Jena School for Microbial Communication
Graduate School of Excellence

Hydrophobic surface binding protein is ligand for recognition of *L. corymbifera* by human phagocytes: Identification of receptor and diagnostic markers.

PhD Student: Jaime David, Acosta España M.D.
Supervisors: PD. Dr. Kerstin Voigt; Prof. Marc Thilo Figge

Abstract:

Members of the fungal order Mucorales are environmental fungi that infect humans with risk factors such as decreased cellular immunity, diabetic ketoacidosis, use of corticosteroids, onco-hematological diseases, COVID-19, among others. These patients with mucormycosis have a late diagnosis (figure 1), consequently with a high risk of mortality, especially in invasive infections. Early diagnosis is crucial since it defines a surgical debridement of the infected tissue and antifungal treatment (Prakash et al., 2019; Patel et al., 2020; Hoenigl et al., 2022).
Rhizopus delemar, *Mucor lusitanicus* and *Lichtheimia corymbifera* were selected because they were most frequently identified as causative agents of mucormycosis in immuncompromised patients. The whole genomes of all three species were entirely sequenced providing a prerequisite for proteomic analyses. Hence, we have subjected *R. delemar*, *M. lusitanicus* and *L. corymbifera* to extensive proteomic analyses targeting the spore surface proteome (surfome) and the secretome (population of secreted proteins). A wide variety of CotH and other proteins were detected in comparative secretome analyses under different cultivation conditions and was compared with the secretome to identify spore surface proteins, which are in addition secreted (Gebremariam et al., 2014).

The results are discussed in the light to identify spore surface proteins, which play important roles in the interaction with phagocytes. This was confirmed in a study by Gebremariam et al., who found that knocking out CotH3 makes the fungus noninvasive and reduces *Rhizopus* virulence in mice, while polyclonal antibodies raised against CotH3 peptides protected diabetic ketoacidotic (DKA) and neutropenic mice from mucormycosis when compared to mice given control preimmune serum (Gebremariam et al., 2019). In this way, antibodies against CotH3 as a possible immunotherapy in the future.

Although adjunctive therapy in patients with mucormycosis is important, in infected patients it has been widely demonstrated that early initiation of antimicrobial therapy is essential (Im et al., 2022). For this reason, the expression of surface and secreted proteins in Mucorales is fundamental, especially to analyze these proteins as probable early biomarkers in diagnosis of mucormycosis, that have already been described for other invasive fungal diseases such as aspergillosis (Hamam et al., 2021).

The immunological response to different pathogens depends on the antigens, thus surface antigens can have several functions that range from adhesion and even in many

pathogens they can become superantigens. But on the host side, the reaction to these antigens can vary in patients. Epithelial and immunological cell receptors in the recognition of pathogens (PRRs) through antigens and pathogen-associated molecular patterns (PAMPS) (Medzhitov, 2007; Schmid-Siegert et al., 2017).

It is important to mention that Toll-like receptors (TLR), which recognize evolutionarily conserved patterns in invasive microorganisms, are in fact the best studied family of pattern recognition receptors (PRR) among the entire large group of receptors. TLRs activate a variety of proinflammatory and antimicrobial responses in response to these patterns and thus play an important role in the first line of defense against pathogens, while also stimulating adaptive immune responses. Single nucleotide polymorphisms (SNPs) in multiple human TLR proteins have been linked to increased susceptibility to infection, according to a growing body of evidence (Skevaki et al., 2015).

For example, genetic variations in TLR-1 with changes in the amino acid Asn248Ser have shown susceptibility in allogeneic hematopoietic stem cell transplant recipients to invasive aspergillosis (Kesh et al., 2005). However, this may have clinical applicability as it can be used as a marker of susceptibility in patients who have multiple risk factors that predispose to invasive fungal disease. This has not yet been described for Mucorales infections in humans, which could be a screening tool for risk groups for mucormycosis. And if described, they would serve to identify at-risk patients even before disease outbreaks, which could allow closer surveillance and early treatment.

Finally, from the patient side, new biomarkers for the diagnosis of mucormycosis may change the landscape and help reduce the high risk of mortality. Therefore, the study of surface proteins in Mucorales and the analysis of possible SNPs in TLRs in clinical samples from previously infected patients can be transformed into rapid tests that allow appropriate treatment, which may have an impact on the survival of these patients. Assessing the risk of infection associated with SNPs in the different TLRs. For this reason, it is important to study clinical samples from patients with mucormycosis, because if specific SNPs in the TLRs were identified, this could be a biomarker that can be used to assess the risk of infection. This would allow closer monitoring of these patients, early diagnosis, and even optimal therapy.

Reference

Gebremariam, T., Alkhazraji, S., Soliman, S. S. M., Gu, Y., Jeon, H. H., Zhang, L., et al. (2019). Anti-CotH3 antibodies protect mice from mucormycosis by prevention of invasion and augmenting opsonophagocytosis. *Science advances* 5. doi:10.1126/SCIADV.AAW1327.

Gebremariam, T., Liu, M., Luo, G., Bruno, V., Phan, Q. T., Waring, A. J., et al. (2014). CotH3 mediates fungal invasion of host cells during mucormycosis. *The Journal of clinical investigation* 124, 237–250. doi:10.1172/JCI71349.

Hamam, J., Navellou, J. C., Bellanger, A. P., Bretagne, S., Winiszewski, H., Scherer, E., et al. (2021). New clinical algorithm including fungal biomarkers to better diagnose probable

invasive pulmonary aspergillosis in ICU. *Annals of Intensive Care* 11, 1–9. doi:10.1186/S13613-021-00827-3/TABLES/2.

Hoenigl, M., Seidel, D., Carvalho, A., Rudramurthy, S. M., Arastehfar, A., Gangneux, J.-P., et al. (2022). The emergence of COVID-19 associated mucormycosis: a review of cases from 18 countries. *The Lancet Microbe* 0. doi:10.1016/S2666-5247(21)00237-8.

Im, Y., Kang, D., Ko, R. E., Lee, Y. J., Lim, S. Y., Park, S., et al. (2022). Time-to-antibiotics and clinical outcomes in patients with sepsis and septic shock: a prospective nationwide multicenter cohort study. *Critical Care* 26, 1–10. doi:10.1186/S13054-021-03883-0/FIGURES/4.

Kesh, S., Mensah, N. Y., Peterlongo, P., Jaffe, D., Hsu, K., van den Brink, M., et al. (2005). TLR1 and TLR6 polymorphisms are associated with susceptibility to invasive aspergillosis after allogeneic stem cell transplantation. *Annals of the New York Academy of Sciences* 1062, 95–103. doi:10.1196/ANNALS.1358.012.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature 2007 449:7164* 449, 819–826. doi:10.1038/nature06246.

Patel, A., Kaur, H., Xess, I., Michael, J. S., Savio, J., Rudramurthy, S., et al. (2020). A multicentre observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India. *Clinical Microbiology and Infection* 26, 944.e9-944.e15. doi:10.1016/J.CMI.2019.11.021/ATTACHMENT/6050CEF9-56E4-43D3-8053-2A746BE27604/MMC3.DOCX.

Prakash, H., Ghosh, A. K., Rudramurthy, S. M., Singh, P., Xess, I., Savio, J., et al. (2019). A prospective multicenter study on mucormycosis in India: Epidemiology, diagnosis, and treatment. *Medical mycology* 57, 395–402. doi:10.1093/MMY/MYY060.

Schmid-Siegert, E., Richard, S., Luraschi, A., Mühlethaler, K., Pagni, M., and Hauser, P. M. (2017). Mechanisms of surface antigenic variation in the human pathogenic fungus pneumocystis jirovecii. *mBio* 8. doi:10.1128/MBIO.01470-17/ASSET/219C93E3-5D81-4B35-8934-5040C60AD5DB/ASSETS/GRAPHIC/MBO0051735680005.JPEG.

Skevaki, C., Pararas, M., Kostelidou, K., Tsakris, A., and Routsias, J. G. (2015). Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases. *Clinical and Experimental Immunology* 180, 165. doi:10.1111/CEI.12578.

Figure 1.- Diagnosis of mucormycosis and new perspectives