The Sweet Side of Fungal Infections: Structural Glycan Diversity and Its Importance for Pathogenic Adaptation

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Abstract: Fungal infections are the most common secondary infections in debilitated individuals in a state of chronic disease or immunosuppression. Despite this, most fungal infections are neglected, mainly due to the lower frequency of their more severe clinical forms in immunocompetent individuals with a healthy background. However, over the past few years, several cases of severe fungal infections in healthy individuals have provoked a change in the epidemiological dynamics of fungal infections around the world, both due to recurrent outbreaks in previously infrequent regions and the greater emergence of more pathogenic fungal variants affecting healthy individuals, such as in the Cryptococcus genus. Therefore, before the arrival of a scenario of prevalent severe fungal infections, it is necessary to assess more carefully what are the real reasons for the increased incidence of fungal infection globally. What are the factors that are currently contributing to this new possible epidemiological dynamic? Could these be of a structural nature? Herein, we propose a discussion based on the importance of the virulence factors of glycoconjugate composition in the adaptation of pathogenic fungal species into the current scenario of increasing severity of these infections.

Keywords: fungal infection; glycoconjugates; immune system; cytokines

Currently, fungal infections are a major cause of comorbidity globally [1]. Indeed, pathogenic fungi contribute to a large portion of deaths in immunocompromised individuals or as a secondary disease [2]. Historically, the pathogenic species of the genus Cryptococcus, Cryptococcus neoformans and Cryptococcus gattii were fungi known to chronically establish a silent infection with difficult diagnosis in the early stages of disease progression [3]. This is mainly complicated by the low spectrum of effective antifungal drugs for the most serious infections [4], especially when associated with the spread of the pathogenic microorganism to critical host tissues such as the central nervous system where it can lead to severe fungal meningoencephalitis [3,6]. In particular, there are indications that the dynamics of infections caused by Cryptococcus spp. have been modified over the
years. Previously, this pathogenic fungus was mostly associated with warmer climate areas where its environmental incidence was higher [7], being found in forests and plant material in tropical or subtropical morphoclimatic domains [8]. However, there has been a surge of cases over the years in previously uncommon places [9–11]. In addition, the highest global incidence of cryptococcosis has always been associated with regions with a history of human immunodeficiency virus (HIV) infections, such as sub-Saharan Africa [12]. The prevalence of a virus that causes severe immunodepression, as is the case with acquired immunodeficiency syndrome (AIDS), creates space for opportunistic infections to thrive in weakened hosts [13]. Consistently, the AIDS crisis at the end of the 20th century was crucial for the increased number of secondary infections caused by \textit{C. neoformans} [14]. This phenomenon demonstrated the great epidemiological proportions that a fungal infection can reach, and exposed the world’s lack of preparation to deal with any severe opportunistic fungal infection outbreaks. In addition to the species of the genus \textit{Cryptococcus}, debilitated individuals are also affected by several other genera of fungi [15], such as the genus \textit{Aspergillus}, which is responsible for cases of severe pneumonia in immunologically weakened hosts, promoting a high mortality rate and high number of hospitalizations worldwide [16]. Furthermore, some pathogenic species of fungus can affect healthy individuals, causing serious infections, for example, the species of \textit{C. gattii} [17]. Interestingly, this species has stood out for its epidemiological dynamics, with more cases reported in urban environments, increasingly distancing itself from its primary incidence in wild habitats [18–21]. In addition, there is the example of the genus \textit{Candida} spp. which, despite being a commensal fungi on body surfaces, can cause serious superficial infections in the skin and mucous membranes of both immunocompromised and immunocompetent individuals [22] and may even cause severe forms of systemic dissemination [23]. Moreover, it is also worth factoring in the COVID-19 pandemic and its more recent outbreaks. The high mortality among COVID-19 patients also suffering from fungal co-infections emphasizes the necessity to take those pathologies with great concern [24]. Multiple countries related an increase in the cases of fungal co-infections in severe COVID-19 patients [25]. The majority of the cases were associated with pulmonary aspergillosis [26], pneumocystis pneumonia [27], cryptococcal pneumonia [28], \textit{C. laurentii} endophthalmitis [29] and \textit{C. neoformans} meningoencephalitis in an immunocompetent patient [30], and also some were related to the outbreaks of the rare mucormycosis infection in India, which is often life-threatening [31]. The susceptibility in these patients can be justified mainly by the use of corticosteroids, which increases the risk of a secondary infection by suppressing an already debilited immune system, opening a space for fungal infection in patients with a weakened antifungal immunity [32]. Indeed, in some cases, critically-ill COVID-19 patients appear to be at low risk for fungal infections when compared to those who have undergone corticosteroid treatment [33].

However, a question arises in the midst of all these infections caused by fungi: what are the real contributing factors leading to a greater spread of pathogenic fungal species? Are their differences of an environmental nature or due to evolutionary traits? Indeed, the intrinsic susceptibility of permissive individuals to fungal infection alone does not justify the increase in fungal dissemination, since immunocompetent individuals can be affected by many of these infections [34]. In fact, there are several factors that systematically contribute to minimize the geographic restrictions of fungi and increase the prevalence of these diseases. It is believed that global warming contributes to the change in dynamics of animal populations, including fungus-carrying species, as is the case for \textit{Cryptococcus} spp., which is strongly associated with birds. [35]. The deforestation of wild environments with the ever-expanding limits of modern agricultural activities and the extensive reforestation of invasive tree species such as the \textit{Eucalyptus} also play a pivotal role in favoring the dispersion of environmental fungal species to regions outside their original niche [36]. This geographical displacement pressure can result in evolutionary adaptation of pathogenic fungal species in order to establish themselves environmentally. Therefore, it is also suggested that the evolutionary distance of fungal species is also due to the dissemination into different geographic regions [37]. This dynamic is critical for the differences in the
molecular composition of these different intra-species or intra-genus variations of fungi [38]. The evolutionary pressure generated by the need for adaptation of fungal species may contribute to the emergence of increasingly resistant strains. For instance, a hypervirulent strain of *C. deuterogattiii* (R265), genetically originated from Brazil [39], gained distinction by causing the infamous epidemiological outbreak of severe cryptococcosis in human hosts and in animal wildlife on the Pacific North Coast in Canada [40], which continues to spread along the US west coast and into California [41]. In addition, a high radiation tolerance for some strains of *Cryptococcus* in the Chernobyl Exclusion Zone—UA—has been described. Such specimens exhibit highly melanized yeast morphology associated with resistance mechanisms against environmental stress [42]. Hence, there is likely a strong relationship between the adaptation of the fungus species and the modifications of the molecular constituents, mainly in the fungal virulence factors.

A substantial portion of the main virulence factors of fungi are composed of glycoconjugates [43–46]. These polysaccharides, mostly found in the cell wall, play an important structural role, and many are conserved among different species of fungi [47]. One of the main components present in the fungal cell wall is the β-glycans, with the β-(1,3)-glycan being the most abundant in *C. albicans* species and β-(1,6)-glycan in *C. neoformans* species [48,49]. These components provide structural stability to the fungal cell and a way of interacting with host cells. For instance, disrupting the binding of β-(1,3)-glycan to dectin-1 and CD11b inhibits macrophage phagocytosis [50]. Interestingly, it has already been observed that glycans of higher molecular weight elicit greater activation of macrophages, thus demonstrating that the potential for cell activation is related to glycoconjugate size [51]. The same behavior has already been reported on another important component for the structuring of the fungal cell, the chitin molecule, composed of long chains of N-acetylglucosamines [52]. Although large chitin fragments are inert, intermediate and small ones are capable of inducing cytokine production in vivo and in vitro. Furthermore, different sized fragments are capable of interacting with different host receptors activating distinct signaling pathways causing intermediate fragments to upregulate TNF-α release, while smaller fragments stimulates both TNF-α and IL-10, indicating that mismatched sized chitin fragments lead to different immune system behavior [53]. Interestingly, the percentage of chitin in the cell wall of fungi varies greatly according to their morphology, ranging from 1% to 2% in yeasts, and reaching up to 15% in filamentous fungi [54]. Indeed, the chitin levels vary through fungi environmental adaptation, as is the case of the Aspergillus species. This pathogenic fungus enhances its chitin production in either hypoxic environments or higher glucose conditions, and this is directly related to its ability of regulating metabolism through the hexosamine biosynthetic pathway [55–57]. Thus, the increase in the chitin composition is due a metabolic shift that culminates in fungal resistance to external stress both in the environment and during pathogenesis [58].

In addition to these components associated with the cell wall, there are mannan glycoconjugates found in *C. albicans* and galactomannans present in *A. fumigatus* [59]. Mannans have a more fluid structure in relation to β-glucans and chitin, covering the outermost part of the cell wall, thus, despite not greatly influencing the structural stiffness of the fungal cell, they block the interaction of the inner β-glucans with immune system receptors [60]. Actually, different strains of *C. albicans* that are deficient on the N- or O- mannan glycosylation process present low binding affinity to innate immune cells, such as neutrophils, resulting in a low phagocytic capacity and ensuing lack of pathogen restraint [61]. Indeed, during infection, *C. albicans* also has the capability to regulate its mannan remodeling through environmental induction, thus controlling its β-glucan exposure and modulating immune recognition [62,63]. Galactomannans are polymers composed of mannans and galactofuranoses that are also found anchored to glycosylphosphatidylinositol (GPI) molecules in the cell membrane of *A. fumigatus* [64]. This component is involved in the activation of phagocytes via DC-SIGN and Dectin-2 receptors, which may lead to the production of pro-inflammatory cytokines such as TNF-α [65,66]. In addition to these glycoconjugates, there are the glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal)
molecules found uniquely in the polysaccharide capsule of Cryptococcus spp. [67]. This genus of pathogenic fungus has the unique feature of producing these virulence factors that are considered the most important ones for the pathogenic Cryptococcus species [68], mainly because of their pronounced immunomodulatory potential in the immune system cells [69]. It has been shown that GXM with larger dimensions elicit greater production of nitric oxide by macrophages and that fractions of different molecular weights of GXM are functionally distinct for the induction of cytokine production in vitro and in vivo [70,71]. Both GXM and GXMGal induce phagocyte apoptosis and the expression of Fas and Fas-L receptors that are associated with the activation of cell death induction pathways [72]. Interestingly, GXM elicits immunoregulatory responses, inducing IL-10 production in immune cells [73], downregulation of molecules associated with antigenic presentation in phagocytes, such as MHCII and CD86 [74], inhibition of TNF-α, IL-1β and IFN-γ production [75,76] and inhibition of the release of neutrophil extracellular traps (NET) [77]. Conversely, GXMGal elicits a pro-inflammatory profile and activation of antifungal responses with the induction of NET release [77], increased expression of MHCII and CD86 in phagocytes and the induction of IL-6 and IL-17 production, important cytokines for triggering antifungal immunity [78]. Furthermore, a possible association can be made with the increased expression of galectin-3 found in individuals with Cryptococcus spp. infection with the presence of galactose in the backbone structure of the capsular components of the fungus, so it ends up contributing to the fight against infection by cellular activation via the interaction of the capsular components with galectin-3 [79]. The range of interactions of fungal glycoconjugates with the immune system cells is quite diverse. Therefore, some of these effects on cytokine production, receptor expression and modulation of some components of the immune system are summarized in Table 1.

Table 1. Immunomodulatory role of some pathogenic fungi glycoconjugates.

| Pathogen          | Glycoconjugate              | Immunomodulatory Role | References |
|-------------------|------------------------------|-----------------------|------------|
| Cryptococcus spp. | glucuronoxylomannan         | ↑NO; ↑apoptosis; ↑Fas;↑Fasl; ↑IL-10; ↓MHCII; ↓CD86; ↓TNF-α; ↓IL-1β; ↓IFN-γ; ↓NET release | [72–77]    |
|                   | glucuronoxylomannogalactan  | ↑apoptosis; ↑Fas; ↑Fasl; ↑NET release; ↑MHCII; ↑CD86; ↑IL-6; ↑IL-17 | [72,77,78] |
| Pneumocystis spp. | β-glucan                    | ↑IL-23; ↑IL-6; ↑IL-17; ↑IL-22; ↑IL-8; ↑MIP-2; ↑IL-1β; ↑TNF-α; ↑Fas; ↑Fasl | [80–82]    |
| C. albicans       | mannan                      | ↑IL-12; ↑IL-6; ↑TNF-α; ↑IL-17; ↑IL-2; ↑IL-4; ↑IFN-γ | [83–86]    |
| Aspergillus spp.  | galactomannan               | ↑TNF-α; ↑IL-6; ↑IL-1Ra | [66,67]    |
|                   | β-glucan                    | ↑IL-1α; ↑IL-1β; ↑IL-22; IL-4, IL-13; ↑IFN-γ; ↑IL-17A | [88,89]    |

Some glycan molecules from pathogenic fungi and their effects on cytokine production, receptor expression and modulation of immune components are summarized with referenced articles. ↑ upregulation, ↓ downregulation.

All these possible interactions with the immune system, especially when dealing with the direct interaction with glycoconjugates, suggest the possibility of adaptive mechanisms to pathogenic fungi that may emerge and favor their virulence. For example, the existence of hypervirulent strains of Cryptococcus spp., as in the example of the hypervirulent strain of C. gattii JP02, which has fewer O-acetylations in its GXM than the hypervirulent strain H99 of C. neoformans, has the characteristic which causes JP02 to disfavor a pro-inflammatory cytokine profile [90]. In addition to O-acetylations, xylosylations that are considered molecular patterns associated with binding affinity with the immune system, complement proteins and antibodies, presenting distinct abundance profiles amongst different strains of Cryptococcus [91,92]. Our research group has recently described a high rate of O-acetylations in β-galactofuranose residues of the GXMGal structure of C. neoformans grown in capsular growth-inducing medium, demonstrating the high presence of important binding sites.
in this polysaccharide structure that confer potential immunobiological activity [93,94]. Furthermore, the glycoinositolphosphorylceramides (GIPC) biosynthetic pathway, a class of glycolipids, produces essential molecules for the viability and virulence of Cryptococcus spp., specifically the presence of groups with xylose branches in the structure of GIPCs [95]. Thus, as it has already been described that mutant strains of C. neoformans deficient in the final production of Xylose, NE178 and NE321 are unable to complete the assembly of GIPCs [96]. Therefore, these modifications only reinforce the importance of the fine regulation of the fungal glycoconjugates synthesis and the interference that these components have on the virulence of these pathogenic fungi. Furthermore, a better understanding of the changes in the adaptation of the virulence factors allows the development of new pharmaceutical strategies to combat fungal diseases, since there is currently a low drug spectrum for the treatment of fungal infections [97]. Finally, the answer to the initial question is based precisely on the fact that fungi rely on the adaptation of their biochemical machinery, as the synthesis of their virulence factors depends on different environmental conditions, both as a free-living organism or as a pathogen. Thus, changes in the morphology and structural composition of fungal species tend to contribute to the diversity and pathogenicity of the various existing strains of pathogenic fungi.

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References
1. Salazar, F.; Brown, G.D. Antifungal Innate Immunity: A Perspective from the Last 10 Years. J. Innate Immun. 2018, 10, 373–397. [CrossRef]
2. Caballero Van Dyke, M.C.; Wormley, F.L., Jr. A Call to Arms: Quest for a Cryptococcal Vaccine. Trends Microbiol. 2018, 26, 436–446. [CrossRef] [PubMed]
3. Salazar, A.S.; Keller, M.R.; Olsen, M.A.; Nickel, K.B.; George, I.A.; Larson, L.; Powderly, W.G.; Spec, A. Potential missed opportunities for diagnosis of cryptococcosis and the association with mortality: A cohort study. EClinicalMedicine 2020, 27, 100563. [CrossRef]
4. Iyer, K.R.; Revie, N.M.; Fu, C.; Robbins, N.; Cowen, L.E. Treatment strategies for cryptococcal infection: Challenges, advances and future outlook. Nat. Rev. Microbiol. 2021, 19, 454–466. [CrossRef] [PubMed]
5. Pagliano, P.; Esposito, S.; Ascione, T.; Spera, A.M. Burden of fungal meningitis. Future Microbiol. 2020, 15, 469–472. [CrossRef]
6. Diniz-Lima, I.; da Rosa, P.R.; da Silva-Junior, E.B.; Guimarães-de-Oliveira, J.C.; de Freitas, E.O.; de Oliveira Nascimento, D.; Morrot, A.; Nimrichter, L.; Prevati, J.O.; Mendonça-Prevati, L.; et al. X-linked immunodeficient (XID) mice exhibit high susceptibility to Cryptococcus gattii infection. Sci. Rep. 2021, 11, 18397. [CrossRef]
7. Akins, P.T.; Jian, B. The Frozen Brain State of Cryptococcus gattii: A Globe-Trotting, Tropical, Neurotropic Fungus. Neurocrit. Care 2019, 30, 272–279. [CrossRef]
8. Garelnabi, M.; May, R.C. Variability in innate host immune responses to cryptococcosis. Mem. Inst. Oswaldo Cruz 2018, 113, e180060. [CrossRef]
9. McGill, S.; Malik, R.; Saul, N.; Beets, S.; Secombe, C.; Robertson, I.; Irwin, P. Cryptococcosis in domestic animals in Western Australia: A retrospective study from 1995–2006. Med. Mycol. 2009, 47, 623–639. [CrossRef]
10. Kidd, S.E.; Hagen, F.; Tscharke, R.L.; Huyhn, M.; Bartlett, K.H.; Fyle, M.; Macdougall, L.; Boekhout, T.; Kwon-Chung, K.J.; Meyer, W. A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc. Natl. Acad. Sci. USA 2004, 101, 17258–17263. [CrossRef]
11. Bruner, K.T.; Franco-Paredes, C.; Henao-Martinez, A.F.; Steele, G.M.; Chastain, D.B. Cryptococcus gattii Complex Infections in HIV-Infected Patients, Southeastern United States. Emerg. Infect. Dis. 2018, 24, 1998–2002. [CrossRef] [PubMed]
12. Edwards, H.M.; Cogliati, M.; Kwenda, G.; Fisher, M.C. The need for environmental surveillance to understand the ecology, epidemiology and impact of Cryptococcus infection in Africa. *FEMS Microbiol. Ecol.* 2021, 97, fiab093. [CrossRef] [PubMed]
13. Limper, A.H.; Adenis, A.; Le, T.; Harrison, T.S. Fungal infections in HIV/AIDS. *Lancet Infect. Dis.* 2017, 17, e334-e343. [CrossRef]
14. Rajasingham, R.; Smith, R.M.; Park, B.J.; Jarvis, J.N.; Govender, N.P.; Chiller, T.M.; Denning, D.W.; Loyse, A.; Boullware, D.R. Global burden of disease of HIV-associated cryptococcal meningitis: An updated analysis. *Lancet Infect. Dis.* 2017, 17, 873–881. [CrossRef]
15. Armstrong-James, D.; Meintjes, G.; Brown, G.D. A neglected epidemic: Fungal infections in HIV/AIDS. *Trends Microbiol.* 2014, 22, 120–127. [CrossRef] [PubMed]
16. Bongomin, F.; Gago, S.; Oladele, R.O.; Denning, D.W. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. *J. Fungi* 2017, 3, 57. [CrossRef]
17. Herkert, P.F.; Hagen, F.; Pinheiro, R.L.; Muro, M.D.; Meis, J.F.; Queiroz-Telles, F. Ecoepidemiology of Cryptococcus gattii in Developing Countries. *J. Fungi* 2017, 3, 62. [CrossRef]
18. Damasceno-Escoura, A.H.; de Souza, M.L.; de Oliveira Nunes, F.; Pardi, T.C.; Gazotto, F.C.; Florentino, D.H.; Mora, D.J.; Silva-Vergara, M.L. Epidemiological, Clinical and Outcome Aspects of Patients with Cryptococcosis Caused by Cryptococcus gattii from a Non- endemic Area of Brazil. *Mycopathologia* 2019, 184, 65–71. [CrossRef]
19. Hurst, S.; Lysen, C.; Cooksey, G.; Vugia, D.J.; Litvintseva, A.P.; Lockhart, S.R. Molecular typing of clinical and environmental isolates of Cryptococcus gattii species complex from southern California, United States. *Mycoses* 2019, 62, 1029–1034. [CrossRef]
20. Diaz, J.H. The Disease Ecology, Epidemiology, Clinical Manifestations, and Management of Emerging Cryptococcus gattii Complex Infections. *Wilderness Environ. Med.* 2020, 31, 101–109. [CrossRef]
21. Mortenson, J.A.; Bartlett, K.H.; Wilson, R.W.; Lockhart, S.R. Detection of Cryptococcus gattii in selected urban parks of the Willamette Valley, Oregon. *Mycopathologia* 2013, 175, 351–355. [CrossRef] [PubMed]
22. Kohler, J.R.; Hube, B.; Puccia, R.; Casadevall, A.; Perfect, J.R. Fungi that Infect Humans. *Microbiol. Spectr.* 2017, 5, 5-3. [CrossRef] [PubMed]
23. Lee, D.J.; O'Donnell, H.; Routier, F.H.; Tiralongo, J.; Haselhorst, T. Glycobiology of Human Fungal Pathogens: New Avenues for Drug Development. *Cells* 2019, 8, 1348. [CrossRef] [PubMed]
24. Bhatt, K.; Agoll, A.; Patel, M.H.; Garimella, R.; Devi, M.; Garcia, E.; Amin, H.; Domingue, C.; Guerra Del Castillo, R.; Sanchez-Gonzalez, M. High mortality co-infections of COVID-19 patients: Mucormycosis and other fungal infections. *Discoveries* 2021, 9, e126. [CrossRef] [PubMed]
25. Chiurlo, M.; Mastrangelo, A.; Ripa, M.; Scarpellini, P. Invasive fungal infections in patients with COVID-19: A review on pathogenesis, epidemiology, clinical features, treatment, and outcomes. *New Microbiol.* 2020, 44, 71–83.
26. Lai, C.C.; Yu, W.L. COVID-19 associated with pulmonary aspergillosis: A literature review. *J. Microbiol. Immunol. Infect.* 2021, 54, 46–53. [CrossRef]
27. Alano, A.; Delliere, S.; Voicu, S.; Bregatne, S.; Megarbane, B. The presence of Pneumocystis jiroveci in critically ill patients with COVID-19. *J. Infect.* 2021, 82, 84–123. [CrossRef]
28. Alegre-Gonzalez, D.; Herrera, S.; Bernal, J.; Soriano, A.; Bodro, M. Disseminated Cryptococcus neoformans infection associated to COVID-19. *Med. Mycol. Case Rep.* 2021, 34, 35–37. [CrossRef]
29. Deepa, M.J.; Megharaj, C.; Patil, S.; Rani, P.K. Cryptococcus laurentii endogenous endophthalmitis post COVID-19 infection. *BMJ Case Rep.* 2022, 15, e246637. [CrossRef]
30. Ghanem, H.; Sivasubramanian, G. Cryptococcus neoformans Meningoencephalitis in an Immunocompetent Patient after COVID-19 Infection. *Case Rep. Infect. Dis.* 2021, 2021, 5597473. [CrossRef]
31. Siikala, A.; Lass-Floerl, C.; Klimko, N.; Ibrahim, A.; Roilides, E.; Petrickos, G. Challenges in the diagnosis and treatment of mucormycosis. *Med. Mycol.* 2018, 56, 93–101. [CrossRef] [PubMed]
32. Bayram, N.; Ozsaygili, C.; Sav, H.; Tekin, Y.; Gundogan, M.; Pangal, E.; Cicak, A.; Ozcan, I. Susceptibility of severe COVID-19 patients to rhino-orbital mucormycosis fungal infection in different clinical manifestations. *Jpn. J. Ophthalmol.* 2021, 65, 515–525. [CrossRef] [PubMed]
33. Fekkar, A.; Lampros, A.; Mayaux, J.; Poignon, C.; Demeret, S.; Constantin, J.M.; Marcelin, A.G.; Monsel, A.; Luyt, C.E.; Blaise, M. Occurrence of Invasive Pulmonary Fungal Infections in Patients with Severe COVID-19 Admitted to the ICU. *Am. J. Respir. Crit. Care Med.* 2021, 203, 307–317. [CrossRef]
34. Lionakis, M.S.; Iliev, I.D.; Hohl, T.M. Immunity against fungi. *JCI Insight* 2017, 2, e93156. [CrossRef] [PubMed]
35. Litvintseva, A.P.; Mitchell, T.G. Population genetic analyses reveal the African origin and strain variation of Cryptococcus neoformans var. grubii. *PLoS Pathog.* 2012, 8, e1002495. [CrossRef]
36. Acheson, E.S.; Galanis, E.; Bartlett, K.; Mak, S.; Klinkenberg, B. Searching for clues for eighteen years: Deciphering the ecological determinants of Cryptococcus gattii on Vancouver Island, British Columbia. *Med. Mycol.* 2018, 56, 129–144. [CrossRef]
37. Farrer, R.A.; Desjardins, C.A.; Sakthikumar, S.; Guja, S.; Saif, S.; Zeng, Q.; Chen, Y.; Voelz, K.; Heitman, J.; May, R.C.; et al. Genome Evolution and Innovation across the Four Major Lineages of Cryptococcus gattii. *MBio* 2015, 6, e00868-15. [CrossRef]
38. Cuomo, C.A.; Rhodes, J.; Desjardins, C.A. Advances in Cryptococcus genomics: Insights into the evolution of pathogenesis. *Mem. Inst. Oswaldo Cruz* 2018, 113, e170473. [CrossRef]
39. Souto, A.C.; Bonfetti, L.X.; Ferreira-Paim, K.; Trilles, L.; Martins, M.; Ribeiro-Alves, M.; Pham, C.D.; Martins, L.; Dos Santos, W.; Chang, M.; et al. Population Genetic Analysis Reveals a High Genetic Diversity in the Brazilian Cryptococcus gattii VGII Population.
and Shifts the Global Origin from the Amazon Rainforest to the Semi-arid Desert in the Northeast of Brazil. *PLoS Negl. Trop. Dis.* 2016, 10, e0004885. [CrossRef]

40. Bartlett, K.H.; Cheng, P.Y.; Duncan, C.; Galanis, E.; Hoang, L.; Kidd, S.; Lee, M.K.; Lester, S.; MacDougall, L.; Mak, S.; et al. A decade of experience: *Cryptococcus gattii* in British Columbia. *Mycopathologia* 2012, 173, 311–319. [CrossRef]

41. Applem Clancey, C.; Ciccone, E.J.; Coelho, M.A.; Davis, J.; Ding, L.; Betancourt, R.; Glaubiger, S.; Lee, Y.; Holland, S.M.; Gilligan, P.; et al. *Cryptococcus deuterogattii* VGI Infection Associated with Travel to the Pacific Northwest Outbreak Region in an Anti-Granulocyte-Macrophage Colony-Stimulating Factor Autoantibody-Positive Patient in the United States. *MBio* 2019, 10, e02733-18. [CrossRef] [PubMed]

42. Dadachova, E.; Bryan, R.A.; Howell, R.C.; Schweitzer, A.D.; Aisen, P.; Nosanchuk, J.D.; Casadevall, A. The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. *Pigment. Cell Melanoma Res.* 2008, 21, 192–199. [CrossRef] [PubMed]

43. Baker, L.G.; Specht, C.A.; Donlin, M.J.; Lodge, J.K. Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. *Eukaryot. Cell* 2007, 6, 855–867. [CrossRef] [PubMed]

44. Bhattacharjee, A.K.; Bennett, J.E.; Glaudemans, C.P. Capsular polysaccharides of *Cryptococcus neoformans*. *Rev. Infect. Dis.* 1984, 6, 619–624. [CrossRef]

45. Cadieux, B.; Lian, T.; Hu, G.; Teti, G.; Liu, V.; Murphy, M.E.; Creagh, A.L.; Kronstad, J.W. The Mannoprotein Cig1 supports iron acquisition from heme and virulence in the pathogenic fungus *Cryptococcus neoformans*. *J. Infect. Dis.* 2013, 207, 1339–1347. [CrossRef]

46. Cherniak, R.; Sundstrom, J.B. Polysaccharide antigens of the capsule of *Cryptococcus neoformans*. *Infect. Immun.* 1994, 62, 1507–1512. [CrossRef]

47. Snarr, B.D.; Qureshi, S.T.; Sheppard, D.C. Immune Recognition of Fungal Polysaccharides. *Front. Immunol.* 2020, 10, 2993. [CrossRef] [PubMed]

48. Garcia-Rubio, R.; de Oliveira, H.C.; Rivera, J.; Trevijano-Contador, N. The Fungal Cell Wall: *Candida*, *Cryptococcus*, and *Aspergillus* Species. *Front. Microbiol.* 2019, 10, 2993. [CrossRef] [PubMed]

49. Banks, I.R.; Specht, C.A.; Lodge, J.K. A chitin synthase and its regulator protein are critical during infection and drug treatment. *Curr. Opin. Microbiol.* 2010, 13, 59–66. [CrossRef] [PubMed]

50. Giles, S.S.; Dagenais, T.R.; Botts, M.R.; Keller, N.P.; Hull, C.M. Elucidating the pathogenesis of spores from the human fungal pathogen *Aspergillus fumigatus*. *Microbiol. Spectr.* 2019, 7, 1–25. [CrossRef] [PubMed]

51. Cleary, J.A.; Kelly, G.E.; Husband, A.J. The effect of molecular weight and beta-1,6-linkages on priming of macrophage function in mice by (1,3)-beta-D-glucan. *Immunol. Cell Biol.* 1999, 77, 395–403. [CrossRef] [PubMed]

52. Banks, I.R.; Specht, C.A.; Donlin, M.J.; Jerik, K.J.; Levitz, S.M.; Lodge, J.K. A chitin synthase and its regulator protein are critical for chitosan production and growth of the fungal pathogen *Cryptococcus neoformans*. *Eukaryot. Cell* 2005, 4, 1902–1912. [CrossRef] [PubMed]

53. Da Silva, C.A.; Chalouni, C.; Williams, A.; Hartl, D.; Lee, C.G.; Elias, J.A. Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. *J. Immunol.* 2009, 182, 3573–3582. [CrossRef]

54. Shepardson, K.M.; Ngo, L.Y.; Aimananda, V.; Latge, J.P.; Barker, B.M.; Blesser, S.J.; Ikawakura, Y.; Hohl, T.M.; Cramer, R.A. Hypoxia enhances innate immune activation to *Aspergillus fumigatus* through cell wall modulation. *Microbes Infect.* 2013, 15, 259–269. [CrossRef] [PubMed]

55. Lockhart, D.E.A.; Stanley, M.; Raimi, O.G.; Robinson, D.A.; Boldovjakova, D.; Squair, D.R.; Ferenbach, A.T.; Fang, W.; van Aalten, D.M.F. Targeting a critical step in fungal hexosamine biosynthesis. *J. Biol. Chem.* 2020, 295, 8678–8691. [CrossRef] [PubMed]

56. Latge, J.P.; Beauvais, A.; Chamilos, G. The Cell Wall of the Human Fungal Pathogen *Aspergillus fumigatus*: Biosynthesis, Organization, Immune Response, and Virulence. *Annu. Rev. Microbiol.* 2017, 71, 99–116. [CrossRef]

57. Walker, L.A.; Gow, N.A.; Munro, C.A. Fungal echinocandin resistance. *Fungal Genet. Biol.* 2010, 47, 117–126. [CrossRef]

58. Gow, N.A.R.; Latge, J.P.; Munro, C.A. The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiol. Spectr.* 2017, 5, 1–25. [CrossRef]

59. Gow, N.A.; Hube, B. Importance of the Candida albicans cell wall during commensalism and infection. *Curr. Opin. Microbiol.* 2012, 15, 406–412. [CrossRef]

60. Sheth, C.C.; Hall, R.; Lewis, L.; Brown, A.J.; Odds, F.C.; Erwig, L.P.; Gow, N.A. Glycosylation status of the C. albicans cell wall affects the efficiency of neutrophil phagocytosis and killing but not cytokine signaling. *Med. Mycol.* 2011, 49, 513–524. [CrossRef] [PubMed]

61. Hall, R.A. Adapting to change: Interactions of Candida albicans with its environment. *Future Microbiol.* 2017, 12, 931–934. [CrossRef] [PubMed]

62. Wheeler, R.T.; Kombe, D.; Agarwala, S.D.; Fink, G.R. Dynamic, morphotype-specific Candida albicans beta-glucan exposure during infection and drug treatment. *PLoS Pathog.* 2008, 4, e1000227. [CrossRef] [PubMed]

63. Costach, C.; Coddeville, B.; Latge, J.P.; Fontaine, T. Glycosylphosphatidylinositol-anchored fungal polysaccharide in *Aspergillus fumigatus*. *J. Biol. Chem.* 2005, 280, 39385–39842. [CrossRef]
65. Serrano-Gomez, D.; Dominguez-Soto, A.; Ancochea, J.; Jimenez-Heffernan, J.A.; Leal, J.A.; Corbi, A.L. Dengdritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of Aspergillus fumigatus conidia by dendritic cells and macrophages. J. Immunol. 2004, 173, 5635–5643. [CrossRef]

66. Loures, E.V.; Rohm, M.; Lee, C.K.; Sanitos, E.; Wang, J.P.; Specht, C.A.; Calich, V.L.; Urban, C.F.; Levitz, S.M. Recognition of Aspergillus fumigatus hyphae by human plasmacytoid dendritic cells is mediated by dectin-2 and results in formation of extracellular traps. PloS Pathog. 2015, 11, e1004643. [CrossRef]

67. Decote-Ricardo, D.; LaRocque-de-Freitas, I.F.; Rocha, J.D.B.; Nascimento, D.O.; Nunes, M.P.; Morrot, A.; Freire-de-Lima, L.; Previato, J.O.; Mendonca-Previato, L.; Freire-de-Lima, C.G. Immunomodulatory Role of Capsular Polysaccharides Constituents of Cryptococcus neoformans. Front. Med. 2019, 6, 129. [CrossRef]

68. Bosc, I.; Reese, A.J.; Ory, J.J.; Janbon, G.; Doering, T.L. A yeast under cover: The capsule of Cryptococcus neoformans. Eukaryot. Cell 2003, 2, 655–663. [CrossRef]

69. McClelland, E.E.; Bernhardt, P.; Casadevall, A. Estimating the relative contributions of virulence factors for pathogenic microbes. Infect. Immun. 2006, 74, 1500–1504. [CrossRef]

70. Fonseca, F.L.; Nohara, L.L.; Cordero, R.J.; Frases, S.; Casadevall, A.; Almeida, I.C.; Nimrichter, L.; Rodrigues, M.L. Immunomodulatory effects of serotype B glucuronoxylomannan from Cryptococcus gatti correlate with polysaccharide diameter. Infect. Immun. 2010, 78, 3861–3870. [CrossRef]

71. Albuquerque, P.C.; Fonseca, F.L.; Dutra, F.F.; Bozza, M.T.; Frases, S.; Rodrigues, M.L. Cryptococcus neoformans and its cell wall components induce similar cytokine profiles in human peripheral blood mononuclear cells despite differences in structure. FEMS Immunol. Med. Microbiol. 1999, 26, 309–318. [CrossRef] [PubMed]

72. Villena, S.N.; Pinheiro, C.S.; Nunes, M.P.; Takiya, C.M.; DosReis, G.A.; Previato, J.O.; Mendonca-Previato, L.; Freire-de-Lima, C.G. Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from Cryptococcus neoformans induce macrophage apoptosis mediated by Fas ligand. Cell Microbiol. 2008, 10, 1274–1285. [CrossRef]

73. Chiapello, L.S.; Baronetti, J.L.; Aoki, M.P.; Gea, S.; Rubinstein, H.; Masih, D.T. Immunosuppression, interleukin-10 synthesis and apoptosis are induced in rats inoculated with Cryptococcus neoformans glucuronoxylomannan. Immunology 2004, 113, 392–400. [CrossRef] [PubMed]

74. Zaragoza, O.; Rodrigues, M.L.; De Jesus, M.; Frases, S.; Dadachova, E.; Casadevall, A. The capsule of the fungal pathogen Cryptococcus neoformans. Adv. Appl. Microbiol. 2009, 68, 133–216. [CrossRef] [PubMed]

75. Vecchiarell, A.; Retini, C.; Pietrela, D.; Monari, C.; Tascini, C.; Beccari, T.; Kozel, T.R. Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1 beta secretion from human monocytes. Infect. Immun. 1995, 63, 2919–2923. [CrossRef]

76. Walenkamp, A.M.; Chaka, W.S.; Verheul, A.F.; Vaishnav, V.V.; Cherniak, R.; Coenjaerts, F.E.; Hoepelman, I.M. Pneumocystis carinii cell wall beta-glucan induces polysaccharide of tumor necrosis factor alpha and interleukin-1 beta secretion from human monocytes. Infect. Immun. 1995, 63, 1968. [CrossRef]

77. Almeida, F.; Wolf, J.M.; da Silva, T.A.; DeLeon-Rodriguez, C.M.; Rezende, C.P.; Pessoni, A.M.; Fernandes, F.F.; Silva-Rocha, R.; Martinez, R.; Rodrigues, M.L.; et al. Galectin-3 impacts Cryptococcus neoformans infection through direct antifungal effects. Nat. Commun. 2017, 8, 668. [CrossRef]

78. Carmona, E.M.; Lamont, J.D.; Xue, A.; Wylam, M.; Limper, A.H. Glycosphingolipids mediate pneumocystis cell wall beta-glucan activation of the IL-23/IL-17 axis in human dendritic cells. Am. J. Respir. Cell Mol. Biol. 2012, 47, 50–59. [CrossRef]

79. Carmona, E.M.; Lamont, J.D.; Xue, A.; Wylam, M.; Limper, A.H. Pneumocystis cell wall beta-glucan stimulates calcium-dependent signaling of IL-8 secretion by human airway epithelial cells. Respir. Res. 2010, 11, 95. [CrossRef] [PubMed]

80. Hahn, P.Y.; Evans, S.E.; Kottom, J.T.; Standing, J.E.; Pagano, R.E.; Limper, A.H. Pneumocystis carinii cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. J. Biol. Chem. 2003, 278, 2043–2050. [CrossRef]

81. Nguyen, T.N.Y.; Padungros, P.; Wongrisupphakul, P.; Sa-Ard-Iam, N.; Mahanonda, R.; Matangkasombut, O.; Choo, M.K.; Ritprajak, P. Cell wall mannan of Candida kruzei mediates dendritic cell apoptosis and orchestrates Th17 polarization via TLR2-MyD88-dependent pathway. Sci. Rep. 2018, 8, 17123. [CrossRef] [PubMed]

82. Tada, H.; Nemoto, E.; Shimauchi, H.; Watanabe, T.; Mikami, T.; Matsumoto, T.; Ohno, N.; Tamura, H.; Shibata, K.; Akashi, S.; et al. Saccharomyces cerevisiae- and Candida albicans-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. Microbiol. Immunol. 2002, 46, 503–512. [CrossRef]

83. Van de Veerdonk, F.L.; Marijissen, R.J.; Kullberg, B.J.; Koenen, H.J.; Cheng, S.C.; Joosten, L.; van den Berg, W.B.; Williams, D.L.; van der Meer, J.W.; Joosten, L.A.; et al. The macrophage mannose receptor induces IL-17 in response to Candida albicans. Cell Host Microbe 2009, 5, 329–340. [CrossRef]
86. Savolainen, J.; Kosonen, J.; Lintu, P.; Viander, M.; Pene, J.; Kalimo, K.; Terho, E.O.; Bousquet, J. Candida albicans mannan- and protein-induced humoral, cellular and cytokine responses in atopic dermatitis patients. *Clin. Exp. Allergy* 1999, 29, 824–831. [CrossRef]

87. Wong, S.S.W.; Krylov, V.B.; Argunov, D.A.; Karelin, A.A.; Bouchara, J.P.; Fontaine, T.; Latge, J.P.; Nifantiev, N.E. Potential of Chemically Synthesized Oligosaccharides to Define the Carbohydrate Moieties of the Fungal Cell Wall Responsible for the Human Immune Response, Using *Aspergillus fumigatus* Galactomannan as a Model. *Msphere* 2020, 5, e00688-19. [CrossRef]

88. Ratitong, B.; Marshall, M.; Pearlman, E. beta-Glucan-stimulated neutrophil secretion of IL-1alpha is independent of GSDMD and mediated through extracellular vesicles. *Cell Rep.* 2021, 35, 109139. [CrossRef]

89. Lilly, L.M.; Gessner, M.A.; Dunaway, C.W.; Metz, A.E.; Schwiebert, L.; Weaver, C.T.; Brown, G.D.; Steele, C. The beta-glucan receptor dectin-1 promotes lung immunopathology during fungal allergy via IL-22. *J. Immunol.* 2012, 189, 3653–3660. [CrossRef]

90. Urai, M.; Kaneko, Y.; Ueno, K.; Okubo, Y.; Aizawa, T.; Fukazawa, H.; Sugita, T.; Ohno, H.; Shibuya, K.; Kinjo, Y.; et al. Evasion of Innate Immune Responses by the Highly Virulent *Cryptococcus gattii* by Altering Capsule Glucuronoxylomannan Structure. *Front. Cell Infect. Microbiol.* 2015, 5, 101. [CrossRef]

91. Belay, T.; Cherniak, R. Determination of antigen binding specificities of *Cryptococcus neoformans* factor sera by enzyme-linked immunosorbent assay. *Infect. Immun.* 1995, 63, 1810–1819. [CrossRef] [PubMed]

92. Kozel, T.R.; Levitz, S.M.; Dromer, F.; Gates, M.A.; Thorkildson, P.; Janbon, G. Antigenic and biological characteristics of mutant strains of *Cryptococcus neoformans* lacking capsular O acetylation or xylosyl side chains. *Infect. Immun.* 2003, 71, 2868–2875. [CrossRef] [PubMed]

93. Previato, J.O.; Vinogradov, E.; Maes, E.; Fonseca, L.M.; Guerardel, Y.; Oliveira, P.A.V.; Mendonca-Previato, L. Distribution of the O-acetyl groups and beta-galactofuranose units in galactoxylomannans of the opportunistic fungus *Cryptococcus neoformans*. *Glycobiology* 2017, 27, 582–592. [CrossRef]

94. Previato, J.O.; Vinogradov, E.; Silva, M.A.E.; Oliveira, P.A.V.; Fonseca, L.M.; Maes, E.; Mendonca-Previato, L. Characterization of the 6-O-acetylated lipoglucuronomannogalactan a novel *Cryptococcus neoformans* cell wall polysaccharide. *Carbohydr. Res.* 2019, 475, 1–10. [CrossRef]

95. Heise, N.; Gutierrez, A.L.; Mattos, K.A.; Jones, C.; Wait, R.; Previato, J.O.; Mendonca-Previato, L. Molecular analysis of a novel family of complex glycoinositolphosphoryl ceramides from *Cryptococcus neoformans*: Structural differences between encapsulated and acapsular yeast forms. *Glycobiology* 2002, 12, 409–420. [CrossRef]

96. Gutierrez, A.L.; Farage, L.; Melo, M.N.; Mohana-Borges, R.S.; Guerardel, Y.; Coddeville, B.; Wieruszeski, J.M.; Mendonca-Previato, L.; Previato, J.O. Characterization of glycoinositolphosphoryl ceramide structure mutant strains of *Cryptococcus neoformans*. *Glycobiology* 2007, 17, 1C. [CrossRef] [PubMed]

97. Nivoix, Y.; Ledoux, M.P.; Herbrecht, R. Antifungal Therapy: New and Evolving Therapies. *Semin. Respir. Crit. Care Med.* 2020, 41, 158–174. [CrossRef]