Review Article

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# Pathogenesis of Mucormycosis: The Spores on a Sail.

**Abstract:**
Fungi of the order Mucorales are capable to infect human beings and animals. Mucormycosis is a well-known, life-threatening infection, primarily occurring in immune-compromised conditions like diabetic ketoacidosis, organ transplantation, increased serum iron and neutropenia. The mortality and morbidity rate due to Mucormycosis is rapidly rising despite of surgical debridement and antifungal aids. In this article, we will review (i) the existing knowledge about fungi (ii) interaction of Mucorales with host immune system (iii) assessment of virulence factors of Mucorales which play a key role in infection like high-affinity iron permease FTR1, spore coat protein CotH, reductase permease system. Since Mucormycosis infection is angioinvasive in nature, emphasis is placed on the ways by which organisms acquire iron from the host and its interaction with blood vessels. The present mini-review attempts to spread awareness regarding difficult to treat infection of mucormycosis and to provide possible targets for future research for therapeutic measures.

**Key Words:** Mucormycosis, Pathogenesis, Virulence Factors, Host Response

## Introduction:

Mucormycosis is a promptly growing opportunistic infection caused by Mucorales, an order of fungi. [1] Mucorales acts as the most prevalent pathogen inflicting disease in humans. In patients suffering from mucormycosis, Rhizopus Oryzae species are found upon exploration. It accounts for about 60-70% of most mucormycosis infections. [2] It has been concluded using studies based on various biopsy reports that Mucormycosis is the third most widespread fungal infection after Candidiasis and aspergillosis. It is additionally common in adults. [3]The reason behind uprising outbreak of mucormycosis is due to increased cases of diabetes mellitus, neutropenia, organ transplant, prolonged corticosteroid therapy, malignant neoplasms. [4] A disparity in the risk factors associated with mucormycosis is seen between developing countries like India, Pakistan and developed countries like the U.S. While uncontrolled Diabetic Mellitus caps the leading cause in developed countries, stem cell transplantation in developing ones. [5,6] Woefully, despite of varying surgical treatments including debridement, antifungal therapy, the mortality rate is high. New strategies which can thwart the infection are the need of the hour. Understanding pathogenesis behind infection can assist in modulating the treatment plan effectively.

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## Transmission:

It usually spread by three modes-

1. Inhalation of fungal spores
2. Consuming infected food
3. Inoculation by infected needles
4. Infected wounds, burns, trauma [4,7]

## Pathogenesis:

The interaction between host and causative organism is extremely significant in disease development. In mucormycosis, the fungal spores and hyphae intrude the human body, severing the defence barrier, infiltrating blood vessels and dissemination. [8]

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## Host Defense:

Macrophages are the initial line of defence that recognizes and responds hastily when the pathogen invades, degrading and ingesting the same. By any possibility, if fungi escape, it evokes neutrophils which undergo migration towards them, ravage and engulf them. Neutrophils fiddle a substantial role in the defence mechanism. They elicit cytokines like tumour necrosis factor [TNF-α], interferon-gamma [IFN-γ], interleukin Ib, who act as proinflammatory cytokines recruiting other inflammatory factors. They also operate toll-like receptors (TLR-2) and a vigorous NF-κB related gene expression. [9,10] Furthermore, Neutrophils generates oxidative metabolites, peptides and perforins. These oxidative metabolites exterminate the fungi by killing spores and hyphae. [11]

In patients with diabetic ketoacidosis with decreased ph and increased blood glucose, neutrophils are incapable to execute their process of chemotaxis and degradation of hyphae by differing mechanisms like oxidative and non-oxidative. In studies, it has been revealed that inhalation of Mucorales spores in usual healthy animals did not result in infection. But the immunocompromised animals or the animals suffering from diabetic ketoacidosis develop mucormycosis post-invasion of spores in their body. [12]

Not solely do phagocytes dysfunction leads to the development of mucormycosis in patients, but there are also many other components associated. Similarly, platelets also hold a key role against the invasion of Mucorales. Platelets on contact with fungal hypha and spores aggregate and induce clot formation, impeding the function of spores. They possess antimicrobial and antifungal properties. They express molecules that bind to endothelial cells, dendritic cells, which gets activated upon binding and suppress Mucorales. They contain granules having cytokines with antifungal properties. [13, 14] Likewise, natural killer cells exhibit antifungal properties and kill the hyphae by various oxidative and non-oxidative methods. They secrete granules having pro-inflammatory cytokines which act against fungi. [15]

## Virulence factors:

Mucorales possess diverse virulence factors which facilitate them to inflict disease in the host. Genomic studies have ascertained that during the evolution of Rhizopus Oryzae, the organism has made itself competent to withstand varying environmental variations by secretion of virulence components. [16] These genetic adaptations have made it plausible for the organism to inundate the host body, survive under hostile conditions, invasion in blood vessels, attains nourishment for its metabolism from the host and evolving as immune to antifungal agents. They are the rmotolerant and stress-tolerant in nature. [17]

One of the virulence characteristics is the acquisition of iron from the host. Iron is the vital element of the human body required for the effective functioning of cells. Mucorales acquire free iron from the host lowering down the immunity rendering the host more susceptible to disease.[18] The organism secretes lytic enzymes like aspartic proteinases along with metabolic alkaloids and toxins like rhizoxin which facilitates its access and spread in the host. Rhizoxin is antimitotic in function. In recent studies, it has been observed that rhizoxin is not secreted by fungi itself but from a symbiotic association with the bacteria Burkholderia. Rhizopus exhibits an active ketone reductase system too, which bestows to its pathogenesis in patients suffering from diabetic ketoacidosis. [19-22]

## Host Pathogen Interaction:

Not many studies have been done associated to epithelium interaction with the spores. After the deterioration of epithelium, the spores adhere to extracellular matrix protein, collagen type IV and laminin of the basement membrane. Mucorales express differing glycolytic and proteolytic enzymes which encroaches the host system after damaging it. [23, 24]

The endocytosed Mucorales bind to endothelium by expressing a peculiar protein known as coat homolog (CotH) protein. These (CotH) proteins are distinct to Mucorales and the virulence of the organism relies upon the abundance of its copies produced. Expressed protein gets adhered to receptors called glucose-regulator protein 78 (GRP78) existing on the surface of the host endothelium.[25] GRP78 is also called BiP/HSPA[5]. It is recognized as a protein elicited during glucose scaracity in the cells. It is a component of the heat shock protein family HSP70, located in the endoplasmic reticulum it is a unique receptor in host cells.[26] GRP78

functions in two ways. It acts as a crucial chaperone, where it supports in compiling, wrapping and organizing protein and deportation of non-sense proteins. Furthermore, it presents as a sensor during the stress of the endoplasmic reticulum. GRP78 and CotH proteins expression is heightened during Rhizopus invasion. Their secretion is also intensified during diabetic ketoacidosis and increased free iron in serum. The adherence of CotH to the receptor proteins boosts the invasion of Mucorales in the host where it results in destruction. [27]

Research studies have implied that in the Mucorales infected mice, an increased expression of GRP78 was found to be present. These mice were more likely to be suffering from diabetic ketoacidosis. Normal healthy mice carrying anti GRP78 serum were found to be protected against diabetic ketoacidosis. These findings suggest an association of diabetic ketoacidosis in patients to Mucorales. [28]

Furthermore, Platelet-derived growth factor receptor [PDGF] was found to be more triggered during this fungal infection. PDGF is known to regulate cell division and functioning. PDGF receptors promote the binding of fungi and cause invasion in blood vessels. [29]

## Iron Uptake Mechanism:

Iron is indispensable for the growth of fungus. In the normal state, Iron is bound to the serum proteins like transferrin, ferritin and lactoferrin. This bound state iron deters the host from lethal consequences of unbound free iron. It is the universal protection mechanism against fungi as scaracity of free iron suppresses the growth of fungi in the human body. In research studies done on animals, it has been established that presence of iron in serum promotes the growth of fungi whereas iron deprivation prohibits the growth. Fungi acquire iron from the host by three mechanisms:

1. Iron from the heme
2. Proton mediated iron transfer
3. Iron chelation [30]

## Reductase Permease System:

Fungi possess a reductase permease system which facilitates them to acquire host iron. Permease has an elevated likeness towards iron have enzyme reductase on the surface which reduces insoluble ferric iron form to soluble ferrous ion. Thus reduced ferrous state iron is captured by a protein complex. The protein complex is made up of multicopper oxidase and a ferrous permease. The genetic studies have revealed the existence of ferric reductase (three), copper oxidase (six) and iron permease (one) combination. FTR1 is the gene credible for coding the high-affinity iron permease during fungal infection. [31, 32] Reduction of gene expression FTR1 or reduced synthesis of the gene declines the fungi impact in animals with mucormycosis. Immunisation in mice utilizing anti-FTR1p, protected them from Mucorale infection. [33]

## Pathway 1: Iron from the heme:

Heme is an affluent source of iron for intruding fungi because of the angioinvasive attribute of disease. Heme is procured in two ways. Through the reductase permease system, ferric iron is acquired from heme. Heme oxygenase is an enzyme that helps in attaining ferric iron in the cytosol when heme is transported intracellularly. [34, 35]

## Pathway 2: Proton mediated Iron transfer:

Patients suffering from Diabetic ketoacidosis are more likely to be invaded by Mucorales causing mucormycosis. An acidic ph of range 7.3-6.8 promotes the growth of R.oryzae. These patients have a heightened level of free iron in serum eliciting acidic conditions. Moreover, the addition of iron from exogenous sources further accentuates the growth of fungi. It has been seen that acidic pH impedes the binding of iron to protein carriers and there is proton mediated transfer of free iron to serum from transferrin. Protons from the acidic pH bind to transferrin allowing iron displacement to the serum which further moves intracellularly by reductase permease system. [36]

The increased serum glucose level in DKA patients also leads to impaired phagocytic function and IFN-γ synthesis.The presence of glucose, iron and ketone bodies provokes the GRP78 and CotH expression which further augments damage by fungi. [37]

## Pathway 3: Iron Chelation:

R. oryzae produces a siderophore, rhizoferrin. Rhizoferrin is incapable of rendering adequate iron to Rhizopus from the serum. Also, Rhizopus lacks an enzyme, non-ribosomal peptide synthase which generates a siderophore. Thus, Mucorales are dependent on host iron like in Diabetic KA patients, enzyme haem oxygenase or reductase system. [38]

Deferoxamine, a siderophore is used in patients when there is excessive iron load. It is an iron chelator and chelates iron from carrier bounds iron with the formation of ferroxamine (iron-deferoxamine complex). This complex adheres to the fungus through receptors Fob 1 and Fob 2 b. By reduction mechanism, iron is liberated from the complex. Thus formed ferrous ion is oxidised to the ferric state by enzyme copper oxidase which is then transported intracellularly by permease FTR1. [39]

## Conclusion:

Immunocompromised patients, patients undergone organ transplantation or on deferoxamine therapy are extremely prone to the development of Mucormycosis. Virulence factors of pathogen in a host at risk elicit numerous pathologies in patients due to angioinvasion. Augmenting host immunity, counteracting virulence factors can assist in counteracting the Mucorales. FTR1 is an important gene for the survival of R. Oryzae and inhibition of it by passive immunisation can procure encouraging results in future against life-threatening mucormycosis.

## References:


1. Hibbett DS, Binder M, Bischoff JF, et al. A higher-level phylogenetic classification of the Fungi. Mycol Res 2007; 111:509–47.
2. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clin Microbiol Rev 2000; 13:236–301.
3. Dignani MC. Epidemiology of invasive fungal diseases on the basis of autopsy reports. F1000Prime Rep. 2014;6:81.
4. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. Clin Microbiol Rev 2005; 18:556–69.
5. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis. 2005;41(5):634–53.
6. Chakrabarti A, Singh R. The emerging epidemiology of mould infections in developing countries. Curr Opin Infect Dis. 2011;24(6):521–6.
7. Sun HY, Singh N. Mucormycosis: its contemporary face and management strategies. Lancet Infect Dis. 2011;11(4):301–11.
8. Petrikkos G, Tsioutis C. Recent Advances in the Pathogenesis of Mucormycoses. Clin Ther. 2018;(6):40, 894–902.
9. Roilides E, Kontoyiannis DP, Walsh TJ. Host defenses against zygomycetes. Clin Infect Dis. 2012;54(Suppl 1):S61–6.
10. Chamilos G, Lewis RE, Lamaris G, Walsh TJ, Kontoyiannis DP. Zygomycetes hyphae trigger an early, robust proinflammatory response in human polymorphonuclear neutrophils through toll-like receptor 2 induction but display relative resistance to oxidative damage. Antimicrob Agents Chemother 2008; 52:722–4.
11. Ibrahim AS, Voelz K. The mucormycete-host interface. Curr Opin Microbiol. 2017;40:40–5.
12. Chinn RY, Diamond RD. Generation of chemotactic factors by Rhizopus oryzae in the presence and absence of serum: relationship to hyphal damage mediated by human neutrophils and effects of hyperglycemia and ketoacidosis. Infect Immun 1982; 38:1123–9.
13. Perkhofer S, Kainzner B, Kehrel BE, Dierich MP, Nussbaumer W, Lass-Flörl C. Potential antifungal effects of human platelets against zygomycetes in vitro. J Infect Dis. 2009;200(7):1176–9.
14. Speth C, Rambach G, Lass-Flörl C. Platelet immunology in fungal infections. Thromb Haemost. 2014;112(4):632–9.
15. Schmidt S, Tramsen L, Perkhofer S, Lass-Flörl C, Hanisch M, Röger F, et al. Rhizopus oryzae hyphae are damaged by human natural killer (NK) cells, but suppress NK cell mediated immunity. Immunobiology. 2013;218(7):939–44.
16. Ma L-J, Ibrahim AS, Skory C, et al. Genomic analysis of the basal lineage fungus rhizopus oryzae reveals a

whole-genome duplication. Madhani HD, ed. PLoS Genet. 2009;5:e1000549.

17. Lamaris GA, Ben-Ami R, Lewis RE, Chamilos G, Samonis G, Kontoyiannis DP. Increased virulence of Zygomycetes organisms following exposure to voriconazole: a study involving fly and murine models of zygomycosis. J Infect Dis. 2009;199(9):1399–406.
18. Ibrahim AS, Spellberg B, Edwards J Jr. Iron acquisition: a novel perspective on mucormycosis pathogenesis and treatment. Curr Opin Infect Dis 2008; 21:620–5.
19. Jennessen J, Nielsen KF, Houbraken J, et al. Secondary metabolite and mycotoxin production by the Rhizopus microsporus group. J Agric Food Chem 2005; 53:1833–40.
20. White JD, Blakemore PR, Green NJ, et al. Total synthesis of rhizoxin D, a potent antimitotic agent from the fungus Rhizopus chinensis. J Org Chem 2002; 67:7750–60.
21. Partida-Martinez LP, Hertweck C. Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 2005; 437:884–8.
22. Farley PC, Sullivan PA. The Rhizopus oryzae secreted aspartic proteinase gene family: an analysis of gene expression. Microbiology 1998;144:2355–66.
23. Schoen C, Reichard U, Monod M, Kratzin HD, Rüchel R. Molecular cloning of an extracellular aspartic proteinase from Rhizopus microsporus and evidence for its expression during infection. Med Mycol. 2002;40(1):61–71.
24. Spreer A, Rüchel R, Reichard U. Characterization of an extracellular subtilisin protease of Rhizopus microsporus and evidence for its expression during invasive rhinoorbital mycosis. Med Mycol. 2006;44(8):723–31.
25. Chibucos MC, Soliman S, Gebremariam T, Lee H, Daugherty S, Orvis J, et al. An integrated genomic and transcriptomic survey of mucormycosis-causing fungi. Nat Commun. 2016;7:12218.
26. Liu M, Spellberg B, Phan QT, et al. The endothelial cell receptor GRP78 is required for mucormycosis pathogenesis in diabetic mice. J Clin Invest 2010; 120:1914–24.
27. Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res 2007; 67:3496–9.
28. Liu M, Spellberg B, Phan QT, Fu Y, Fu Y, Lee AS, et al. The endothelial cell receptor GRP78 is required for mucormycosis pathogenesis in diabetic mice. J Clin Invest. 2010;120(6):1914–24.
29. Kovalenko M, Rönnstrand L, Heldin CH, Loubtchenkov M, Gazit A, Levitzki A, et al. Phosphorylation site-specific inhibition of plateletderived growth factor beta-receptor autophosphorylation by the receptor blocking tyrphostin AG1296. Biochemistry. 1997;36(21):6260–9.
30. Kerrels V, et al. Ferritin-associated iron induces neutrophil dysfunction in hemosiderosis. J Lab Clin Med. 1999;133(4):353–61.
31. Stearman R, Yuan DS, Yamaguchi-Iwai Y, Klausner RD, Dancis A. A permease-oxidase complex involved in high-affifinity iron uptake in yeast. Science 1996; 271:1552–7.
32. Knight SA, Vilaire G, Lesuisse E, Dancis A. Iron acquisition from transferrin by Candida albicans depends on the reductive pathway. Infect Immun 2005; 73:5482–92.
33. Jung WH, Sham A, Lian T, Singh A, Kosman DJ, Kronstad JW. Iron source preference and regulation of iron uptake in Cryptococcus neoformans. PLoS Pathog 2008; 4:e45.
34. de Locht M, Boelaert JR, Schneider YJ. Iron uptake from ferrioxamine and from ferrirhizoferrin by germinating spores of Rhizopus microsporus. Biochem Pharmacol. 1994;47(10):1843–50.
35. Ibrahim AS, Gebremariam T, Lin L, Luo G, Husseiny MI, Skory CD, et al. The high affinity iron permease is a key virulence factor required for Rhizopus oryzae pathogenesis. Mol Microbiol. 2010;77(3):587– 604.
36. Artis WM, Fountain JA, Delcher HK, Jones HE. A mechanism of susceptibility to mucormycosis in diabetic ketoacidosis: transferrin and iron availability. Diabetes. 1982;31(12):1109–14.
37. Liu M, Lin L, Gebremariam T, Luo G, Skory CD, French SW, et al. Fob1 and Fob2 Proteins Are Virulence Determinants of Rhizopus oryzae via Facilitating Iron Uptake from Ferrioxamine. PLoS Pathog. 2015;11(5):e1004842.
38. Thieken A, Winkelmann G. Rhizoferrin: a complexone type siderophore of the Mucorales and entomophthorales (Zygomycetes). FEMS Microbiol Lett 1992; 73:37–41
39. de Locht M, Boelaert JR, Schneider YJ. Iron uptake from ferrioxamine and from ferrirhizoferrin by germinating spores of Rhizopus microsporus. Biochem Pharmacol. 1994;47(10):1843–50.