Another major consequence of this germination process is the recruitment of the neutrophils to the site of infection. Like alveolar macrophages, neutrophils are professional phagocytes whose function is essential to kill inhaled conidia as well as germinating conidia. Killing is caused by two different mechanisms: In the case of conidia, it is an intracellular killing with granules fusing to the phagolysosome; in the case of hyphae, neutrophils are able to secrete antifungal compounds from their arsenal of toxic molecules present in granules. However, with the exception of the ROS, the nature of the molecules responsible for the killing remains unknown to date. A recent discovery associated with the clustering of the neutrophils with hyphae and other immune cells has been the discovery of neutrophil extracellular traps (NETs) consisting of nuclear DNA decorated with antifungal proteins (Urban et al. 2006). However, the efficacy of hyphal killing by this extracellular mechanism remains controversial (Bruns et al. 2010; McCormick et al. 2010).

In the absence of sufficient pulmonary defenses, because of either immunosuppression that renders the human host neutropenic or cortisone injection to fight graft versus host disease that results in deficient phagocytic defense, conidia establish themselves in the lung and



adapt their physiology to overcome the stress imposed by the host environment. Because the optimum temperature for conidial germination and growth is between 37°C and 45°C, they germinate readily into hyphae by 6–8 h in mammalian tissue (McDonagh et al. 2008). During this early stage of germination, *A. fumigatus* utilizes a highly coordinated gene expression program that allows adaptation to nutrient deprivation, iron limitation, hypoxia, and other unfavorable growth conditions in the host environment (McDonagh et al. 2008). Excessive hyphal growth and dissemination follow in the profoundly neutropenic host, leading to increased risk of IA resulting in thrombosis and hemorrhage (Fig. 1) (Hohl and Feldmesser 2007; Dagenais and Keller 2009).

CLINICAL PRESENTATIONS

Aspergillus species are relatively unique among pathogens as they are responsible for a gamut of infections extending across the clinical spectrum to include primary allergic reactions, saprophytic involvement, and invasive disease. The type of *Aspergillus* infection generally depends on the immunologic background of the infected host, with immunodeficient patients developing invasive disease, whereas immunoreactive patients develop allergic disease. The most frequently encountered clinical presentations involve the lungs, such as acute or chronic IA and allergic bronchopulmonary aspergillosis (ABPA). Other common infections caused by *Aspergillus* include acute invasive or chronic sinusitis, cutaneous aspergillosis, aspergilloma, and cerebral aspergillosis. In some immunocompetent patients, inhalation of conidia could result in nonpathogenic saprophytic colonization; however, in the immunocompromised patient this conidial acquisition will likely result in establishment of invasive disease (Tomee and van der Werf 2001). The large burden of *Aspergillus* infections is sustained by immunocompromised individuals, including those who have undergone HSCT, SOT, cancer patients, patients with various congenital immune deficiencies such as CGD, and patients treated for autoimmune diseases (Richardson 2005).

Although cancer patients undergoing treatment constitute the a majority of patients at risk for *Aspergillus* infections, patients with CGD have an estimated 33% lifetime risk of IA, and IA may in fact be the first manifestation of CGD. Although disease in a majority of patient groups is caused by either *A. fumigatus* or *A. flavus*, most cases of *A. nidulans* infection have been reported in patients with CGD (Cohen et al. 1981; Henriot et al. 2013). In addition to differences in the epidemiology of specific *Aspergillus* isolates, the clinical presentation in patients with CGD is characterized by an insidious onset of fatigue, fever, increased sedimentation rate, and pneumonia (Segal et al. 1998). In early disease, there is an acute neutrophilic response in which the neutrophils surround hyphae. However, in patients with CGD, the hyphae remain intact owing to impaired neutrophil-mediated killing of hyphae.

Fungal sinusitis can manifest as allergic, saprophytic, or invasive disease. Invasive *Aspergillus* sinusitis is likely underdiagnosed because of its variable clinical presentation and difficulty in establishing the diagnosis (Denning 1998; Ho and Yuen 2000; Marr et al. 2002b), possibly owing to a decreased inflammatory response in affected patients.

Aspergillus infections most commonly involve the lungs, but disease can disseminate via the bloodstream and involve distant organs. One of the most frequent sites of dissemination is the central nervous system (CNS) (Groll et al. 1996). There are studies that have estimated that CNS aspergillosis may be found in 40%–50% of patients with IA and acute leukemia or allogeneic stem cell transplant (Saugier-Weber et al. 1993; Jantunen et al. 2000).

Chronic aspergillosis is less frequently encountered than acute disease and the patient population affected is typically distinct as well. A degree of immune suppression does exist in these patients and exposure to corticosteroids is common. Underlying conditions include corticosteroid use for chronic lung disease, diabetes mellitus, and alcoholism (Denning 1998).

ABPA occurs in asthmatic and cystic fibrosis patients and is a pulmonary disease caused by types I and III hypersensitivity reactions to

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A. fumigatus allergens. Exposure and inhalation of *Aspergillus* conidia result in saprophytic (noninvasive) colonization of the bronchial airways, which then triggers an IgE-mediated allergic inflammatory response. Over time, bronchial obstruction develops and patients can present with productive cough, wheezing, and chest pain; fever and malaise are also presenting symptoms (Stevens et al. 2003). Treatment depends on relieving inflammation via an extended course of systemic corticosteroids and antifungal agents to decrease the fungal burden and diminish the inciting stimulus for inflammation (Stevens et al. 2000; Caputo 2003; Conway et al. 2004).

EPIDEMIOLOGY

IA was the most common mold infection in a review of approximately 5500 patients who underwent HSCT; although >7% of HSCT recipients had mold infections, *Aspergillus* infections were the most common, followed by *Fusarium*, *Mucorales*, and *Scedosporium* infections (Marr et al. 2002a). A large prospective French study analyzed 424 cases of IA and found an incidence of 0.9% in autologous and 8.1% in allogeneic HSCT patients, respectively (Lortholary et al. 2011), highlighting a consistent finding that IA occurs much more readily in the allogeneic HSCT recipient. Among SOT recipients, the highest incidence of IA was for heart transplant recipients (4.8%), followed by lung (4.1%), and significantly dropping for liver (0.8%) and kidney (0.3%), findings that have also continued throughout most other larger studies.

The Transplant Associated Infection Surveillance Network (TRANSNET) study evaluated HSCT and SOT recipients from 23 U.S. medical centers (2001–2005) and included evaluation of a total of 642 cases of IA (Baddley et al. 2010). The 12-month cumulative incidence of IA in all HSCT recipients was 1.6%, compared to 0.63% in SOT recipients. The 12-week all-cause mortality was 57.5% among HSCT recipients and 34.4% among SOT recipients.

There is a well-characterized bimodal distribution of aspergillosis in HSCT recipients that correlates with pre-engraftment neutrope-

nia (median of 16 d after transplantation) and the peak of graft-versus-host disease (GVHD) (median of 96 d after transplantation) (Wald et al. 1997). This likely relates to the two major mechanisms of protection against IA: alveolar macrophages and granulocytes. Most patients (86%) with autologous transplants were diagnosed with IA while neutropenic, whereas patients with allogeneic transplants were at greatest risk after engraftment or during impairment of cell-mediated immunity attributable to cytomegalovirus or GVHD (Wald et al. 1997).

DIAGNOSTIC STRATEGIES

The diagnosis of IA is often not straightforward. The “gold standard” of tissue biopsy is often considered too invasive and complicated by bleeding in high-risk patients. Aspergillosis diagnosed by blood culture is rare (Girmentria et al. 2001). In general, the *Aspergillus* hyphal mass that develops in the lumen during angioinvasion remains in place until the force of blood flow causes hyphal breakage, which then allows the mass to circulate. The likelihood of a blood culture capturing these irregularly and infrequently discharged units is small. The difficulty in detecting *A. fumigatus* in blood culture stands in contrast to other angioinvasive filamentous fungi (e.g., *Fusarium* species, *Paecilomyces lilacinus*, *Scedosporium prolificans*, *Acremonium* species) that have the ability to discharge a steady series of unicellular conidia into the bloodstream that are more likely to be captured in a blood sample. This ability to sporulate in tissue and blood has been termed adventitious sporulation (Schell 1995). As *A. terreus* also displays adventitious sporulation, histopathologic examination of these spores can allow rapid, presumptive identification of *A. terreus*. Therefore, a positive blood culture with *A. terreus* or another mold that shows adventitious sporulation should not be ignored, whereas a positive blood culture with other *Aspergillus* species should be further evaluated.

Serological testing for antibodies to *Aspergillus* antigens is helpful to diagnose aspergillosis or allergic bronchopulmonary aspergillosis in immunocompetent individuals, but

unfortunately serology plays little role for diagnosis in the immunocompromised patient because *Aspergillus* growth does not correlate with an increase in anti-*Aspergillus* antibody titers (Latgé 1999).

Galactomannan (GM) is a major cell-wall component of *Aspergillus* and it is known that the highest concentrations of GM are released in the terminal phases of the disease (Latgé 1999). An enzyme-linked immunosorbent assay (ELISA) technique was introduced using a rat anti-GM monoclonal antibody, EB-A2, which recognizes the 1→5-β-D-galactofuranoside sidechains of the GM molecule (Maertens et al. 1999). A sandwich ELISA technique was introduced in 1995 (Stynen et al. 1995), and by using the same antibody as both a capture and detector antibody in the sandwich ELISA (Platelia *Aspergillus*, Bio-Rad, France), the threshold for detection can be lowered to 1 ng/ml. This technique is used in the current commercially available GM assay for diagnosis of IA. In a meta-analysis conducted to characterize the clinical utility of GM assay, 27 studies were identified and overall the GM assay had a sensitivity of 71% and a specificity of 89% for proven cases of IA as defined by the specific clinical criteria (Pfeiffer et al. 2006). In patients with hematologic malignancy or who have undergone HSCT, the assay was most useful but in SOT recipients, the sensitivity and specificity were 22% and 84%, respectively. Serial testing at least twice a week is recommended (Denning et al. 1997). Unfortunately, the galactomannan assay has decreased sensitivity in the setting of a patient receiving anti-*Aspergillus* (mold-active) antifungals, although the specificity for detection does not change (Marr et al. 2004). Although GM has been extensively validated in patients with hematologic malignancy and those who have undergone HSCT, for unclear reasons, GM appears less useful in SOT recipients. False-positive results with GM can hamper its clinical utility and are seen in patients concurrently receiving some β-lactam antibacterials such as piperacillin-tazobactam, amoxicillin, and amoxicillin-clavulanate.

(1,3)-β-D-glucan is an integral cell-wall component and, in contrast to GM, is not nor-

mally released from the fungal cell (Latgé 1999). Factor G, a coagulation factor of the horseshoe crab, is a highly sensitive natural detector of (1,3)-β-D-glucan (Obayashi et al. 1995). The “G test,” which detects (1,3)-β-D-glucan via a modified limulus endotoxin assay, is useful for detection of fungi such as *Aspergillus*, *Candida*, and even *Cryptococcus*; however, it does not identify the genus of the fungi (Obayashi et al. 1995). A meta-analysis of cohort studies of (1,3)-β-D-glucan for IA revealed that using a single test resulted in a pooled sensitivity of 57% with a specificity of 97% (Lamoth et al. 2012). A study comparing galactomannan, polymerase chain reaction, and (1,3)-β-D-glucan on patients with hematological disorders and the receiver-operating characteristic (ROC) analysis showed an area under the ROC curve that was greatest for the galactomannan assay, using two consecutive positive results. This suggests that the galactomannan was the most sensitive at predicting the diagnosis of IA in high-risk patients with hematological disorders (Kawazu et al. 2004).

TREATMENT

Overall success in treating IA is dependent on numerous factors, not simply the choice of a specific antifungal therapy. As with all immunocompromised patients, detailed knowledge of host factors, underlying disease, concomitant infections, and degree and duration of immunosuppression are keys to overall treatment. It is well known that immune reconstitution is paramount to successful IA therapy, whereas continued exposure to certain immunosuppressive medications, such as corticosteroids, worsens IA. Any antifungal prophylaxis used before the diagnosis of IA could also have an effect on the ultimate choice of targeted therapy. Although the diagnostic work-up needs to be aggressive, it should never delay antifungal therapy in the setting of true concern for IA. As such, the cornerstone of antifungal therapy for IA is prompt and aggressive institution of antifungal therapy, based not only on diagnostic tools but also on clinical suspicion of infection. Antifungal resistance is slowly and steadily increasing among

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Aspergillus isolates and continues to have specific geographic trends that could influence antifungal choice. There is also the question of using antifungal monotherapy or combination antifungal therapy, and, if so, which classes of agents. Finally, although immune reconstitution is paramount, the role and real benefit of adjunctive immunotherapy remains somewhat unclear.

Voriconazole is the preferred antifungal agent for the primary therapy of IA. The global clinical trial that led to its approval was a prospective comparative study showing that voriconazole had a superior response rate in the primary treatment of IA compared to amphotericin B, 52.8% versus 31.6%, respectively (Herbrecht et al. 2002). These and other studies led to the very clear guideline recommendations of the Infectious Diseases Society of America to use voriconazole for the primary treatment of IA (Walsh et al. 2008) in all clinical sites. Although voriconazole is the guideline-recommended first line agent for primary therapy of IA, it is not completely clear when the alternative agents, such as amphotericin B lipid formulations or echinocandins or the other triazoles (posaconazole or itraconazole), should be used in place of voriconazole. The most common reason for using an alternative agent is refractory disease following primary antifungal therapy and the need to transition to salvage therapy.

CONCLUSION

The clinical diseases caused by *Aspergillus* species are diverse and often dependent on host immune status. Although there are hundreds of recognized *Aspergillus* species, it was originally thought that only a handful caused human disease. However, the breadth of infecting species is increasing as more and more sibling species are identified. Accurate identification is not only an interesting taxonomic phenomenon, but clinically relevant as many of these newly characterized species have unique and potentially dire antifungal susceptibility patterns. The *Aspergillus* field is rapidly advancing on all fronts, including basic biology and laboratory identification, fundamental pathogene-

sis, and fungal–host interactions, as well as diagnostic and therapeutic challenges for improved clinical outcome. This bodes well for future advances to impact patient care and survival.

ACKNOWLEDGMENTS

J.A.S. and K.J.K.-C. are supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

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Cold Spring Harb Perspect Med published online November 6, 2014 originally published online November 6, 2014

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