Medical Mycology 2009, **47** (Supplement 1), S261–S270

informa
healthcare

Differences in pathogenicity and clinical syndromes due to *Aspergillus fumigatus* and *Aspergillus flavus*

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Most of the information available about *Aspergillus* infections has originated from the study of *A. fumigatus*, the most frequent species in the genus. This review aims to compare the pathogenicity and clinical aspects of *Aspergillosis* caused by *A. fumigatus* an *A. flavus*. Experimental data suggests that *A. flavus* is more virulent than *A. fumigatus*. However, these were mostly models of disseminated *Aspergillus* infection which do not properly mimic the physiopathology of invasive aspergillosis, a condition that is usually acquired by inhalation. In addition, no conclusive virulence factor has been identified for *Aspergillus* species. *A. flavus* is a common cause of fungal sinusitis and cutaneous infections. Chronic conditions such as chronic cavitary pulmonary aspergillosis and sinuses fungal balls have rarely been associated with *A. flavus*. The bigger size of *A. flavus* spores, in comparison to those of *A. fumigatus* spores, may favour their deposit in the upper respiratory tract. Differences between these species justify the need for a better understanding of *A. flavus* infections.

Keywords Pathogenicity, aspergillosis, *Aspergillus flavus*, *Aspergillus fumigatus*, secondary metabolites

Introduction

Infections caused by *Aspergillus* species have grown in importance in recent years. This probably results from a higher number of patients being at risk, including transplant recipients, neutropenic individuals, allergic patients and those treated with corticosteroids or other immunosuppressive regimens. Despite a better understanding of the epidemiology of *Aspergillus* infections, important diagnostic limitations persist. Accordingly, the mortality for invasive aspergillosis remains very high. As most of the *Aspergillus* infections are caused by *A. fumigatus*, the majority of studies have focused on this species, and our understanding of other *Aspergillus* species is far from satisfactory.

Received 31 January 2008; Accepted 4 June 2008
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Aspergillus flavus is the second leading cause of invasive and non-invasive aspergillosis [1]. In addition, it is the main *Aspergillus* species infecting insects, and it is also able to cause diseases in economically important crops, such as maize and peanuts, and to produce potent mycotoxins. Curiously, some *Aspergillus* syndromes are rarely associated with *A. flavus*. The aim of this review is to summarize the available data comparing the pathogenicity of these two medically important thermotolerant fungi, *A. fumigatus* and *A. flavus*. In addition, clinical syndromes particularly associated with *A. flavus* are presented.

Geographic variations in *Aspergillus* species

Although not completely understood, climate and geographic conditions are very important determinants of the prevalence and distribution of *Aspergillus* species in the air we breathe. The marked predominance of *A. fumigatus* on clinical samples may simply reflect its environmental predominance over other *Aspergillus* species. However, important geographic variations in

DOI: 10.1080/13693780802247702

the distribution of *Aspergillus* species occur all over the globe. For instance, *A. flavus* is particularly prevalent in the air of some tropical countries [2–5]. In countries like Saudi Arabia and Sudan, with semi-arid and arid dry weather conditions, *A. flavus* is frequently described as a leading cause of invasive aspergillosis [6–8]. *A. flavus* seems also to be a prevalent species in India, Pakistan, Qatar and Iran [1]. An early study from Sudan [3] showed that *A. flavus* represented 30% of all aspergilli recovered from the air in June, when the weather is hot, dry and dusty. Conversely, *A. flavus* was sporadically recovered in winter months. Although *A. flavus* is very prevalent in regions where the climate is dry and hot, the presence of a humid and hot climate – as occurs to many parts of India – may also predispose to *A. flavus* infections. In addition, conditions of elevated humidity and temperature have also been associated with *A. flavus* contamination of crops and production of aflatoxin [1].

In Europe, whilst *A. flavus* and *A. niger* were the most frequent airborne aspergilli recovered in a study performed in Barcelona [9], another investigation conducted in Madrid showed *A. fumigatus* to be the most prevalent species (54%) [10]. This might be explained by the existing climatic differences between these two Spanish cities. Also interesting is the fact that in the study performed in Madrid, *A. niger* and *A. flavus* were found to be more heavily influenced by meteorological parameters than *A. fumigatus* was [10]. Comparing the presence of *Aspergillus* species in the air in London, Paris, Lyon and Marseille, Mallea *et al*. [11] found that *A. glaucus* and *A. versicolor* predominate in Southern France, whilst *A. fumigatus* represented >35% of the isolates recovered from Paris and London.

Conidial size, surface and pigments

Aspergillus species produce conidia (asexual spores) that can easily be dispersed in the soil and air. Uptake of conidia by a susceptible host is usually the initial event in *Aspergillus* diseases with alveolar macrophages acting as first-line defence. While the size of *A. fumigatus* conidia ranges from 2 to 3.5 μm, *A. flavus* produces conidia ranging from 3 to 6 μm. This difference in size is of great importance, allowing *A. fumigatus* conidia to reach the pulmonary alveoli much easier than do those of *A. flavus*. This probably also explains why *A. fumigatus* is the main agent of invasive pulmonary aspergillosis, while *A. flavus* is an important aetiology of *Aspergillus* sinusitis and a frequent cause of cutaneous and wound aspergillosis [1]. No data seems to exist on the importance of speed of sedimentation of different sizes of *Aspergillus* spores, which might also be important for the transmission of the pathogen.

In addition to conidial size, the outermost cell wall layer of *Aspergillus* conidia may also be of importance. The outer conidial surface contains rodlets that are associated with hydrophobic properties. These structures may confer resistance to extreme atmospheric conditions and facilitate airborne dispersion of *Aspergillus* conidia. Mutants lacking the gene RodA – encoding the protein responsible for rodlet structure – display enhanced sensitivity to alveolar macrophage killing [12]. Accordingly, rodlets are believed to be virulence factors [13–16]. However, deletion of the RodA gene had no impact on virulence in a murine model of pulmonary infection [17]. Conidia from Δ*rodA* mutants do not properly bind to proteins with hydrophobic pockets, such as albumin or collagen – instead, binding occurs to other host proteins like laminin and fibrinogen [14]. Therefore, this mechanism does not seem to be essential for *Aspergillus* pathogenicity.

Melanin is a large group of dense hydrophobic pigments present in the cell wall of many fungi, adjacent to the rodlet layer [18]. The colour of the pigment is usually dark brown or black, but many other colours have also been observed [19]. Melanin synthesis has been linked to virulence in fungal organisms such as *Cryptococcus neoformans* [20] and *Sporothrix schenckii* [21,22]. The pigment seems to confer protection to the conidia against environmental damage from UV radiation. In addition, it seems to protect against phagocytosis *in vitro* and *in vivo* [23]. Melanin may also reduce complement opsonization by 'camouflaging' binding sites, which for instance can reduce C3 ability to bind conidia [19,24]. Mutant albine *Aspergillus* strains have shown reduced virulence in comparison to wild type strains in models of experimental aspergillosis, with albine conidia being more susceptible to the oxidative mechanisms of monocytes and polymorphonuclear leukocytes [18,23–25]. Differences in melanization between *A. fumigatus* and *A. nidulans* were demonstrated by exposing these fungi to tricyclazole, a fungicidal inhibitor of the THN-reductase enzyme, involved in melanin synthesis via DHN-melanin pathway [24]. Exposure to tricyclazole resulted in inhibition of conidial pigmentation in *A. fumigatus* but not *A. nidulans*, showing that pigmentation involves different pathways in these species. Studying melanisation in *A. fumigatus* strains by immunofluorescence techniques, Youngchim *et al*. [26] demonstrated that anti-melanin antibodies avidly attached to *Aspergillus* conidia. The strength of this binding decreased with conidial germination to become null after hyphae

formation. These data suggest that melanin could be more important as a facilitating factor for fungal survival in the external environment than for virulence in the host. Also again, no much data is there for *A. flavus*. Since several non-pathogenic fungi are also known to produce melanin, this pigment is probably not essential for the occurrence of invasive fungal diseases in humans [19].

Adhesion of *Aspergillus* conidia to the lung epithelia

The adhesion of *Aspergillus* conidia to proteins present in the lung cell basal lamina is considered an important initial step in the development of invasive aspergillosis. Important proteins in this context include fibronectin [27,28], laminin [28–30], type IV collagen [28,29], fibrinogen, complement, albumin, and surfactant proteins [13]. In a comparison involving several *Aspergillus* species, conidia of *A. niger*, *A. fumigatus* and *A. flavus* were found to bind significantly better to fibrinogen than *A. terreus* conidia [31]. In another investigation [32], *A. fumigatus* conidia were found to bind significantly better to the basal lamina and fibronectin than those of *A. flavus*. Studying the mechanisms involved in conidial binding, the authors realized that negatively charged carbohydrates occurring on the conidiospore cell wall played a role in the adhesion of the conidia to host basal lamina.

Phagocytosis

Alveolar macrophages represent the first line of defence against pulmonary aspergillosis. Accordingly, therapy with corticosteroids – which may cause important interference with the ability of macrophages to kill resting conidia – is a major risk factor for invasive aspergillosis. Most *in vitro* studies of interactions between macrophages and *Aspergillus* species have been done on *A. fumigatus* [33–35] and very little is known about *A. flavus* [36,37]. Previous studies revealed that monocyte-derived human macrophages exhibited lower phagocytic capacities against non-*A. fumigatus* aspergilli, especially in *A. nidulans* and *A. niger*, when compared with *A. fumigatus*. In addition, polymorphonuclear leukocytes induced significantly less hyphal damage to both *A. flavus* and *A. nidulans* than to *A. fumigatus* [36]. Perkhofer *et al*. [37] further investigated phagocytosis and intracellular killing for resting conidia of a wide range of *Aspergillus* species by human monocytes-derived macrophages. No differences between clinical and environmental isolates were observed. Similar results were obtained for clinical isolates of *A. fumigatus* and *A. flavus*, with mean killing indexes at 120 minutes ranging from 13.7–77.8% and 14.2–42.2%, respectively. However, some marked isolate-related differences occurred.

Germination rate and thermotolerance

Araujo and Rodrigues [38] showed that germination rates at 37°C differed significantly for the most common pathogenic *Aspergillus* species. Using the same inoculum of *Aspergillus* spores in RPMI 1640 medium, *A. fumigatus* germinated faster than *A. flavus*, which in turn germinated faster than *A. niger*. Interesting results were also obtained when germination rate was evaluated at different temperatures. The percentage of germination markedly increased 3- to 10-fold for both *A. fumigatus* and *A. flavus* when temperature was increased from 20°C to 30°C, and again 2- to 3-fold from 30°C to 37°C. However at 41°C germination of *A. fumigatus* was still enhanced, while germination of *A. flavus* decreased by 45% (as compared with 37°C). The study suggested that temperature plays a crucial role in selecting and promoting pathogenic species of *Aspergillus*, with *A. fumigatus* being the species most able to adapt to extreme changes in environmental conditions. Nonetheless, it remains to be elucidated if the same phenomenon also occurs *in vivo*. As demonstrated in earlier studies [39], high conidial densities were associated with lower *in vitro* germination rates.

In contrast to *A. fumigatus*, *Neosartorya fischeri* is only rarely identified as a human pathogen. Since phenotypic characterization has shown that both *A. fumigatus* and *N. fischeri* can grow at 42°C, *A. fumigatus* may possess other genetic determinants besides thermotolerance that allow it to establish a successful *in vivo* infection [40].

Interactions with the endothelial cells

In a previously reported model of interaction of *A. fumigatus* with primary cultures of human umbilical vein endothelial cells it was observed that after 16 h of interaction hyphae caused injury to the endothelial cell monolayers [41]. Further studies using two clinical isolates of *A. flavus* (AFL8 and AFL24) using this *in vitro* model showed that both isolates caused the same amount of injury as observed for *A. fumigatus* [Lopes-Bezerra, personal communication]. Although invasion of the blood vessels is a key feature of invasive aspergillosis, no comparative data was found for *A. flavus* and *A. fumigatus* on the potential for causing angioinvasion.

The role of albumin

Albumin accounts for around 50% of plasma proteins and is involved in several physiological processes. Rodrigues *et al*. investigated the effect of human albumin upon conidial germination and hyphal development of *Aspergillus* species [42]. Although albumin was shown to significantly promote germination of *A. fumigatus*, the germination of both *A. flavus* and *A. niger* was reduced in presence of albumin. *A. flavus* germination was reduced by 20 and 25% in the presence of 2 and 4% of human albumin, respectively. Similar effects were obtained with the use of bovine albumin. The formation of conidiophores and maturation of *A. fumigatus* conidia were also faster in the presence of human albumin.

Fungal secondary metabolites and toxines

Aspergillus species have been shown to produce several secondary metabolites during invasive hyphal growth in tissues [12]. Many of such substances have been identified as being important in the process of fungal assimilation of nutrients from the host, and include fungal enzymes and toxins. It remains however a subject of debate whether any of these metabolites actually represent a virulence factor. Differently from what was described for other fungi such as *C. neoformans* [43], no single gene virulence factors has been identified for *Aspergillus* species. In addition, very little is known about *A. flavus*, in comparison to its counterpart *A. fumigatus*.

In order to cause invasive infections, filamentous fungi require the activity of extracellular enzymes to degrade the structural barriers in the host [44,45]. These enzymes include nucleases, oxidases, catalases, phosphatases, peptidases and proteases, that are produced to degrade complex macromolecules in order to provide nutrients for the fungus. Fungal proteases may also induce local airway inflammation by activating inflammatory pathways via epithelial cells [46].

Since elastin constitutes about 28% of lung tissue, fungal extracellular elastolytic proteases are supposed to play a role in the pathogenesis of invasive aspergillosis. Kothary *et al*. inoculated mice with elastase-producing and non-producing environmental isolates of *A. fumigatus* [47]. While non-producer isolates caused no destruction to the mice alveoli, isolates that produced elastase killed animals within 48–96 h, which was associated with substantial alveolar necrosis. Similar elastase activity has been observed when clinical and environmental isolates of *A. fumigatus* have shown to produce similar amounts of elastase [48]. In another investigation, the *in vitro* elastolytic activity of *A. flavus* was found to be much lower than of *A. fumigatus* [49]. Some intra-species variation however occurred, with one *A. flavus* isolate producing exceptionally high levels of elastolytic activity.

Other proteases have been detected during *Aspergillus* infection, including the alkaline serine protease, the metalloprotease and an aspartic protease. The exact importance of these enzymes in pathogenesis is uncertain, and this subject has been recently reviewed [50]. For instance, deletion of the coding sequences was associated with no phenotypic modification, and the corresponding mutants retained their virulence in murine infection models, with histopathological studies showing similar extent of mycelial growth in the lungs of parental and mutant strains [51–57]. The significance of the recently identified sedolisins is also unclear [58]. The role of fungal enzymes involved in the propionyl-CoA detoxification has recently been investigated for *A. fumigatus* [50] and *A. nidulans* [59]. When evaluated in a steroid-immunosuppressed murine model of *A. fumigatus* infection, a methylcitrate synthase mutant displayed reduced virulence, suggesting that this protease may be involved in pathogenicity. Molecular studies have been so far unable to identify a single *Aspergillus* enzyme that is undoubtedly associated with virulence in humans. Additionally, very little is known about the importance of proteases in the pathogenesis of *A. flavus* infections. Actually, most studies about proteases secreted by *Aspergillus* of the *flavus* group concerned *A. oryzae* and *A. sojae*, used in the food industry [60].

Amongst the several secondary metabolites produced by *A. flavus* are aflatoxins, the most toxic and potent carcinogenic natural compounds ever characterized [1]. Aflatoxin may contaminate crops prior to harvest or during storage, putting humans and other mammals at risk. In addition, aflatoxins may also depress phagocytosis, intracellular killing and spontaneous superoxide production by macrophages [61]. Experimental animal models failed to establish a role for aflatoxin as a virulence factor, since some virulent strains of *A. flavus* do not produce aflatoxin [62,63].

Gliotoxin is one of the most abundant metabolites produced by *A. fumigatus* during invasive hyphal growth. This toxin exerts a broad spectrum of immunosuppressive effects *in vitro*, including inhibition of cytokine production, antigen presentation and production of reactive oxygen species by macrophages, and reduced cytotoxicity in T-cells [64]. Low concentrations of gliotoxin (0.2 μg/ml) may also impair respiratory ciliary function, which is an important defence host mechanism against aspergillosis [65]. In parallel, other

Aspergillus toxins like fumagillin and helvolic acid require much higher concentrations to inhibit the cilia. Gliotoxin has been detected in the blood of patients with invasive aspergillosis [66], and mice administered with gliotoxin showed marked immunosuppression rending them at risk for invasive aspergillosis [67]. Therefore, gliotoxin has been proposed as a potential virulence factor for *A. fumigatus*. However, a study found no difference in the frequency or degree of gliotoxin production when invasive aspergillosis patients were stratified by the EORTC criteria [68]. Similar results were observed for patients with proven invasive aspergillosis or *Aspergillus* colonization, suggesting that gliotoxin may have a limited role in the pathogenicity of invasive aspergillosis, particularly in infections caused by species other than *A. fumigatus*.

Data for gliotoxin production for *A. flavus* is scant, and some experts will even argue that *A. flavus* does not produce any gliotoxin at all. In a recent study, gliotoxin production was detected in >95% *A. fumigatus* strains and in only 13% *A. flavus* strains [64]. Similar results were obtained in another investigation, in which 93% and 4% of clinical isolates of *A. fumigatus* and *A. flavus* were found to be gliotoxin-producers, respectively [69]. Not only gliotoxin production seems to be infrequent for *A. flavus* isolates, but gliotoxin levels for *A. flavus* are about 80-times lower when compared to what is observed for *A. fumigatus*. For instance, in one investigation [64] mean gliotoxin concentration in the culture supernatants for clinical and environmental strains of *A. fumigatus* ranged from 5-6 μg/ml, while mean levels for *A. flavus* were 0.001 μg/ml only for *A. flavus*. The impact of gliotoxin production might also differ for *A. fumigatus* and *A. flavus*. While lack of gliotoxin production in *A. fumigatus* significantly reduces cytotoxicity on macrophage-like P388D1 cells and CD8 T-cells, absence of gliotoxin does not seem to influence cytotoxicity in *A. flavus*. Although high concentrations of gliotoxin can also be detected in infected lung tissues [66], no data seems to exist for *A. flavus* infections.

Calcineurin is a Ca^{++}-calmodulin-dependent phosphatase that is important in cell signalling [70]. This protein is a critical mediator of calcium signalling and numerous cell stress responses in eukaryotic organisms, including fungi. In *A. fumigatus*, calcineurin seems necessary for filamentous growth [71]. An *A. fumigatus* mutant lacking the calcineurin A catalytic subunit exhibits defective hyphal morphology resulting in decreased filamentation. Another study revealed that deletion of the calcineurin gene reduced *A. fumigatus* virulence in mice [72]. Also, calcineurin inhibitors such as tacrolimus and cyclosporine have shown to create gross and microscopic morphological changes in *A. fumigatus* colonies [73]. Although the calcineurin gene seems important for *A. fumigatus* pathogenicity, its role has not been clarified for infections caused by other *Aspergillus* species.

Virulence in animal models

Animal models play a central role in identifying virulence factors. Maybe the best evidence showing higher virulence for *A. flavus* isolates in comparison to *A. fumigatus* comes from studies involving mice. One example is the classical study by Ford and Friedman, published in 1967 [62]. The study evaluated cumulative mortality rates of normal mice inoculated intravenously with 10^6 viable spores from various *Aspergillus* species. Although *A. flavus* killed all animals within 5 days of infection, only 40% of mice infected with *A. fumigatus* were dead 20 days after the inoculation. Curiously *A. oryzae* – which has GRAS ('generally regarded as safe') status – proved to be as virulent as *A. flavus*. None of the *Aspergillus* species studied caused death when only 10^2 spores were inoculated. However, when a 10^4 inoculum was used, *A. flavus* was still able to kill 38% of animals. Immunosuppression with cortisone, although greatly enhancing disease, was not necessary to ensure infection in mice. In another publication [74], normal mice were intravenously inoculated with 10^4 *Aspergillus* spores. *A. flavus* and *A. fumigatus* killed 35% and 25% of animals, respectively, while *A. terreus* caused 5% mortality only.

More recently, studies in cyclophosphamide-immunosuppressed CD-1 mice have demonstrated that a much lower inoculum is required to kill animals when these are intravenously infected with *A. flavus* spores, in comparison to *A. fumigatus* [75–77]. While the LD_{90} (lethal dose killing 90% of animals) for *A. flavus* ranged from 2.2×10^5 to 2.6×10^5 CFU/ml, a 4- to 50-fold higher LD_{90} occurred for *A. fumigatus* (1×10^6 to 1.2×10^7). For *A. terreus*, the LD_{90} was about 40- to 100-fold higher (1×10^7 to 2×10^7) than the observed LD_{90} for *A. flavus*. It is noteworthy however that none of these studies have directly compared virulence by testing more than one *Aspergillus* species in the same experiment. Fig. 1 shows the results of a study in which the virulence of different *Aspergillus* species was compared in an immunosuppressed mice model [Warn P, personal communication]. While mean LD_{90} (dose per gram) for isolates of *A. fumigatus* was 9,566, LD_{90} for *A. flavus* was 1,440 (~7-fold less). A much higher inoculum was required for *A. niger* and *A. terreus*, suggesting reduced virulence.

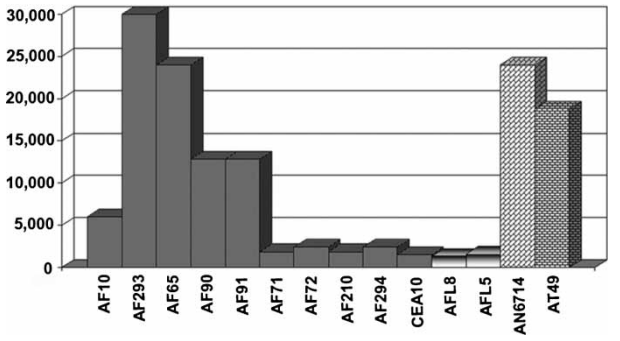

Fig. 1 Comparative virulence of *Aspergillus* species in outbred CD-1 Swiss mice. The y-axis shows the lethal dose required to kill 90% of animals (LD_{90}, dose per gram). All mice received 200 mg/kg of cyclophosphamide 3 days before intravenous injection with *Aspergillus* spores. *Aspergillus* species studied included *A. fumigatus* (strains AF10, AF293, AF65, AF90, AF91, AF71, AF72, AF210, AF294, and CEA10), *A. flavus* (AFL8 and AFL5). *A. niger* (AN6714), and *A. terreus* (AT49). At least 10 animals were infected with each *Aspergillus* isolate – results on the graphic represent mean values.

Similar results have been observed in a study in which the invertebrate wax moth larvae were used as an alternative host model of invasive aspergillosis [Slatter J, personal communication]. As shown in Fig. 2, survival rate for uninfected larvae was about 90% on day 7. A lower virulence was observed for isolates of *A. terreus*, in comparison to *A. fumigatus*. Similarly to the studies involving mice, *A. flavus* demonstrated a higher virulence in larvae, in comparison to the other *Aspergillus* species. All larvae infected by *A. flavus* died within 2 days of infection.

One important limitation of the studies above is that they all represent models of disseminated *Aspergillus* infections. Although the data strongly suggests that *A. flavus* is a more virulent species than *A. fumigatus*, no direct comparison seems to exist using models of inhaled infection. Intranasal inoculation mimics the natural route of infection and would also be a more appropriate route than intravenous inoculation [13,14].

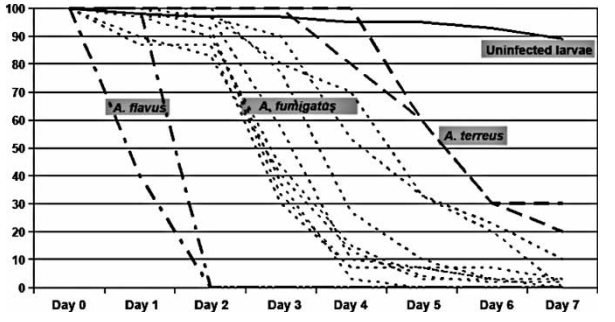

Fig. 2 Survival rate for moth larvae infected with different *Aspergillus* species. Larvae were observed for 7 days at 37°C. All larvae received an inoculum of 2×10^6 CFU/ml (2×10^4/larvae).

As mentioned before, the bigger size of *A. flavus* conidia may be an important factor limiting the ability of these spores to reach the alveoli.

In order to establish a proper model of *A. flavus* sinus or pulmonary infection, bigger animals such as rabbits may be required, instead of mice or rat [78].

Evidence from clinical trials

No data regarding species-related mortality was provided in the two largest trials on invasive aspergillosis [79,80]. This is probably due to the limited number of patients included with non-*A. fumigatus* infections in these studies. For instance, although 277 patients were included in the voriconazole *versus* amphotericin B trial [79], only seven patients had documented *A. flavus* infection. In the AmBiLad trial [80] no details were given regarding causative species. Therefore, no conclusion regarding differences in virulence among *Aspergillus* species can be reached from these studies.

Clinical syndromes

Aspergillus sinusitis

Due mainly to its conidia size, *A. flavus* is more likely to be recovered from the upper respiratory tract than *A. fumigatus* [1]. *A. flavus* may be involved in all forms of *Aspergillus* sinusitis, but there is a particular one that deserves special attention: chronic granulomatous sinusitis. This is a curious syndrome of chronic slowly progressive sinusitis associated with proptosis that has been also called indolent fungal sinusitis and primary paranasal granulomas [81,82]. Florid granulomatous inflammation is the histological hallmark of this condition, and virtually all cases are caused by *A. flavus*. Again, almost all reports come from the Sudan, Saudi Arabia, and the Indian subcontinent. There are a limited number of reports in the USA, which appear to almost exclusively affect African-Americans [1]. Patients tend to be immunocompetent and involvement of the central nervous system frequently occurs.

Although *A. fumigatus* is the most frequent *Aspergillus* organism causing allergic fungal sinusitis [83], *A. flavus* is a frequent aetiology in some geographic areas, particularly the Middle East and India [84–88]. *A. flavus* is also not a frequent cause of sinuses fungal balls (aspergillomas), with most reported cases occurring in India, Sudan and other tropical countries [1].

Pulmonary infections

As mentioned before, *A. flavus* is the second leading cause of invasive pulmonary aspergillosis. Although *A. fumigatus* causes the vast majority of allergic bronchopulmonary aspergillosis (ABPA) cases, most of the series in which *A. flavus* has also been implicated were originated in India [89–91]. Interesting cases of ABPA occurring as an occupational disease in individuals without asthma have also been reported in Japan [92]. These were usually caused by *A. oryzae* and affected workers involved in the production of soybean products.

For unknown reasons, *A. flavus* rarely causes chronic cavitary aspergillosis (CCPA) or lung fungal balls [93]. If remains to be elucidated if *A. flavus* is less able than *A. fumigatus* in causing chronic conditions such as CCPA.

Cutaneous and wound infections

Most cases of cutaneous aspergillosis involve *A. flavus* [1]. The same is also true for tongue aspergillosis [94,95], which tends to affect neutropenic patients with intense mucositis or oral ulcers. In a recent review of postoperative aspergillosis, *A. flavus* was identified in 41.2% of wound aspergillosis cases confirmed by culture [96]. In some reports, infections have been associated with the dissemination of *A. flavus* spores within the surgical room. *A. flavus* is also the main cause of *Aspergillus* osteomyelitis following trauma [97–99].

Keratitis

Fungal keratitis occurs predominantly in tropical and warm climates. At least 80% of *Aspergillus* keratitis cases are associated with *A. flavus* [6]. The major predisposing condition to *A. flavus* keratitis is trauma, generally with plant material [1].

Outbreaks

Outbreaks of aspergillosis involving the skin, oral mucosa or subcutaneous tissues are more frequently associated with *A. flavus* than any other *Aspergillus* species [1,100].

Conclusion

Although *A. flavus* seems more virulent than *A. fumigatus*, the evidence for this assumption is based mainly on experimental models of disseminated infection. Comparative studies in which animals are primarily infected via the respiratory tract are lacking and required. In addition, the available data shows highly variable results depending on the *Aspergillus* isolate studied, suggesting intra-species variation in virulence. It seems however that *A. fumigatus* is more able than *A. flavus* to adapt to extreme changes in environmental conditions, which includes the human body.

The size of *A. flavus* conidia is a very important determinant of the clinical presentations of aspergillosis caused by this species. Accordingly, *A. flavus* is particularly significant in infections involving the paranasal sinuses, skin, mucosas and the eyes. The prevalence of *A. flavus* in the environment depends greatly on climate conditions. It remains to be seen if the phenomenon of global warming will lead to an increase in *A. flavus* infections in the clinical practice.

Acknowledgements

I would like to thank Dr Peter Warn and Joanne Slatter, who kindly provided me with their data on animal and larvae models. I am also in debt with Dr Kwon-Chung, for giving helpful expert advice and Dr. Lopes-Bezerra, for her data on endethelial cells.

Declaration of interest: The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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This paper was first published online on iFirst on 24 July 2008.