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Artículo de investigación

Aspergillus fumigatus: a mere bioaerosol or a powerful biohazard?

Aspergillus fumigatus: un simple bioaerosol o un poderoso riesgo biológico?

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Abstract

Aspergillus fumigatus conidia constitute a common and widespread bioparticle in the air, which in immunocompromised and immunosuppressed patients, can produce a wide range of complaints ranging from mild allergenic reactions to severe diseases. One of the most relevant *Aspergillus*-related diseases is the invasive aspergillosis (IA), because the morbidity and mortality remain high, despite all efforts. The poor outcomes of IA treatment are frequently associated with host status (metabolic changes, malnutrition and poor immune response), a late diagnosis, and lack of adequate antifungal therapy, namely due to the resistance to liposomal amphotericin B and triazole therapy. The present work covers aspects of *A. fumigatus* biology and its role in allergy and in the development of invasive aspergillosis, additionally topics of diagnosis and therapeutic approach are also reviewed.

Keywords: *Aspergillus fumigatus*, cell death, invasive aspergillosis, diagnosis, treatment

Resumen

Los conidios de *Aspergillus fumigatus* son biopartículas comunes en el aire, que pueden causar una amplia variedad de patologías en pacientes inmunocomprometidos y inmunosupresos, que van desde las reacciones alérgicas leves a graves patologías. Una de las enfermedades más relevantes producidas por especies del género *Aspergillus* es la aspergilosis invasiva (AI), ya que la morbilidad y la mortalidad sigue siendo elevada, a pesar de todos los esfuerzos. Los escasos resultados al tratamiento utilizado en la AI, se asocian frecuentemente con estado del paciente (alteraciones metabólicas, malnutrición, deficiente respuesta inmunológica), retraso en el diagnóstico y falta de una terapia antifúngica adecuada, debido sobre todo a la resistencia terapéutica a la anfotericina B y a los triazoles. El presente trabajo abarca aspectos de la biología de *A. fumigatus* y su papel en la alergia y desarrollo de la aspergilosis invasiva, además de temas de diagnóstico y enfoque terapéutico.

Palabras clave: *Aspergillus fumigatus*, muerte celular, aspergilosis invasiva, diagnóstico, tratamiento

INTRODUCTION

The genus *Aspergillus*, comprising more than 200 species, has a remarkable impact on public health both beneficial, as the backbone of several biotechnological purposes ranging from traditional fermentation to production of recombinant proteins, and adversely, as plant and human pathogens or even as mycotoxins producers (Gugnani, 2003; Scazzocchio, 2009). Among the nearly 20 human pathogenic species, *Aspergillus fumigatus* is the prime causal agent of human infections,

followed by *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus nidulans* that is frequently used as model organism to investigate several fundamental problems of biology, such as intron self-splicing or cell cycle regulation (Denning, 1998; Morgan *et al.,* 2005; Scazzocchio, 2009).

Despite being most commonly found in growing or decaying vegetation, *A. fumigatus* presents a widespread environmental distribution, being capable to use several organic subtracts and to adapt to diverse environmental conditions (McCormick *et al.*, 2010), such as hypersaline waters (Butinar *et al.*, 2011). This fungus produces large numbers of conidia (asexual spores) that easily become airborne being efficiently dispersed through the air due to their small size (2-3 μm diameter) and inherent hydrophobicity (O'Gorman, 2011). Also, these conidia persist in the atmosphere for long periods of time due to the complex nature of the cell wall, which protects them from several physical and chemical distresses (Latgé, 2007).

Ways of spore entry, and mechanisms of pathogenesis in humans

Humans inhale several hundreds of conidia daily, being this the main route of infection (Segal, 2009). Nevertheless, other routes, such as through the direct contact with altered skin, eyes, and ears, and by the passage through the gastrointestinal tract have also been documented (Denning, 1998; Kami *et al.*, 2002; Kang *et al.*, 2008; Ben-Ami *et al.*, 2010). Once inhaled, the small size allows the conidia easily to evade the defense mechanisms of the nasal cavity and upper respiratory tract, being able to reach the lung alveoli. In healthy individuals, the immune system is well adapted to prevent these fungal infections, since alveolar macrophages easily detect these inhaled conidia, engulf and destroy them (Latgé, 1999; Brakhage *et al.*, 2010).

Conversely, in immunocompromised patients, *Aspergillus* species can induce allergic diseases (asthma, allergic bronchopulmonary aspergillosis, allergic sinusitis, and alveolitis) and non-invasive aspergillomas, after exposure to airborne conidia (Osherov, 2012). Moreover, in these patients, resting conidia easily escape alveolar macrophage recognition through an hydrophobin layer formed by the RodA that hide up the β-glucan molecules recognized by dectin-1 receptors from the macrophages, blocking conidia engulfment and the generation of a pro-inflammatory response. Also, this protein also silences the neutrophils response to swollen or germinated conidia, not being able of degranulation, releasing DNA and forming neutrophils extracellular traps (Brakhage *et al.*, 2010). *Aspergillus fumigatus* conidia present the ability to growth and sporulate at 37ºC (but also at temperatures higher than 55ºC), a common aspect amongst all human pathogens (Bhabhra & Askew, 2005). The thermotolerance of this fungus, coupled with its capacity to use a broad spectrum of both carbon and nitrogen sources to support its growth (Rhodes, 2006), and the ability to support different sort of environmental stress, have contributed to the important role of *A. fumigatus* as an human pathogen, due to the continuous adaptation of this fungus to the host. Additionally, the cytotoxic effect of secondary metabolites produced by *A. fumigatus* conidia, has been recently reported (Gauthier *et al.*, 2012). Among these metabolites, trypacidin was described as highly toxic to lung cells. Then, the accumulation of conidia in alveoli may expose the pneumocytes to toxic levels of these metabolites (Gauthier *et al.*, 2012).

Pathologies and sensitization to the fungus

One of the mild manifestations of the exposure to *A. fumigatus* conidia in humans is allergy. This is a hypersensitivity reaction initiated by immunologic mechanisms induced by the exposure and sensitization to common environmental antigens, e.g. the proteins present in airborne mould particles (Johansson *et al.*, 2001). Skin prick testing (SPT) constitute a simple and reliable method to determine allergen sensitization in epidemiologic studies (Dreborg & Frew, 1993). No data is available about worldwide sensitization to this fungus, but the prevalence of sensitization to *Aspergillus* ranges between 5 and 29% (Gioulekas *et al.*, 2004; Maurya *et al.*, 2005; Calabria & Dice, 2007), being considered a cause of symptoms aggravation

for rhinitis (Stark *et al.*, 2005) and asthma (Black *et al.*, 2000; Maurya *et al.*, 2005). Currently, 25 allergens have been described for *A. fumigatus*, with molecular weights ranging from 11 to 90 kDa, having the majority of these a known biological activity (Simon-Nobbe *et al.*, 2008). Most of these proteins show specific binding to IgE in patients with asthma and bronchopulmonary aspergillosis.

Among all *Aspergillus*-related diseases, invasive aspergillosis (IA) is perhaps the most devastating complaints mainly seen in severely immunocompromised patients. Since the first description of IA as an opportunistic infection in the early 50', there has been a significant increase in the number of cases documented by autopsy in all developed countries, namely amongst immunocompromised patients (Denning, 1998; Latgé, 2001; Latgé 2003). During the last decade, and despite all efforts, the mortality due to IA remains high being the poor outcomes attained in the treatment of this disease associated with host status, delay of an early diagnosis, and lack of adequate antifungal therapy (Howard *et al.*, 2010; Snelders *et al.*, 2011; Hadrich *et al.*, 2012). This delay in the *A. fumigatus* detection allows the fungus to reach such a population size and tissue destruction in the host that renders recovery impossible, despite of the treatment employed (Rementeria *et al.*, 2005). The majority of IA reported cases are in patients with underlying hematologic malignancy, or those subjected to bone marrow or solid organ transplantation requiring allogenic hematopoietic stem cell transplantation. Additionally, patients receiving high doses of corticosteroids or with acquired immunodeficiency syndrome (AIDS), leukemia, granulomatous diseases or hospitalized with severe illnesses, are also at risk (Morgan *et al.*, 2005; Boucher & Patterson, 2008; Beirão & Araujo, 2013).

Diagnosis, treatment and new perspectives of study

The conventional methods of IA diagnosis (microscopy, histopathology, growth in pure culture and morphologic study of the reproductive structures) are challenging because of nonspecific clinical presentation, difficulty in obtaining samples of infected tissues and non-specificity and delay of radiological imaging (e.g., CT scan) (Senn *et al.,* 2008*)*. To overcome these difficulties, new, fast, culture-independent, and highly specific tools have been developed to perform an early diagnosis. Among these, galactomannan (GM) and (1→3)-β-d-glucan (BG) antigens quantification by enzyme-linked immunosorbent assay (ELISA), and DNA fungal detection by PCR methods have been proposed (Fisher, 2013; Lass-Flörl *et al.*, 2013). However, the performance of these diagnostic tools can be influenced by several factors, such as the existence of a patient underlying disease, the concomitant use of drugs and the prophylactic antifungal strategy employed (Beirão & Araujo, 2013). Molecular tools present the overwhelming advantage of allowing the diagnostic at early stage of fungal diseases. These DNA-based assays are also commonly recommended for the identification of *A. fumigatus* within *Aspergillus* section *Fumigati* and for the evaluation of its genetic diversity, a major concern regarding outbreak controlling (Araujo *et al.*, 2009; Serrano *et al.*, 2011). It has been reported that the combination of several identification strategies can widely improve IA diagnosis (Rogers *et al.*, 2013), reducing the amount of antifungals used in the empirical therapeutic treatment (Fisher, 2013). Recently, a new approach based on single nucleotide polymorphism (SNP) markers has been suggested, as an attractive alternative for simultaneous detection, identification and genotyping of microorganisms from clinical specimens (Caramalho *et al.*, 2013).

Nowadays, four families of antifungals molecules shown anti-*Aspergillus* activity: the polyenes (amphotericin B deoxycholate, and nystatin); the triazoles, (itraconazole, voriconazole, and posaconazole); the echinocandins (caspofungin, micafungin, and anidulafungin); and the allylamines (terbinafine) (Kontoyiannis & Bodey, 2002; Odds *et al.*, 2003; Denning & Hope, 2010; Groll *et al.*, 2010; Shalini *et al.*, 2011). The main constrain associated with IA therapeutics is the increasing of triazole resistance observed in *A. fumigatus* isolates. Triazole resistance has emerged since 2007, especially in northern Europe, and undermines the management of the disease since these antifungals

are the primary agents used for prophylaxis and treatment (Denning *et al.*, 2011; Snelders *et al.*, 2011). Several mechanisms have been proposed to explain triazole resistance. The environmental route of resistance defends that resistant airborne *A. fumigatus* spores developed in the environment due to the use of azoles for crop protection and material preservation (Verweij *et al.*, 2012). Nevertheless, of the nearly 30 agricultural azole fungicides, only a few present cross-resistance with medical triazoles for *A. fumigatus* (Snelders *et al.*, 2011). Other authors defend that triazole resistance results as a consequence of prior patient exposure to several triazole compounds. The long period of drug exposure and the high number of reproducing microorganisms may be indicated as favorable conditions for the development of triazole resistance. However, fungal isolates that develop a triazole resistance during therapy are unlikely to cause further transmission, since person-to-person transmission in *Aspergillus* associated disease is very uncommon (Snelders *et al.*, 2008). Though, the environmental factors driving triazole resistance remain unclear, since only a few *Aspergillus* strains of clinical origin showing triazoles resistance have been studied in detail (Howard *et al.*, 2009). As alternative treatments, a lipid formulation of amphotericin B, or a combination of voriconazole and an echinocandin, have been proposed although there is insufficient data available to support these choices (Verweij *et al.*, 2012).

In order to overcome all constrains induced by triazole resistance, new therapeutical approaches are desired. Continuous attempts have been employed to research new anti-*Aspergillus* drugs, or to find new potential cellular targets (e.g., disruption of cell wall biosynthetic enzymes, blocking of DNA topoisomerase activity, disruption of enzymatic pathways involved in the metabolism of essential amino acids, or of enzymes involved in the synthesis of sphingolipids, polyamines, and proteins) although without promising results, so far (Latgé, 1999).

An alternative approach to overcome with the difficulties to treat IA is the assumption that cell death machineries differ from pathogens to hosts, providing new insights in the investigation that can be exploited in the discovery/development of new antifungals that activate the fungal cell suicide (Hamann *et al.*, 2008; Ramsdale, 2008). Programmed cell death (PCD) (Robson, 2006) appears to be a highly conserved mechanism among living organisms, such as bacteria (Lewis, 2000), protists (Deponte, 2008), yeasts (Madeo *et al.*, 2004), filamentous fungi (Ramsdale, 2008), plants (Pennell & Lamb, 1997), and animals (Jacobson *et al.*, 1997). PCD is involved in many biological processes, including tissue homeostasis, fungal inter- and intra-species interactions, development, aging, and lifespan control (Robson, 2006; Hamann *et al.*, 2008; Ramsdale, 2008). This suicide mechanism can be group into two major categories: programmed mechanisms genetically regulated (autophagy and apoptosis), and mechanisms environmentally driven induced by physical or chemical injuries (necrosis). This type of PCD represents a developmental strategy to remove unwanted, diseased or physiologically or genetically defected cell. The classical hallmarks of apoptosis (type I) are the externalization of phosphatidylserine, the accumulation of DNA strand breaks and release of cytochrome c from the mitochondrial inter-membrane space to the cytosol, accompanied by ultrastructural changes in the mitochondria. Autophagy (type II) is not a well-defined process and represents a 'self-eating' mechanism of damaged cells, being the major cellular pathway for bulk degradation of cytosolic material, promoting viability under nutrient starvation conditions. Contrary to PCD, this process is a lysosome-dependent and a caspase-independent process that involves vacuolization and cell lysis (Lu, 2006; Richie *et al.*, 2007; Hamann *et al.*, 2008; Ramsdale, 2008). Two different apoptotic pathways have been proposed: caspase-dependent or -independent process. Caspases are cysteine-aspartic proteases that cleave their substrates after an aspartate residue found in mammals, while metacaspases are found in other eukaryotes, such as plants, protists, and fungi, being similar to caspases, they contain a caspase-specific catalytic diad of histidine and cysteine, as well as a caspase-like secondary structure (Carmona-Gutierrez *et al.*, 2010). For the moment, two metacaspases have already been reported in *A. fumigatus*, showing a high activity in cultures entering stationary phase (Mousavi &

Robson, 2003), while two caspase-like activities (caspase 3 and caspase 8) have been found *A. nidulans* during sporulation (Thrane *et al.*, 2004).

Preliminary studies of filamentous fungi genome have already identified homologues of mammalian PCD components that appear to be absent in yeasts though experimental studies have yet to confirm their function (Robson, 2006; Richie *et al.*, 2007). In fact, *A. fumigatus* may be different from yeasts by the existence of redundant pathways of PCD that can replace the metacaspases, analogous to what has been described in higher eukaryotes (Richie *et al.*, 2007), proposing this fungi as an alternative models for the study of more complex metazoan cell death pathways (Fedorova *et al.,* 2005).

The production of reactive oxygen species (ROS) and the consequent activation of an antioxidant defense system have been reported as implicated in the development of an apoptotic phenotype in fungi, being typically an early event preceding the appearance of other apoptotic markers (Fröhlich & Madeo, 2000). The role of these molecules is still unknown, nonetheless, it has been proposed that they play a direct role in the accumulation of dsDNA breaks, due to their damaging effects on lipids and DNA, or act only as primary signals during apoptosis (Ramsdale, 2008).

CONCLUSIONS

Aspergillus fumigatus presents a widespread environmental distribution, being capable to use several organic subtracts and to adapt to diverse environmental conditions, due to its thermotolerance, and capacity to resist to several sort of environmental stresses. These are also the characteristics that allow this fungus to adapt and colonize the host. Invasive aspergillosis is perhaps the most devastating *Aspergillus*-related disease, being a matter of concern for both health practitioners and immunocompromised or immunosuppressed patients. Despite all efforts, morbidity and mortality remain really elevated. Therefore, is of primordial importance to improve early diagnosis methods, such as the new SNP-based assay for detection, identification and genotyping of *A. fumigatus* in clinical samples, and to develop new therapeutic approaches, such as the exploitation of fungal apoptosis mechanisms in order to block the germination of the fungal conidia inside its human host.

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