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Aspergillus fumigatus and Its Weapons for Infection

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Abstract:

Aspergillus fumigatus is an opportunistic pathogen causing infection in immunosuppressed patients. Its incidence is increasing since last decade and spreading fatal diseases amongst the weakened immunity patients. Aspergillus fumigatus have number of characteristics like thermotolerance, small conidiospores size, enzymes, allergens, and toxins etc. that help to protect it from host defence mechanism. Pathogenic properties of this fungus and defect in host immunity mechanism may result in dissemination of this pathogen. A better understanding about its pathogenic characteristics will help in better clinical treatment of patients. In this article, we tried to explore the characteristics that enhance its pathogenic calibre to cause infections.

Key words: *Aspergillus fumigatus; immune system; allergens; toxins; proteases; phospholipases*

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Introduction

Aspergillus fumigatus is a pathogenic fungus belonging to the genus Aspergillus. It is a saprophyte, an organism which utilizes non-living matter for their survival, and world-wide in distribution. Its natural habitat is soil and grows on dead organic matter. It is an opportunistic pathogen, which does not infect healthy host cells, but can infect immunocompromised individuals, in whom the infection can be life threatening 1.

A. fumigatus reproduces mainly through air-born conidiospores which are microscopic and free floating in environment. Its small spore size enhances its pathogenicity in host cells and an individual inhales hundreds of conidia on a daily basis but cleared up from lungs due to healthy pulmonary defence mechanisms2.

Infections from Aspergillus species has been increasing since last ten years and is one of the main causes for mortality in immunocompromised patients globally. Rise in mortality rate in patients with weakened immune response, especially due to Aspergillus species, prompted researchers to understand the epidemiology of these microbes3. Inhaled conidiospores cannot be removed from lungs due to weakened immune response and leads to their growth and germination inside host cells4.

Factors such as resistance against high temperature also enhance its dimension for virulence as it can grow quickly at 37ºC whereas optimum temperature for mesophilic fungi ranges between 25-35ºC. It carries characteristics of thermotolerance and thermophilic fungi because it can maintain its growth below 20ºC and can survive up to 60ºC5.

Infection from Aspergillus fumigatus directs development of many diseases such as allergic bronchopulmonary aspergillosis, aspergilloma, or invasive aspergillosis. Incidence of invasive aspergillosis is getting worse since last twenty years and the control measures are not adequate enough to limit its prevalence completely, hence the high mortality rate6.

Light microscopy image of the conidial head bearing chains of asexual conidiospores of A.fumigatus. Image taken from http:// www.primidi.com /images/aspergillus_fumigatus.jpg

Effect of Aspergillus fumigatus on mammals:

There are up to 200 Aspergillus species but only 15-20 of them can cause diseases in humans. Aspergillus fumigatus is the dominant infectious agent amongst them and research is still underway to understand its mechanism of infection appropriately. It contributes only 1% of total conidia produced by Aspergillus species in environment but accountable for up to 50% of infection caused by this species. A. fumigatus has more infectious competence than other species with its special characteristics like microscopic spore size, thermotolerance, and biosynthetic pathway for survival under unfavourable condition in host cells. However, its pathogenicity is secondary to immunity level of the host but important features that induce its pathogenicity are:

1. Thermotolerance

2. Conidial surface.

3. Cell wall.

Thermotolerance: The capacity to grow on high temperature makes it distinct from less thermotolerant Aspergillus species and provides opportunity to grow in mammalian respiratory tract7.

Conidial surface: There are rod-like structures on the outer surface of the conidia which are hydrophobic in nature and help conidia in their dispersion[7].

Cell wall: Immune mechanisms in humans target cell wall of the pathogenic fungi to destroy them and

hence cell wall is the main protective unit for A. fumigatus. Basically, the cell wall is a rigid structure which protects A. fumigatus from phagocyte killing mechanism of host cells. Polysaccharides are the basic components of cell wall and are classified on the basis of solubility in hot alkaline8.

Soluble part consists of α (1, 3)-glucans with some galactomannan (Bernard & Latge, 2001). Insoluble polysaccharide mainly composed of Galactomannan, chitin and β (1, 3)-glucan and is thought to be essential for cell rigidity. Composition of cell wall like formation of pigment is an essential characteristic to guard fungus Device manipulation in the context of post-production activities, in case of gestural control of digital audio effects or sound spatialisation.from host immune response. Melanin is one of the essential elements of cell wall to protect fungi from UV radiation and in maintenance of genomic factors of spores. It increases the defence competence of fungus against host immunity by reducing fungal cell phagocytosis and also by increasing resistance against cell lysis[8].

Aspergillus fumigatus is responsible for wide range of diseases depending on the immune status of individuals. In patients with asthma and cystic fibrosis, it can cause infection in from of allergic bronchopulmonary aspergillosis. This microbe came to attention due to its impeccable strength of causing disease like invasive Aspergillosis (IA). It can be lethal infection for immunocompromised patients, especially with malignancies like leukaemia, solid organ transplant, and individuals receiving long-termed corticosteroid therapy[2].

Biological view of Aspergillus fumigatus:

Studies on Aspergillus fumigatus have postulated that any A. fumigatus can infect, depending on the type of host. It enters into the host by airborne conidia to respiratory system, as the size of conidia is very small (up to 2-3 µm in size) and its small size provides opportunity to enter into alveoli and settle down in lungs. Thermotolerance is one of the strongest characteristics, as it can grow at 37ºC and can survive above 50ºC. A study about germination rate between three species, Aspergillus flavus, A. niger and A. fumigatus, have reported that there was no difference in germination rate at 30ºC but germination rates were different at 37ºC and A. fumigatus exhibited maximum germination rate compared to others2.

Function of immune system on Aspergillus fumigatus:

Previous research suggested that hyphae, conidia, and other organs of Aspergillus fumigatus can be engulfed by nonspecific phagocytic cells of the lungs such as endothelial cells, tracheal and alveolar epithelial cells. Once they are engulfed, they utilize cell resources for germination and expansion in hosts' environment. These studies also defined that under unfavourable conditions Aspergillus controls its activity in host cells. It was observed that phagocytosis of fungal hyphae by nonspecific cells is less significant than conidia but once they are engulfed by host cells, represented a rapid growth and damage to them. It is still unknown which molecules are responsible for the incidence, either molecules present on the surface of fungal hyphae or molecules involved in surface receptors of cells9.

Immunosuppressive treatment supports its infection by reducing killer cell population which are responsible for clearing up of pathogens. It has been illustrated by experiments in mice that exclusion of natural killer cells or delay in its response doubles the mortality rate. Similar experiment with immunocompromised mice indicated that natural killer cells are not essential immunosuppressive mice. There may be other immune factors in immunocompromised mice accountable for removal of pathogenic microorganisms. It introduces a new area of research to find out about these molecules in more in detail which activate or suppress the expression of these cells by fungus9.

It has been demonstrated that pathogen associated molecular patterns (PAMP) are responsible for recognition of fungal invasion by immune system and receptors that distinguish these patterns are known as PRR (e.g., receptors like IL-1R/TLR). Presence of immature dendritic cells in conjunction with other immune cells is observed with PAMP in peripheral tissues, which become mature and recognize information related to pathogenic fungus and show response in form of inflammation. They also report about dominant cytokines, which are essential against infection. This suggests that survival and death of host depends on activation of different kinds of cytokines patterns with respect to fungal constituents. The molecules responsible for activation of these actions have been identified in some fungi and the molecules involved are aspartic

proteases, mannans and phospholipomannans which are pivotal in pathogenicity of C. albicans. It gives an impression that activation of dendritic cells depends on fungal molecules by means of two TLR receptor molecules, TLR-2 and TLR-4. These receptor molecules are also crucial in macrophages activation in mouse during Aspergillus infection but the function of these molecules in A. fumigatus is still unknown. According to Roeder et al., conidial infection activates two types of TLR receptors whereas hyphal infection activates only TLR2 receptor. These studies are suggestive of involvement of some other factors that may be responsible for TLRs activation in identification of A. fumigatus. As per recent studies, TLRs produce escape mechanisms to some of pathogens; especially TLR2 initiation activity stimulates immunosuppression by encouraging production of IL-109.

Zhang et al. defined the significance of surface component to discern active components in activation system of PAMPs, for instance, β-glucan is accountable for production of C3 on yeasts" surface and also regulates expression activity of TLR (especially TLR2 and TLR4) in human macrophages. Aspergillus fumigatus hyphae play an important role in activation and attaching to macrophages with help of these receptors. β-glucan is an essential factor of hyphal cell wall and is secreted by invasive fungus in infected tissues. Polymorphonuclear phagocytes can be activated by these TLR receptors. Some studies reported TLR-2 stimulates substances responsible for preventing growth or killing of fungus and secretion of cytokines responsible for pro-inflammatory activity whereas TLR-4 supports involvement of azurophil granules and IL-10 9.

Molecules involved in virulence of *Aspergillus fumigatus:*

Biological molecules such as toxins, allergens and hydrolytic enzymes play significant role in its pathogenic activity. Hydrolytic enzymes include proteases and phospholipases responsible for catabolism of proteins and phospholipids respectively9.

Toxins: These molecules are secreted by conidia and other parts of fungus. The toxins oozed out from conidia protect it from phagocytosis, macrophages, respiratory burst action, and cytokines action of macrophages. These toxins are resistant against heat with very low molecular weight and are exclusively secreted by pathogenic conidia. Toxins from conidia are different from hyphae and studies are still on the way to uncover it appropriately9.

Aspergillus fumigatus hyphae secrete several toxins like gliotoxin, helvolic acid, and fumagillin; gliotoxin is the most toxic substance amongst them. It has been reported that some aerial conidia can produce gliotoxin and is true only for some of environmental conidia of Aspergillus fumigatus. Gliotoxin is regulated by the genes, which gets activated at the time of secondary metabolism. It can cause apoptosis at its macromolecular concentrations and nanomolar concentrations can hamper activity of ciliary cells and can injure endothelial cells9.

Helvolic acid is the member of a natural steroid antibiotic family, which is known as fusidanes and its higher concentration can support fungus to infect host cells by influencing oxidative burst of macrophages, inducing ciliostasis and epithelial cell rupture. Fumagillin is another toxin, which is an anti-tumour antibiotic and prevents angiogenesis. It acts as inhibitor for endothelial cell proliferation and ciliary function of respiratory epithelium. The activity of these two toxins in pathogenesis is still unknown but its activity in higher concentration may be essential in pathogenesis of Aspergillus fumigatus9.

Allergens: These are responsible for diverse pathological conditions such as bronchopulmonary aspergillosis, allergic rhinosinusitis, asthma, and aspergilloma. Allergens produced by Aspergillus fumigatus are named as Asp f1-Asp f23 and exhibit reaction with IgE of immunodeficient individuals, though their secretion at the time of development of fungus in invasive aspergillosis is still unknown. Some allergens show signs of very close similarity in their sequences with known functional proteins and enzymes but others have their own characteristics. An immune response of Type I hypersensitivity is triggered by all allergens and also forms antibodies IgG and IgE of close resemblance. These characteristics of allergens are utilized as diagnostic tool in identification of disease related to this microbe9

Hydrolytic enzymes: These are essential for fungi like Aspergillus fumigatus in biodegradation of raw

organic materials. To cause an infection, it is necessary to break cell wall barriers of lung epithelium cells which comprised of proteins and phospholipids and the two main enzymes, proteases and phospholipases play a part in degrading proteins and phospholipids9.

a. Proteases.

b. Phospholipases.

Gestural Acquisition

Proteases:

Fungi play a substantial role in biodegradation of waste organic matters present in environment and to accomplish this action; microbes secrete different kinds of extracellular enzymes. *Aspergillus fumigatus* infect mammals through respiratory system and secretes extracellular lytic enzymes to penetrate lung epithelium for pathogenicity. In brief, extracellular enzymes are utilized to dissolve structural defences of host cells and these structural defences are mainly composed of proteins and proteases are responsible for catabolism of proteins. Experimental evidences suggested that clinical strains secrete more proteases than normal strains and supports hypothesis that proteases are responsible for virulence of microorganisms[9].

Elastin and collagen are key components of lung environment, therefore to infect host, enzymes with catabolic activity are vital for pathogenic action. Studies illustrated that strains isolated from patients with IA show greater level of elastase activity than environmental strains and may be the reason for pathogenicity in clinical strains but its activity in environmental strains is still in process of research. Epithelial cells in lungs show changes at the time of infection and seems like these are carried out by alkaline serine proteases. Several studies suggested that proteases encourage detachment activity of epithelial cells and production of inflammatory substances like chemokines and cytokines. Extracellular proteases released by *A. fumigatus* in vivo are alkaline serine proteases, metalloprotease, and aspartic proteases[9].

Alkaline serine proteases:

These molecules participate in allergic reactions and most enzymes of this category show characteristics of antigens, as very unique antibodies have been observed in infected individuals. They are core enzymes released by fungi when it grows in presence of protein and hydrolyses different types of proteins such as collagen, fibrinogen, and casein and also exhibit action on haemoglobin and serum albumin to a lesser extent. Some other types of serine proteases were also found attached to the cell wall, which support fungal hyphae in host cell penetration. Previous studies reported number of other proteases released by Aspergillus fumigatus. Iraneta et al. conducted a study on extracts of Aspergillus fumigatus and found alkaline proteases that showed reaction with Immunoglobulin E (IgE)9.

Metalloprotease:

These are responsible for extracellular collagenolytic activity and involve in one-third of total collagenolytic activity. They also exhibit elastinolytic action but to a lesser extent compared to alkaline serine proteases and the presence of cations such as Zn2+ and Co2+ does not differ their activity. There is only similarity in two metalloproteases which is conserved motif of metalloprotease superfamily carrying Zn. One intracellular metalloprotease has been identified with molecular weight of 82 kDa and has a catabolic property for small cytoplasmic peptides; a characteristics of thimet oligopeptidases family[9].

Aspartic proteases:

These are secreted at the time of infection caused by Aspergillus fumigatus and exhibit property of pepsin family, named as aspergillopepsin. There are two types of aspartic proteases observed, one is produced by the fungus and the second one is attached to cell wall of the fungus. Aspergillopepsin F is an aspartic proteases enzyme produced by germ tubes and hyphae responsible for penetration in host cells. There is no difference in its activity in the presence of lung components. Studies suggested that there is no difference in pathogenicity in presence or absence of aspergillopepsin[9].

Phospholipases:

Phospholipases are group of enzymes responsible for degradation of phospholipids and are pivotal for generation of lipid-derived second messengers[10]. Phospholipids are utilized as substrates by all phospholipases but the site of action on phospholipids is different for each phospholipases enzymes. They are also different in their mode of action and type of regulation. Phospholipases have variety of functions like digestion of nutrients and

formation of bioactive molecules. Phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidyle thanolamine are major constituents of phospholipids in all mammalian cells. Phospholipids are scattered irregularly in all eukaryotic cells, phosphatidylcholine and sphingomyel in outer and phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine in inner leaflet of plasma membrane11. Microbes have developed different means of infection during the course of evolution including production of hydrolytic enzymes that are capable of destroying cell membranes. Host cell penetration is essential for the pathogenicity12.

Classification of Phospholipases:

Phospholipases are classified on the basis of their site of action on phospholipids. There are four categories of phospholipases termed as phospholipase A, B, C and D.

Phospholipases A (PLA):

This enzyme is further divided into two classes i.e. phospholipase A1 (PLA1) and phospholipase A2 (PLA2). PLA1 cleaves the fatty acid ester bond at SN1 position of the glycerol region, whereas phospholipase A2 breaks fatty acid bond at SN2 position. These PLAs are responsible for formation of free fatty acids and 2-acyl lysophospholipid or 1-acyl lysophospholipid respectively13.

Phospholipases B (PLB):

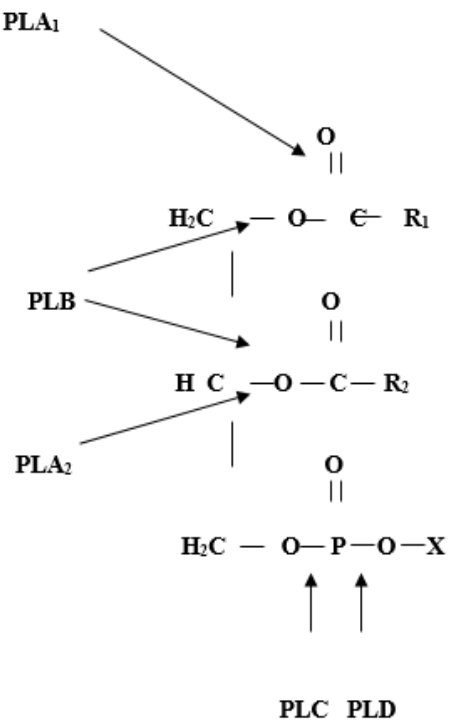
PLB is a special enzyme in phospholipases family because of its ability to hydrolyse both R-1 and R-2 fatty acids and also has lysophospholipase-transacyclase (LPTA) activity. It has been confirmed that PLB has both hydrolase and acyltransferase activities for enzymes isolated from C. albicans, penicillium notatum, and saccharomyces cerevisiae13.

Phospholipases C:

Phospholipase C acts phosphodiester bond of phospholipids just before phosphate group as represented in Figure 2. PLCs generate second messenger molecule for signal transduction in cells. Free intracellular mobilization of Ca2+ and activation of protein kinase C isozymes work as signal transduction pathways for cells. After phospholipase C dependent hydrolysis, phosphatidylinositol 4, 5-bisphosphate (PIP2) generates second messenger molecules inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol. These molecules are responsible for activity of second messenger pathways14.

Phospholipases D (PLD):

Phospholipases D are widely distributed and present in all living organisms like plants, bacteria, fungi and mammals15. Phospholipases D belongs to superfamily of enzymes that catalyses phospholipids and produce secondary messenger molecules. Secondary messenger molecules are crucial for signal transduction between cells and to maintain their integrity. Phospholipases D acts on phospholipids after phosphate group and releases phosphatidic acid and choline. Studies indicated that phosphatidic acid is key molecule generated by PLD action on phospholipids, though choline may have some characteristics of second messenger molecule. It was reported that phospholipases D involved in cell relocation, receptor endocytosis, cytoskeletal reorganization and membrane permeability. In brief, PLD is essential for regulation of cell physiology like cell proliferation and survival16.



The general structure of glycerophospholipid and the site of hydrolysis of phospholipid by different classes of phospholipases. R1 and R2 are fatty acids

chains and X is polar head group such as choline, ethanolamine or inositol.

Conclusion

Aspergillus fumigatus is a major pathogen in genus Aspergillus causing diseases worldwide in immunocompromised patients. It develops diseases like aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis. Invasive aspergillosis (IA) is a severe life-threatening condition caused by its infection. Its specific features like thermotolerance, cell wall, conidial surface, toxins, allergens, proteases, and phospholipases act as its virulent weapons to infect immunocompromised individuals.

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