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Review

Cryptococcus: History, Epidemiology and Immune Evasion

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Abstract: Cryptococcosis is a disease caused by the pathogenic fungi *Cryptococcus neoformans* and *Cryptococcus gattii*, both environmental fungi that cause severe pneumonia and may even lead to cryptococcal meningoencephalitis. Although *C. neoformans* affects more fragile individuals, such as immunocompromised hosts through opportunistic infections, *C. gattii* causes a serious indiscriminate primary infection in immunocompetent individuals. Typically seen in tropical and subtropical environments, *C. gattii* has increased its endemic area over recent years, largely due to climatic factors that favor contagion in warmer climates. It is important to point out that not only *C. gattii*, but the *Cryptococcus* species complex produces a polysaccharidic capsule with immunomodulatory properties, enabling the pathogenic species of *Cryptococccus* to subvert the host immune response during the establishment of cryptococcosis, facilitating its dissemination in the infected organism. *C. gattii* causes a more severe and difficult-to-treat infection, with few antifungals eliciting an effective response during chronic treatment. Much of the immunopathology of this cryptococcosis is still poorly understood, with most studies focusing on cryptococcosis caused by the species *C. neoformans*. *C. gattii* became more important in the epidemiological scenario with the outbreaks in the Pacific Northwest of the United States, which resulted in phylogenetic studies of the virulent variant responsible for the severe infection in the region. Since then, the study of cryptococcosis caused by *C. gattii* has helped researchers understand the immunopathological aspects of different variants of this pathogen.

Keywords: *Cryptococcus gattii*; *Cryptococcus neoformans*; cryptococcosis; infection; virulence factor; immunomodulation

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Citation: Diniz-Lima, I.; Fonseca, L.M.d.; Silva-Junior, E.B.d.; Guimarães-de-Oliveira, J.C.; Freire-de-Lima, L.; Nascimento, D.O.; Morrot, A.; Previato, J.O.; Mendonça-Previato, L.; Decote-Ricardo, D.; et al. *Cryptococcus*: History, Epidemiology and Immune Evasion. *Appl. Sci.* **2022**, *12*, 7086. https://doi.org/10.3390/app12147086

Received: 11 May 2022
Accepted: 12 July 2022
Published: 13 July 2022

1. Introduction

In this review, we approach general aspects of the pathogenic species of the genus *Cryptococcus*, *Cyptococcus gattii*, seeking to explore its history, as well as other aspects related to its physiology and structural morphology. Revisiting the epidemiology of *Cryptococcus* spp., we aim to describe the geographic distribution of the reemerging fungus *C. gattii* and its epidemiological importance as well. Furthermore, we showcase the functional characteristics of the virulence factors of this genus, a topic being constantly re-evaluated in recent years and a fundamental aspect for understanding the physiopathology of the fungus. Although a lot remains unknown when distinguishing the pathological characteristics of the two species *C. neoformans* and *C. gattii*, the most relevant virulence

component in these fungi is the polysaccharide capsule, which despite being vastly researched, is still not completely understood regarding the correlation between its chemical structure and immunomodulatory properties. Therefore, we aim to shed some light on this complex subject while describing the general features of *C. gattii* immunopathology.

2. Classification and Habitat of the *Cryptococcus* spp.

Cryptococcus (Filobasidiella) is a genus of fungus in the Phylum Basidiomycota of the Subphylum, Basidimycotina, of the Order Sporidiales and Family Sporidiobolaceae. It has more than 100 species ubiquitously distributed in the environment, with two species commonly known to cause cryptococcosis in humans, *Cryptococcus neoformans* and *Cryptococcus gattii* [1]. *C. neoformans* is a fungus that is distributed globally, being found in bird feces, trees and soil [2]. For the most part, it affects immunocompromised patients, causing a symptomatic and disseminated infection [3]; however, there are reports of *C. neoformans var grubii* infecting immunocompetent patients [4]. *C. gattii* is seen as a tropical and subtropical fungus, being found in reforestation trees, such as pine and eucalyptus, and in the soil close to these trees [5], and differently from *C. neoformans*, *C. gattii* infection occurs in both immunocompetent and immunocompromised patients [6]. Another species of the same genus, *C. laurentii*, is distributed in a dispersed way in the environment, in soil and plants, even maintaining a role for symbiosis with mycorrhizas, helping in plant metabolism [7]. It is known not to cause infection in humans and immunocompetent animals [8,9]; however, there are reports of infected immunocompromised patients in tropical regions [10–12].

3. First Historical Aspects of *Cryptococcus* spp.

The discovery of the *C. gattii* fungus was carried out after several attempts to classify other species of the same genus throughout the years. It started in 1894 in Germany, when the pathologist Otto Busse and the surgeon Abraham Buschke isolated a yeast similar to *Saccharomyces* from a bone infection in a patient, calling it "*Saccharomycosis hominis*". Later in the same year in Italy, Francesco Sanfelice isolated a similar yeast from peach juice ferment and named it *Saccharomyces neoformans* because of its peculiar colony formation [3,13]. In 1901, in France, Jean-Paul Vuillemin would give new nomenclatures to these yeasts, as they did not form ascospores, which are clusters of spores within a single mother cell called an ascus [14], and did not ferment carbohydrates, both hallmarks of *Saccharomyces*. Thus, the yeast discovered by Busse was named *Cryptococcus hominis* and that of Sanfelice was renamed *Cryptococcus neoformans*. It was only in 1950 that Benham in the United States would recommend the definitive nomenclature for the species (*C. neoformans)*, which would be adopted in later works [15]. In the same year, Evans and authors, for the first time, described an antigenic feature of the fungus in three specific serotypes: A, B and C [16]; only in 1968, Wilson described a fourth serotype, D [3]. Decades later, based on agglutination reactions of the capsular constituents of the fungus, the complex of species of *Cryptococcus neoformans* could be divided into serotypes A, D and hybrid AD, with serotype A corresponding to the *grubii* variant, whereas *C. gattii* has distinguishing serotypes B and C [17,18]. In addition to this classification, the division can also be made from DNA polymorphisms found in these species. In this way, the species *C. neoformans* of serotype A, known as *C. neoformans* variant *grubii*, can be divided into molecular types VNI, VNII and VNB, the latter referring to a specific variant [19], and serotype D, known as *C. neoformans* variant *neoformans* with molecular type VNIV and the hybrid of the two serotypes AD with molecular type VNIII [17]. *C. gattii* has a B serotype with the most common variants VGI and VGII and C serotype with VGIII and VGIV [20]. Although the characterization studies were carried out at the beginning of the century and since then, this pathogen has been known to cause disease in humans [13], *C. neoformans* became more important at the end of the 20th century with the AIDS crisis, where it was responsible for a large part of the morbidity in these patients. Recent data show that about 181,000 deaths from cryptococcal meningitis are recorded annually [21]. *C. gattii* infections are more frequent in tropical and

subtropical regions and even though their incidence has increased globally over the last few decades, they do not comprise the highest incidence globally, with about 80% of cases being caused by *C. neoformans*. However, *C. gattii* is responsible for more severe cases of the disease [3], with studies suggesting these cases may be underdiagnosed, either because the tests used rely on cryptococcal serum antigen, which is very hard to detect in cases of localized pulmonary cryptococcosis, or are unable to discriminate between different species of *Cryptococcus* [22,23].

4. *Cryptococcus gattii*

The first clinical sample finding of *C. gattii* came from the cerebrospinal fluid of a Congolese individual, initially reported as *C. neoformans* in 1894. Only in later years, the yeast with an atypical elliptical shape would be confirmed as *C. gattii* [2]. Initially, *C. gattii* was not classified as a species on it own, but considered a *Cryptococcus neoformans* variant [24]. Differentiation between the yeasts was made using CBD medium, still used to differentiate the species and consisting of an agar solution containing canavanine-glycine-bromothymol blue. *C. neoformans* only uses glycine as a carbon source and does not show favorable growth in the presence of canavanine [25]; however, *C. gattii* grows well on this medium and uses glycine not only as a carbon source, but also as a source of nitrogen, thus, alkalinizing the medium, granting it a bluish tinge due to the pH indicator bromothymol blue [26]. Unlike *C. neoformans*, which is ubiquitously distributed in soil and plant species, *C. gattii* is found specifically in plant material, mainly in urban landscape trees and in tree species associated with tropical and subtropical climates, such as in forests of *Eucalyptus* spp. in Australia [27]. The most common variants of *C. gattii* are the globally distributed VGI and VGII, both responsible for causing most cases of *C. gattii* cryptococcosis in immunocompetent individuals [28,29]. The variants of *C. gattii* are more frequent in warmer morphoclimatic domains, such as the predominant VGII in the Brazilian territory, with findings in the Amazon biome [30–32], clinical isolates from the Brazilian Northeast [33], Central-West [34] and the Brazilian Southeast [35–37], as well as in northern Australia and the islands of Papua New Guinea [8]. However, there is a trend of VGII findings causing outbreaks of *C. gattii* in temperate regions around the world, as has been observed with large numbers of domestic animals infected with *C. gattii* reported in western Australia [38], in outbreaks of *C. gattii* in North America from Vancouver Island in Canada, which resulted in cases of infections both in wildlife and domestic animals [39,40], along with an outbreak of cases in immunocompetent and immunocompromised humans in 1999 [41]. This outbreak also contributed to the expansion of new cases in the Northwest Pacific Coast of the United States of America, in Washington and Oregon, and more recently in the Southeast of the USA [42,43]. These cases obtained their clinical findings with evidence of a genetic profile from the tropical forests of South America [44,45]. However, more recent analyses indicate a high genetic diversity of the population in the Brazilian variant *C. gattii* VGII, changing the origin of this variant from the Amazon rainforest to the Brazilian semi-arid northeast [46]. Thus, the data suggest a change in the dynamics of the global dissemination of *C. gattii*, associating it to the current scenario of climate change and global warming that favors the increase in new cases of *C. gattii* infections by facilitating its spread [47].

5. Structure and Virulence Factors

Although estimates indicate the genetic separation of ancestors of the genus *Cryptococcus* at about 30 to 40 million years ago [48], evidence suggests that about 80 to 100 million years ago, ancestors belonging to the species complex of the genus *Cryptococcus* were physically separated by the drift of the supercontinent Pangea into two continental masses in the region that today corresponds to South America and Africa. This event would have made possible the accumulation of genetic determinants in the genus *Cryptococcus* in different environments that, in the future, would lead to differentiation in the contemporary species *C. neoformans* and *C. gattii* [49–51]. The role that birds currently play in the aerial dispersal

of *Cryptococcus* spp. between different regions is closely linked with the emergence of bird species in the early Cretaceous and the dispersal of the *Cryptococcus* genus between continents in the same geological period [49,52]. However, despite the evolutionary distance between the current species of the genus *Cryptococcus*, the structural determinants that are shared between these species are still well preserved, especially between the pathogenic *C. neoformans* and *C. gattii* [27]. Among these components, the cell wall of *Cryptococcus* spp. itself. has molecules that help the fungus defend against host defenses and environmental stress, in addition to intrinsic characteristics of the yeast, such as resistance to temperatures of 37 °C [53]. A protein that is required for this thermal resistance is a serine/threonine phosphatase called calmodulin, responsible for modulating the yeast response to environmental stresses. Given that calmodulin mutations adversely affect *Cryptococcus* spp. resistance to the physiological temperature of mammals, it is fundamental in enabling its successful growth in the host [54,55]. Other proteins involved in *C. gattii* thermotolerance are superoxide dismutase (SOD2p) [56] and trehalose-6-phosphate synthase (TPS1p and TPS2p) [57]. Another important virulence factor in the fungus is the production of melanin [3], present in the cell wall of the fungus and associated with: resistance to oxidative stress [58–60], resistance to antimicrobial peptides and antifungal components [60–62] and tolerance to environmental radiation, with the example of some *Cryptococcus* variants found surviving in environments with high levels of ionizing radiation, such as on the walls of the reactor of the Chernobyl nuclear disaster-UA [63,64]. Regarding melanin synthesis, laccase (Lac1), a phenol oxidase present in *Cryptococcus* spp., is responsible for this role, depositing this molecule in the yeast cell wall [65,66]. It has already been described that the deposition of melanin in the cell wall of *Cryptococcus* spp. depends on the composition and flexibility of the cell wall; as the *C. gattii* variant R265 has a higher chitosan composition than the *C. neoformans* variant H99, this ends up contributing to a more homogeneous distribution of melanin in the cell wall of *C. gattii* [67]. Chitosan is a deacetylated form of chitin and its presence in the cell wall of *Cryptococcus* spp. provides structure and stability to the other molecules in the cell wall [68]. In addition, differences in the amount of chitosan between different species of *Cryptococcus* have already been seen, with possible implications concerning the the virulence gap between *C. gattii* variant R265 and *C. neoformans*, since *C. gattii* has two- to three-times more chitosan in its cell wall [69]. Other virulence factors have already been described, such as the production of hexitol D-mannitol that helps in the survival of *Cryptococcus* spp. in the host and confers the resistance to oxidative stress [70,71], as well as the production of phospholipases, contributing to yeast homeostasis and virulence [72,73], provoking the rupture of cell membranes in the host [74] and, thus, contributing to the passage of the fungus to critical sites, such as the central nervous system of mammals [75]. In addition to all these components that are considered important virulence factors for both pathogenic species, *C. neoformans* and *C. gattii*, and other species of the same genus, they also produce a capsule containing mostly polysaccharide components that are determinants in the pathogenesis of the fungus [76]. However, due to its complexity, this subject will be addressed in more detail in another topic.

6. Cell Wall

The cell wall of *Cryptococcus* spp., as in other species of fungus, provides protection against environmental stressors, such as ultraviolet radiation, dehydration and variations in pH, temperature and osmolarity [77,78], in addition to mediating adhesion to host cells and protecting against free radicals and oxidant species produced by the cells of defense in the immune system [79,80]. Contrary to what one might think, the cell wall of *Cryptococcus* spp. is not a static barrier, but a dynamic structure that adapts to environmental conditions [81]. Its integrity is regulated by intracellular adapters that orchestrate its remodeling [82,83] and its importance for the survival of the fungus in the host is made clear when the pathogenicity of mutant variants that lack cell wall integrity regulators is compromised [84]. Linked polysaccharides, plasma membrane-associated glycoproteins and pigments, such as melanin, comprise cell wall components, extending physically

into the extracellular medium [77]. These polysaccharides that make up the cell wall of *Cryptococcus* spp. are glycans, more specifically glucose polymers connected by β-(1-3), β-(1-6) and α-(1-6) bonds [85], chitins, which are polymers of *N*-acetylglucosamines, and chitosans [86]. Unlike the glycans and glycoconjugates present in the polysaccharide capsule of *Cryptococcus* spp., which are synthesized by glycosyltransferases in cytoplasmic organelles, cell wall polymers are produced by synthases present in the plasma membrane [87]. Synthases and deacetylases present at sites in the plasma membrane, producing chitin and chitosan, respectively, as the extracellular medium and other synthases form channels with activity of glycosyltransferases, allowing the passage of sugar nucleotides from the cytosol to the unreduced ends of glycans present in the cell wall [88]. Once in the extracellular medium, the glycans are arranged in layers of macrostructures, with the innermost part rich in electron density and composed of chitin, chitosan and β-glycans, the middle part rich in melanin and glycoproteins and the outermost part with lower electronic density being composed of α- and β-glycans [89]. Glycans correspond to about 50 to 60% of the dry weight of the cell wall [76,89]; β-(1-3)-glycans form long polysaccharide chains and are the most abundant component in fungal species acting as a skeleton of the cell wall. The β-(1-6)-glycans are the most abundant component in the cell wall of *C. neoformans*, forming smaller chains that link to glycosylphosphatidylinositol molecule anchors through glycosidic bonds, chitin molecules, chitosan, β-(1-3)-glycans and constituents of the polysaccharide capsule [90,91]. However, the mechanism through which this arrangement occurs is still unknown, especially with regard to the percentage of the glycan composition in the cell wall of *C. gattii*. Furthermore, glycan degradation as a regulatory mechanism is not yet well characterized in *C. neoformans* and *C. gattii*, despite the fact that α-(1-3) and β-(1-3)-glycanase activity has already been identified in cell wall of *C. neoformanss* [92]. Although glycans play an important role in the structure of the cell wall of *Cryptococcus* spp., chitin polymers, β-(1-4)-*N*-acetylglucosamines, are responsible for the rigidity of the fungal cell wall, mainly due to its secondary structure, which is composed of antiparallel pleated beta-sheets stabilized by hydrogen bonds [90]. Interestingly, the percentage of chitin in the cell wall of fungi varies greatly according to their morphology, ranging from 1 to 2% in yeasts, reaching about 15% in filamentous fungi [93]. Other species of fungi have about 60% of their chitin converted into chitosan, β-(1-4)-*N*-glucosamine [94]. However, *C. neoformans* has about three- to five-times more chitosan than chitin in its cell wall and, since chitosan has multiple positive charges, *C. neoformans* yeast acquires greater solubility and flexibility in the environment [68]. In addition to the action of deacetylases, chitin can undergo catalysis by chitinases, three of which, Chi2, Chi4 and Chi22, are associated with the formation of spores during sexual reproduction of *C. neoformans* [95]. In this context, the exact repertoire of enzymes associated with chitin and chitinase skeleton remodeling in *C. gattii* is not yet known. However, a systematic analysis of the proteins associated with the cell wall of *C. neoformans* identified and divided about 29 proteins into three distinct groups: proteases, glycan remodeling enzymes and free radical regulatory enzymes [96]. Some of those proteins bind to β-(1-3)-glycans, being soluble in cell wall components and coming from secretion processes or catalysis by protein lipases associated with glycophosphatidylinositol groups, while another part is integrally associated with the cell wall, covalently linked to β-(1-6)-glycans and anchored to the cytoplasmic membrane by glycophosphatidylinositol groups [96–98] (Figure 1).

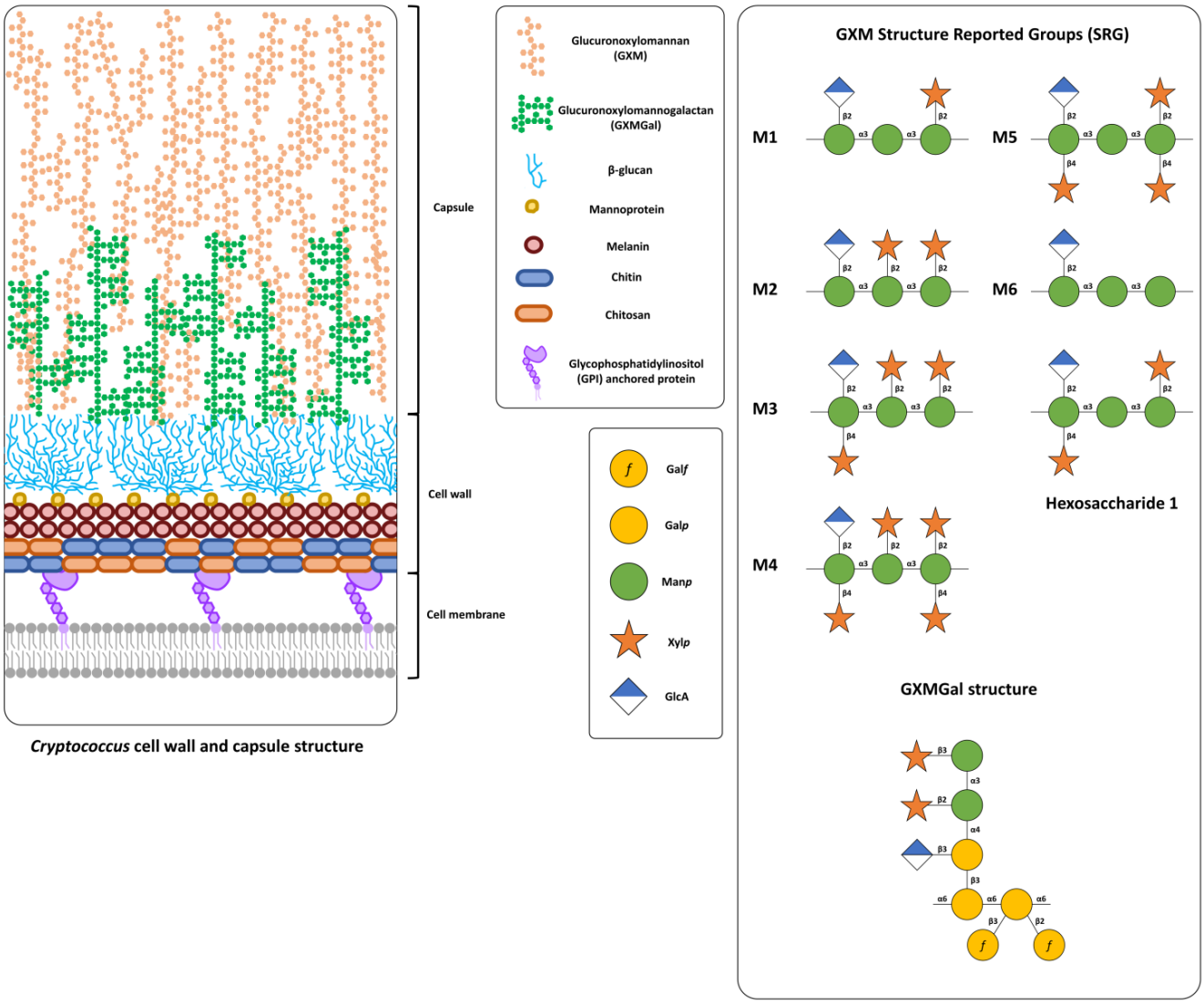

Figure 1. Cell wall and the polysaccharide capsule structure of *Cryptococcus* spp. with description of the molecular structure of the capsule polysaccharides glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal).

7. Polysaccharide Capsule

The most important and best-studied virulence factor of *Cryptococcus* spp. is its polysaccharide capsule, the outermost structure of yeast, which is chemically associated with the fungal cell wall and also permeates the yeast microenvironment [99,100]. Although there are other capsulated fungal species, none are considered pathogenic, only *C. neoformans* and *C. gattii* present such morphology associated with their pathogenicity [76,101]. Its components are constantly exuded into the extracellular medium, which facilitates their collection for purification assays by chromatography and structural characterization by magnetic resonance [102]. Its composition comprises mainly the polysaccharides glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal) [103,104]. GXM corresponds to about 90 to 95% of the polysaccharide capsule, having a molecular weight of 1700 to 7000 kDa, depending on the variant of *Cryptococcus* spp. [105]. The basic unit of GXM is a mannose trisaccharide joined by α-(1-3)-mannose bonds and with β-(1-2)- and β-(1-4)-glucuronosidic and β-(1-2)-xylosidic substitutions, having 6-*O*-acetylations in mannose residues, mainly in those where there are no bonds with the radical groups of glucuronic acid and xylose [106,107]. About two out of three mannoses are 6-*O*-acetylated, with the possibility for glucuronic acid-linked mannoses to also be linked [107,108]. Differences in the proportions

of xylose, mannose and glucuronic acid of GXM separate the different serotypes found in *Cryptococcus* spp. [104,108], suggesting: 1:3:1 and 2:3:1 for serotypes D and A and 3:3:1 and 4:3:1 for serotypes B and C, respectively [108,109]. However, these proportions are still not well established and further research is needed for this purpose. Something that differs from the polysaccharide capsule of *Cryptococcus* spp. of bacterial capsular species is the existence of Structure Reported Groups (SRG), which are seven structures, all different from each other, in the configuration of substitutions in the mannose polymer in the case of GXM, ranging from M1 to M6 [102], with the last called Hexosaccharide 1, described by Nimrichter and authors [110] (Figure 1). GXMGal is a polysaccharide of lower molecular weight, about 100 kDa, and corresponds to about 5 to 10% of the total capsule [108]. The structural characterization of GXMGal is usually conducted from the purification of acapsular variants of *C. neoformans,* such as CAP67, but has only one layer of GXMGal, with the absence of GXM, which facilitates the separation of GXMGal [111,112]. GXMGal is a more complex polysaccharide than GXM, having a backbone composed of galactose polymer with α-(1-6)-galactan bonds, linked by β-(1-3)-galacto-α-(1-4)-manno-α-(1-3)-mannan galactomannan chains, with galactomannan side chain substitutions with β-(1-2)- and β-(1-3)-xylosyl and β-(1-3)-glucuronyl groups [111,113], and rare double galactofuranose residues, associated with the galactan backbone by β-(1-2)- and β-(1-3)- bounds at the same galactose residue [114,115] (Figure 1). GXMGal is the most abundant polysaccharide in the capsule of *C. neoformans* due to its lower molecular weight compared to GXM. Nonetheless, attempts to visualize GXMGal by microscopy fail since this polysaccharide covers a thin layer on the surface of non-encapsulated variants [105]. There are indications that GXMGal recovers the innermost parts of the capsule of *C. neoformans*, since the removal of the outermost layers by radiation indicates the absence of galactose in these samples [116]. In addition to the major polysaccharide components GXM and GXMGal, there is a small percentage of mannoproteins, less than 1% [117,118], which are located in the innermost part of the polysaccharide capsule of *Cryptococcus* spp. close to the cell wall [119]. These mannoproteins were recently evaluated in proteomic analysis in the species *C. neoformans* and *C. gattii*, with about 46 and 36 predicted, respectively, for each species [120]. There are few data regarding the composition of mannoproteins of *Cryptococcus* spp., especially regarding *C. gattii*. However, some mannoproteins have already been associated with high immunogenic capacity [113], being partially linked to the dynamics of capsular components [118,121] and to the virulence of the yeast, as is the case of Cig1 in *C. neoformans*, responsible for capturing iron, with the deletion of its CIG1 gene, associated with impaired cell and capsular growth [122,123]. It is also the case for MP98, a chitin deacetylase present in the capsule and associated with the integrity of the cell wall [68], and in *C. gattii,* the recently described Krp1, associated with the construction of the polysaccharide capsule [120]. *C. gattii* has also been described as capable of altering its capsular structure, increasing its virulence and host evasion; there is a description of modifications in two *O*-acetylations of GXM from *C. gattii* that confer this characteristic on the virulent strain JP02 [124]. Interestingly, morphological characteristics in the capsular dimension of *C. gattii* are associated with the greater virulence of this species in relation to others of the genus *Cryptococcus*, since it has the ability to change its size during infection, facilitating its intracellular life stage and passage through the blood–brain barrier [125]. Clearly, virulence factors of glycoconjugate composition are proposed to be crucial for adaptation of pathogenic fungal species, such as in the *Cryptococcus* genus [126].

7.1. Glucuronoxylomannan (GXM)

The glucuronoxylomannan polysaccharide is the major component in the *Cryptococcus* spp. capsule and its effects on the immune system are diverse, converging mainly on an immunoregulatory response [100]. GXM is diffused in body tissues during a cryptococcal infection, with the highest concentrations being found in the lungs, largely due to the persistence of the fungus during pulmonary infection, while the lowest levels are in the spleen, blood and cerebrospinal fluid [127]. Studies investigating the immunomodulatory

effects in the presence of GXM use non-encapsulated strains as a control, since they do not have GXM [128,129]. This polysaccharide still has an antiphagocytic character, since it generates electrostatic repulsion due to its negative charge, reducing the interaction between effector cell and pathogen [130]. This is exemplified by the reduction in cell adhesion of non-capsulated yeasts; when they are experimentally coated with GXM, the phagocytosis is drastically reduced [131]. A characteristic that GXM brings to the table is the affinity for specific anti-GXM antibodies generated in the course of an infection and the influence that these antibodies have in increasing phagocytosis in yeasts by macrophages [132,133]. GXM increases affinity not only depending on specific antibodies, but also on the action of complement proteins, such as C3, which reinforces the importance that complement receptors have in fighting infection [134]. These receptors are not only found in macrophages but also in B cells [135], and their presence is associated with the maintenance of the B-1 lymphocyte population [136]. In addition, the complement receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18) have already been described as contributing to the phagocytosis of *C. neoformans*, independently of the action of antibodies [137]. In addition to binding soluble molecules, GXM has already shown interaction with receptors of innate immunity cells, mainly in the incorporation of the polysaccharide into the cell interior, mediated by: CD18 receptor on neutrophils [138], FcγRII and CD206, important in the antigenic presentation of dendritic cells during cryptococcosis [139]. Although these receptors are not associated with GXM clearance during infection [140], their accumulation within phagocytes leads to the modulation of intracellular cytokine production, such as the regulation of TNF-α by the interaction with CD14 and TLR4 via GXM binding [141], and GXM-CD14 interaction that induces IL-8 production in alveolar macrophages [142]. The negative modulation of GXM in the production of cytokines associated with the inflammatory response has already been observed, as was the reduction in secreted TNF-α, IL-1β and IFN-γ [143,144] and the increase in IL-10 associated with an anti-inflammatory profile [145]. Furthermore, high levels of IL-10 are associated with the presence of GXM in cerebrospinal fluid, and this has already been described as a predictor of death from cryptococcal meningoencephalitis [146]. Another mechanism generated by the interaction with GXM is the induction of cellular apoptosis [145,147]. This mechanism has already been described as being triggered on the production of nitric oxide induced by GXM [148], and the action of caspase-8 via the induction of Fas and FasL receptor expression [109,149]. Furthermore, GXM is able to suppress the expression of MHCII, reducing antigen presentation by dendritic cells and, consequently, the proliferation of T lymphocytes. However, GXM also non-specifically prevents T cell proliferation [150–152]. Another mechanism observed is the inhibition in the production of neutrophil extracellular traps (NET) by the presence of GXM in an in vitro model, which further reinforces the immunoregulatory potential of this polysaccharide [128]. Interestingly, that is evidence that fractions of different molecular weights of GXM from *C. neoformans* elicit different immunomodulatory activities in vitro. Nevertheless, the direct relationship between molecular weight and immunomodulatory effect has not yet been established, especially with regard to GXM fractions from *C. gattii*, which have very little data available [153]. However, other aspects have been studied, such as the induction of apoptosis, which is already a well-described mechanism for *C. neoformans* [154]. Similarly, Chiapello and colleagues still describe this mechanism in *C. gattii* as dependent on the production of nitric oxide by phagocytes [148]. Interestingly, Villena and colleagues proposed that macrophages that internalize apoptotic bodies are sufficient to contribute to immunoregulation, as they trigger the release of anti-inflammatory mediators, which facilitate infection [109].

7.2. Glucuronoxylomannogalactan (GXMGal)

The polysaccharide glucuronoxylomannogalactan was several times described as galactoxylomannan due to its first structural characterizations showing the presence of galactose, xylose and mannose [111]. However, in later studies, it was found that it had a galactose main chain as opposed to mannose and the residue associated with the galac-

tose side chain, previously described as β-(1-3)-xylosyl, was actually a β-(1-3)-glucuronyl bond, thus, adding glucuronic acid to the structure of this polysaccharide. Therefore, a new nomenclature was proposed, identifying it as a glucuronoxylomannogalactan [155]. Furthermore, a high rate of *O*-acetylations was recently revealed in the structure of GXMGal from *C. neoformans* in a capsular growth-inducing medium, demonstrating the higher presence of important binding sites in this polysaccharide that may confer potential immunobiological activity [115]. One of the characteristics that differentiates the species of *C. neoformans* and *C. gattii* with regard to the immunoregulatory potential of GXM is the differences in the *O*-acetylations of this polysaccharide, suggesting a relationship with the greater virulence of *C. gattii* [124], on the GXMGal of *C. gattii;* the real implications of its potential are still unknown. Another important structural adjustment proposed is the presence of two galactofuranosides, linked by β-(1-2)- and β-(1-3)- bonds to the galactose residues of the main chain of GXMGal from *C. neoformans*, which suggests greater complexity in the structure of this polysaccharide than previously observed [156]. Some immunomodulatory effects of GXMGal have already been observed, such as the activation of cellular apoptosis induced by Fas and FasL, as already observed in GXM [109] and unlike GXM, GXMGal induces NET release by neutrophils instead of inhibiting [128], which shows a clear indication of immunoactivating activity in GXMGal. Regarding GXMGal apoptosis-inducing properties, T lymphocyte glucoreceptors responsible for the interaction with this polysaccharide, such as CD7, CD43 and CD45, have already been described within this mechanism, inducing indirectly and directly by the activation of Bid and Caspase-9 [157]. An interesting finding in this context is the relationship discovered by GXMGal in the activation of immunological paralysis, through the depletion of B lymphocyte populations, due to its pro-apoptotic properties during the course of infection by *C. neoformans* [158,159]. GXMGal has already been described as a potent inducer of dendritic cell activation in the context of a Th17 profile in vitro and, in addition, treatment with GXMGal before infection by *C. neoformans* promotes protection against the fungus by a mechanism dependent on the production of IL-6 and IL -17 [160]. The production of IL-6 induced by GXMGal has also been observed in monocytes and appears to be independent of the action of TNF-α [161]. In addition, it has already been described that GXMGal binds to CD18 receptors on neutrophils [138], inducing iNOS expression in the RAW macrophage line as well as nitric oxide (NO) production [109,162]. However, some immunoregulatory properties of GXMGal have already been reported, such as the induction of a Treg profile in function and in number of cells [163], the inhibition of IL-17A production in T lymphocytes from patients with rheumatoid arthritis [164] and reduced production of IL-1β and IL-12 [165]. Data on the immunomodulatory effects of GXMGal are diverse and demonstrate the little knowledge elucidated about the real functions and mechanisms that this polysaccharide can elicit; in addition, there are no data on the structure of this polysaccharide in *C. gattii*, demonstrating the importance of further research in order to unravel it, as it must result in possible differences in immunobiological effects between different species of *Cryptococcus* spp.

8. Cryptococcosis

Cryptococcosis is an infectious disease caused by fungal species of the genera *Cryptococcus*, *C. neoformans* and *C. gattii*. They are globally distributed, although *C. gattii* is generally seen as a tropical or subtropical fungus [1,48,166]. Cryptococcosis affects immunocompromised individuals, causing severe lung disease and meningoencephalitis, with *C. gattii* particularly capable of infecting immunocompetent hosts [6,27]. As well as *C. neoformans*, *C. gattii*, despite indiscriminately affecting immunocompetent individuals, has risk factors associated with infection, such as diabetes [167,168], history of cancer [169], use of corticosteroids, chronic lung disease, age over 50 years and smoking habits [170,171], in addition to the ease of dissemination of the fungus in immunocompromised patients, as already described for *C. neoformans* [3,172]. There is a tendency for cases of cryptococcosis to be associated as a secondary infection in already debilitated patients, as in the case of HIV-positive patients, with about 11 to 17% of these patients having already been diagnosed with cryptococcosis

in different studies around the world [173,174]. The prevalence of death from cryptococcal meningoencephalitis in these patients reaches 15% and the global incidence of cryptococcosis in 2014 was around 223,000 cases [21]. Data on cryptococcosis are alarming since more and more immunocompetent human individuals are affected by the disease. Immunocompetent patients already account for about 60% of cases of cryptococcosis, with about 67% having associated cryptococcal meningoencephalitis [172,175]. In both studies, about 24 to 25% of the patients were asymptomatic, which indicates the possibility of misdiagnosis in cases of *C. gattii* cryptococcosis. In addition to affecting immunocompetent individuals, this fungus has increased its area of influence, with several cases reported in regions not previously associated with its clinical diagnosis [176,177]. In northern Brazil, about 23% of clinical isolates from patients affected by cryptococcosis are *C. gattii* [30], and the VGII variant *C. deuterorogattii* was described as the most prevalent in immunocompetent individuals in a study in the southeastern region of Brazil [36]. *C. gattii* infection is associated with permanence in areas where the fungus is present with the persistence of contact with the host [178]. Normally, this fungus is associated with plant and forest material in rural and wild environments; thus, environmental isolates are increasingly found more in urban areas, suggesting the establishment of the fungus in cities and a possible correlation with the persistence of the fungus and the emergence of new clinical findings, especially the higher risk variant, VGII [8,31,179].

9. Disease Development

During its life cycle outside the host, the fungus found in the environment produces spores of about 1 to 5 µm in diameter, which are easily aspirated by mammalian hosts [180]. These infectious propagules lodge inside the pulmonary alveoli and proceed to the life cycle, promoting cell division and proliferating inside the lungs, triggering severe pneumonia [181]. The incubation time of *C. neoformans* has already been described as around 110 months, being characterized by a period of dormancy before the establishment of the infection and the appearance of clinical symptoms in debilitated patients, whereas *C. gattii* has reported much shorter periods, with an average of 6 to 7 months, indicating that this fungus is more consistent with a primary infection [182,183]. During the course of cryptococcosis, hosts may be completely asymptomatic before the clinical picture deteriorates and progresses into acute pneumonia [180]. The symptoms during infection are very wide ranging and are associated with pneumonia, such as chest pain, malaise, dyspnea, fever, cough, weight loss, night sweats and hemoptysis [184].

10. *Cryptococcus* Dissemination

After its establishment in the lung tissue, the capsular and cell body growth of the fungus occurs, a mechanism that is closely associated with the impediment of phagocytosis and formation of titanized yeasts. Titan cells have been found in both *C. neoformans* and *C. gattii* [185,186]. These yeasts have a size greater than 20 µm in cell body diameter and have a greater-than-normal capsular thickening, which gives the yeast greater external coverage with its structural virulence factors [187,188]. A characteristic that the fungus has during infection is the tropism for the central nervous system [189], and associated with this, the titanization of the yeast is a morphological modification that is linked to favoring the spread to this region of the organism; however, *C. gattii* is less likely to disseminate to the CNS as compared to *C. neoformans*, and *C. gattii* is more likely to present as pneumonia [171,190]. After the establishment of the yeast in the lungs, the fungus can spread to other organs in the organism, resulting in the progression to systemic cryptococcosis [191] (Figure 2). This extrapulmonary spread favors the passage of the fungus to the central nervous system and can be mediated by the action of phagocytes present in the pulmonary alveoli, residing in the site of infection in the lungs [189]. There are several mechanisms proposed for this dissemination, the "*Trojan Horse*" being one of the most observed [192]. In this dissemination, the yeast is internalized by resident phagocytes and migrates through transcytosis through the epithelial barrier; thus, the intact yeast inside the cell can migrate

to the various tissues that the phagocyte finds in the body, including the central nervous system, going through the phagocyte by lytic or non-lytic extrusion, also called vomocytosis. Both *C. neoformans* and *C. gattii* have already presented this type of transport [193]. Yeast can be either phagocytosed or actively enter the cell body, by mechanisms not yet well elucidated [194] and, in addition, the cell can migrate through the pulmonary endothelium and blood–brain barrier by paracellular passage (between cells) and transcellular passage (through the cell) [195,196] (Figure 2). Interestingly, even rare, transplacental migration can occur, with cases already well documented on this topic [197]. Although the transfer of yeast by the organism mediated by phagocytes is well described, there are other mechanisms by which *Cryptococcus* spp. can cross physiological barriers, such as transcytosis by epithelial cells, already described as mediated by the binding of GXM with the CD14 receptor and palmitic acid from the yeast by binding to the pulmonary surfactant protein D, both mechanisms starting with the facilitation of cell adhesion [198,199]. A similar mechanism has been associated with the binding of hyaluronic acid on CD44, also facilitating cell adhesion and the metalloproteinase Mpr1 engaging Annexin A2 in the transfer of yeast transcellularly across the blood–brain barrier [200,201]. In addition to these mechanisms, *Cryptococcus* yeast produces urease and induces the release of IL-33, which leads to the dissociation of tight junctions in the epithelium, facilitating paracellular passage [202,203]. Urease, as well as other proteolytic enzymes and Plb1, are capable of destroying the epithelial cells in the blood–brain barrier, favoring the passage of yeast [204,205] (Figure 2). Despite the mechanisms of dissemination of yeasts, dependent on their intrinsic factors, the dissemination of *Cryptococcus* through immune cells is particularly important, mainly due to the subversion of cellular activity that the capsular constituents of yeast promote [189]. In this way, phagocytes have already been demonstrated important in the dissemination of the fungus to the central nervous system [206]. In general, the exopolysaccharides exuded by the internalized yeast accumulate inside the cell and, in a way that has not yet been elucidated, suppress the intracellular defense mechanisms [207].

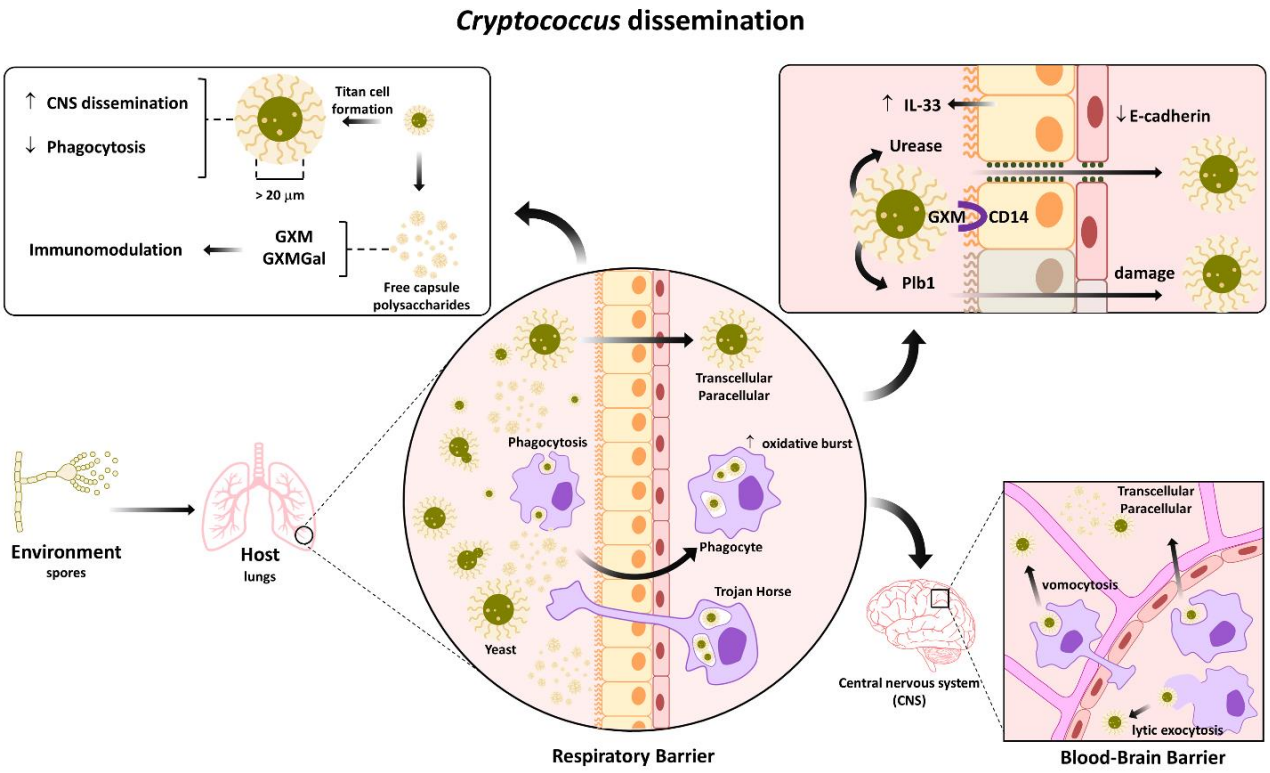

Figure 2. First morphological changes in *Cryptococcus* spp. inside the host and its dissemination mechanisms through the respiratory and blood–brain epithelial barriers.

11. Cryptococcosis Resistance

Before any subversion occurs, it is necessary that the yeast survives inside the cell without harm to its functional integrity. Therefore, an intrinsic characteristic of *Cryptococcus* spp. is the ability to survive in more acidic pH, including inside the phagolysosomes of the phagocyte–host [208]. This survival ends up being advantageous for the yeast also regarding the access to iron for its metabolism, since transferrins capture this micronutrient in neutral pH; thus, a more acidic pH increases the availability of iron intracellularly for the yeast [209], with iron being a determining factor for the virulence and dissemination of the fungus by the host [210]. In this context, in addition to surviving in more acidic pH, the yeast of *Cryptococcus* spp. can prevent phagolysosomal maturation to some degree [211] and, specifically, internalized yeasts of *C. deurogattii* R265 are able to prevent phagolysosomal fusion into dendritic cells by subverting phagosomal actin cytoskeleton rearrangement in the early stages of phagolysosomal maturation, preventing acidification and enzyme entry in the digestive vacuole by forming a kind of actin cage that protects the yeast intracellularly, a phenomenon that has not yet been observed in other species of *Cryptococcus* and that culminates in the immunological paralysis of the cell [212]. The subversion of macrophages by the infection caused by *Cryptococcus* spp. has already been described in terms of activation profile, since molecular factors of yeast are already described for inducing a differentiation to the M2 profile of macrophages [213]. This profile is associated with less control in fungal dissemination and less oxidative capacity to eliminate yeast intracellularly [214]. In addition, the result of a Th2-induced lymphocyte profile is also associated with reduced infection control [187,215]. In this sense, an immunological alteration caused by the opportunistic infection of *Cryptococcus* spp. is the state of Immune Reconstitution Inflammatory Syndrome (IRIS) [216], it occurs in individuals diagnosed with Acquired Immunodeficiency Syndrome (AIDS) or immunosuppression that paradoxically generates exacerbated inflammation in response to an opportunistic infection, such as cryptococcosis. This phenomenon is still not completely understood and is associated with the worsening of an individual's health status, having been observed in infections caused by *C. gattii* [27]. An efficient treatment for patients affected by IRIS and cryptococcal meningitis is the use of corticosteroids to alleviate the inflammatory response, being effective in the treatment of infections by *C. gattii* [217]. In general, the treatment for cryptococcosis lasts about 6 months, varying from case to case. In asymptomatic and mild cases, treatment with fluconazole is indicated and in more cases, pneumonia and meningitis indicate liposomal amphotericin B and flucytosine, in addition to continuous treatment with fluconazole [218]. In the case of *C. gattii*, initial in vitro experiments identified a lower susceptibility of *C. gattii* to fluconazole in relation to *C. neoformans*, with treatment with amphotericin B and flucytocin being more effective for *C. gattii* infection [219]. Specifically, *C. gattii* appears to show an adaptive heteroresistance to fluconazole, which leads to recurrence of disease severity during infection [220].

12. Immune Response against Cryptococcosis

12.1. The First Contact

When initially established in the lungs, the yeasts of *Cryptococcus* spp. come into contact with the first immunological barrier, the surfactants of the pulmonary [221]. Collectins, specifically SP-A and SP-D, have already demonstrated binding activity by *Cryptococcus* spp., presenting affinity for yeast sugar residues, which increases yeast affinity for phagocytes present by opsonization [222,223]. However, it has already been shown that *C. neoformans* appears to be resistant to the action of SP-A [224]. Further, this fungus subverts the action of the SP-D receptor as previously described by using it for its transcellular traffic [198] and, curiously, the action of SP-D is associated with worsening cryptococcosis, leading to mortality in an animal model by increasing IL-5 levels and promoting pulmonary eosinophilia during infection [225] (Figure 3). In addition, when contacting soluble surfactants in the pulmonary alveoli, the yeast encounters several cells responsible for innate immunity, such as alveolar macrophages and resident dendritic cells,

which are responsible for the initial anti-cryptococcal cellular response, mainly through the recognition of yeast molecular patterns, phagocytosis and release of cytokines in the lung microenvironment [226,227].

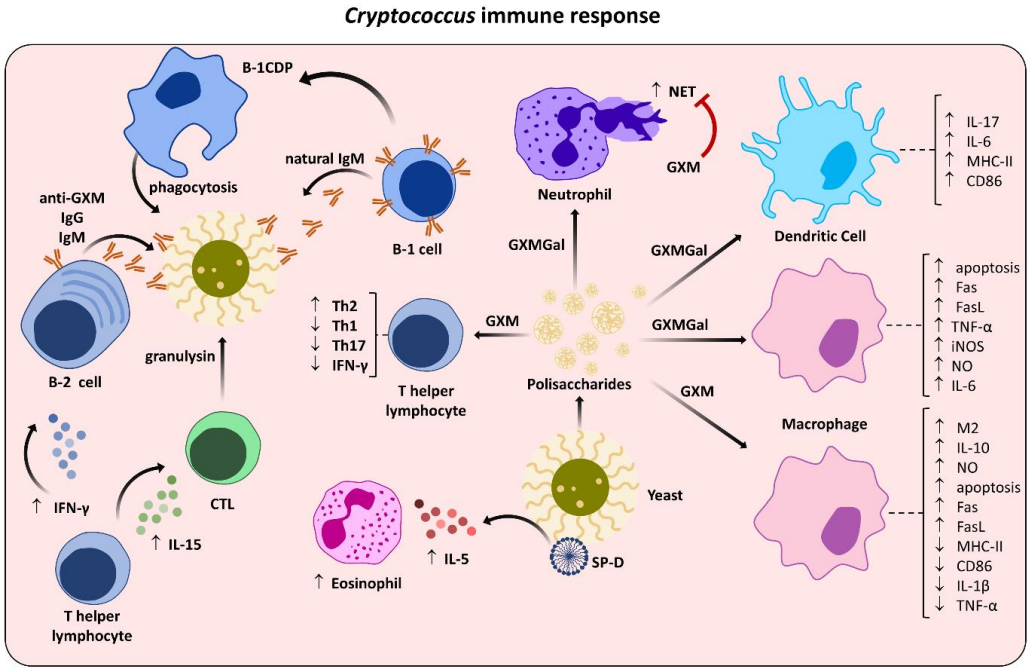

Figure 3. Immune response against *Cryptococcus* spp. and its capsular polysaccharides immunomodulatory effects.

12.2. Immunomodulation

Recognition of the molecular patterns associated with the fungus by immune cells is crucial for an efficient anti-cryptococcal response [227]. Da Silva-Junior and colleagues described this aspect using TLR9-deficient mice. When its deficiency in a model of cryptococcosis was caused by *C. gattii*, it was associated with a lower Th1 and Th17 profile and a higher presence of titanized cells, characterizing a lack of disease control [185]. Mannose and *β*-glycan receptors also mediate the incorporation of *C. neoformans* yeast by phagocytosis and production of cytokines with a pro-inflammatory profile, as in the production of TNF-α [228]. Furthermore, complement receptors CR3 and CR4 are involved in the recognition of *C. neoformans*, mediating phagocytosis independently of antibody action, as previously seen when binding to GXM [137]. Receptors, such as dectin-1 and CD11b, are associated with a rapid interaction with *C. neoformans* spores *β*-glycans, facilitating alveolar macrophage phagocytosis [229]. However, dectin-1 does not appear to be necessary for host defense against *C. neoformans*. This is most likely due to the blockade of *β*-glycan binding sites by GXM, since the spores that are non-encapsulated rapidly bind to the receptor exerting cellular activation, unlike encapsulated yeasts [230]. Receptors that alter the cytokine profile and mediate *C. neoformans* yeast–cell adhesion are the SCARF1 and CD36 scavenger receptors, which increase IL-1*β* and CXCL2 production [231], TLR2 and MyD88 adapter, which increase the production of TNF-α, IL-12 and IFN-γ [232] and mannose receptors that induce the proliferation of $CD4^+$ T lymphocytes [233]. In addition, non-encapsulated strains of *C. neoformans* activate the inflammasome by NLRP3 and fungal capsular constituents appear to inhibit this process [234]. Likewise, inhibition in the production of IL-8 and CXCL1 has also been observed in endothelial cells in contact with *C. neoformans* due to the presence of the polysaccharide capsule in the fungus [235]. The cytokine microenvironment is also compromised not only by the *Cryptococcus* spp. intrinsic factors, as already observed, but by extrinsic ones. For instance, during infection caused by *C. gattii*, the microbiota seem to be important for limiting the progression of the disease, with germ-free animals being more susceptible to this infection and with lower production

of IFN-γ, IL -1β and IL-17, which leads to a lower infection control [236]. Interestingly, the production of IFN-γ appears to be more potent during macrophage phagocytosis induction of *C. neoformans* and less responsive for the phagocytosis of *C. gattii* yeasts, with *C. gattii* also showing a reduced capacity for inducing IL-12 in vitro when compared to *C. neoformans* [237]. Within the *C. gattii* species complex itself, there are differences in the in vitro cytokine induction potential, with the hypervirulent R265 variant *C. deuterorogattii* showing the lowest inductions of pro-inflammatory cytokines, such as TNF-a, IL-1b and IL-6, which, in theory, suggests a greater escape from the pro-antifungal conditions of the inflammatory microenvironment during an infection [238].

12.3. Cell Response against the Infection

The internalized fungus survives well at the acidic pH of the phagolysosome and subverts its maturation machinery, as already described [211]. In this sense, some analyses were carried out with regard to autophagy markers during an infection caused by *C. neoformans* and it was noted that unlike in other pathogenic yeast species, cryptococcosis caused by the selective intracellular pathogen *C. neoformans* is less severe in the absence of autophagy [239]. Furthermore, NLRC4 inflammasome-mediated activation of caspase-1 during *C. gattii* cryptococcosis increases pyroptosis in phagocytes and affects their phagocytic capacity by inhibiting the yeast intracellular parasitism [240]. Ueno and colleagues even describe that *C. gattii* has a greater resistance to oxidative stress generated by the antifungal activity of neutrophils than *C. neoformans* [241]. In addition to the control of cryptococcosis by the innate immune response, the adaptive response also plays a key role. During cryptococcosis, where the cytotoxic role of $CD8^+$ T lymphocytes in infection control has already been reported, interestingly, this mechanism is dependent on activation by $CD4^+$ T lymphocytes and the production of IL-15, a cytokine responsible for the increase in the cytotoxic activity of $CD8^+$ T lymphocytes in the immune microenvironment [242]. Furthermore, it is well described, not only for *C. gattii* but also for other pathogenic fungal species, that targeting the Th1/Th17 activation axis is important for fighting infection and for the better clearance of the fungal load in the host, with the production of cytokines, such as IFN-γ and IL-17, associated with this phenotype [221] (Figure 3). Therefore, T lymphocyte populations mediate infection control by acting directly on the yeast and directing the antifungal immunity by controlling the cytokine profile in the immune microenvironment; however, the mechanisms orchestrated during the immunopathogenesis of cryptococcosis are still discreetly elucidated, especially in the context of infection caused by *C. gattii*. Along these same lines, the role that the B lymphocyte population plays during this infection is still poorly understood. However, there are some data that show the importance of this population of cells in the production of antibodies. For instance, it has been observed that B lymphocytes produce IgM antibodies that have the ability to induce the Th1 profile and assist in the recruitment of macrophages and phagocytosis during pulmonary *C. neoformans* cryptococcosis [243]. Specifically, natural IgM produced by B-1 lymphocytes appears to mediate antifungal immunity in the early stages of infection caused by *C. neoformans* [244,245]. However, not only the production of natural IgM seems to be related to the reduction in the predisposition to the formation of titan yeasts, but the population of B-1 lymphocytes seems to limit this phenotype that is associated with the worsening of the *C. gattii* and *C. neoformans* infection [187,190,246]. This demonstrates the importance that the B lymphocyte population as a whole seems to have in anticryptococcal immunity, especially when it is associated with the clinical description of a higher incidence of *C. neoformans* cryptococcosis in human individuals who have compromised B lymphocyte populations [247,248]. The populations of B lymphocytes are responsible for maintaining the humoral response in the body, helping to control infections caused by a countless range of pathogens [249]. Among them, the subpopulation of B-1 lymphocytes has a decisive role in the control of fungal infections, being recently studied, during the progression of *C. gattii* and *C. neoformans*

infection [245,246]. B-1a and B-1b populations have been reported to produce natural antibodies against the capsular components of *Streptococcus pneumoniae*, attenuating the infection [250]. In addition to humoral activity, B-1 lymphocytes are capable of acting innately, differentiating themselves in a population of mononuclear phagocytes and fighting the infection caused by *C. neoformans* in a nitric-oxide-dependent manner; this population is called B-1-Cell-Derived Phagocytes (B-1CDP) [251] (Figure 3). However, it was demonstrated in the infection of *Paracoccidioides brasiliensis* that B-1 cells also secrete IL-10 and induce activation of regulatory T cells, facilitating disease progression [252,253]. This contribution of IL-10 production by B-1 cells was also observed in *Leishmania* and *Francisella* infections [254–256]. Therefore, B-1 cells seem to perform their effector function differently in different infections. Furthermore, XID mice have already been described as having increased susceptibility to *C. neoformans* cryptococcosis [257], and recent data using the same animal model suggest that in the aspect of natural antibody production, the role of IgM produced by B-1 cells seems to be related to the control of infection of *C. neoformans* and *C. gattii*, preventing its spread from the lungs to the brain and restricting the size of yeasts [187,246]. However, despite having observed the important role that B lymphocytes play in the onset of cryptococcosis, using B lymphocyte depletion and transfer strategies in the infection model caused by *C. neoformans* [244,245], B-1 cell population transfer strategies have not yet been used to demonstrate the effect of its reconstitution and depletion during the experimental model of *C. gattii* infection. Only a few trials have been conducted with unsatisfactory results using the same strategy in an experimental model of *C. neoformans* infection [246]. Therefore, B lymphocyte immunity is potentially decisive for the control of cryptococcosis and there are still multiple facets to be explored in this field of study during infection caused by *C. gattii*. Nevertheless, both *C. gattii* and *C. neoformans* present distinct features when comparing together their molecular components, epidemiological aspects and immune response; therefore, we summarize some differences between the pathogenic species of *Cryptococcus* in Table 1.

Table 1. Some differences between *Cryptococcus* pathogenic species.

Characteristic	*Cryptococcus neoformans*	*Cryptococcus gattii*	References
Geographic distribution	Found globally in bird feces, trees and soil.	Found in tropical and subtropical areas closely to pine and eucalyptus trees *.	[2,5]
Infection	Mostly immunocompromised hosts; secondary infection; incubation period around 110 months.	Mostly immunocompetent hosts; primary infection; incubation period from 6 to 7 months.	[3,6,182,183]
Serotypes	A, D and AD	B and C.	[17,18]
Genotypes	(A) VNI and VNII; (D) VNIV; (AD) VNIII; VNB.	(B) VGI and VGII; (C) VGIII and VGIV.	[17,19,20]
Cryptococcosis globally	Corresponds around 80% of infections.	Corresponds < 20% of infections.	[3]
Culture medium growth	Only uses urea as a nitrogen source, does not grow well in the presence of canavanine.	Can use glycine as a nitrogen source, grows well in the presence of canavanine.	[25,26]
Cell wall	H99 strain presents lower chitosan composition; less homogenous melanin distribution.	R265 strain presents higher chitosan composition; more homogenous melanin distribution.	[67]
Capsule composition, mannoprotein components, and structural difference	Proportion of (xylose:mannose:glucuronic acid) by serotypes: (D) 1:3:1 and (A) 2:3:1; mannoproteins: 46 predicted; Cig1; MP98; H99 strain GXM presents more *O*-acetylations.	Proportion of (xylose:mannose:glucuronic acid) by serotypes: (B) 3:3:1 and (C) 4:3:1; mannoproteins: 36 predicted; Krp1; JP02 strain GXM presents less *O*-acetylations.	[68,108,109,120,123,124]
Cryptococcus dissemination	More likely to disseminate to the CNS **; less severe disease than *C. gattii*.	More likely to present as pneumonia; a more severe disease.	[171]

Table 1. *Cont.*

Characteristic	*Cryptococcus neoformans*	*Cryptococcus gattii*	References
Immune and pharmacological resistance	Cannot fully prevent phagolysosomal maturation; susceptibility to fluconazole.	R265 strain can highly prevent phagolysosomal maturation in dendritic cells leading to immune paralysis; adaptive heteroresistance to fluconazole and best treatment combining also amphotericin B and flucytocin.	[212,219]
Immune response	During phagocytosis macrophage IFN-γ production is more potent when compared to *C. gattii*; less severe cryptococcosis in the absence of autophagy	Reduced capacity for inducing IL-12 in vitro when compared to *C. neoformans*; R265 strain presents a lower production of TNF-a, IL-1b and IL-6; greater resistance to neutrophil oxidative stress than *C. neoformans*	[237–239,241]

* *C. gattii* infections are more frequent in tropical and subtropical regions, even though their incidence has increased globally over the last few decades. ** Although *C. neoformans* can cause a severe meningoencephalitis in immunocompromised individuals, it also establishes a severe form of pneumonia.

13. Concluding Remarks

In summary, cryptococosis is a disease of great clinical importance, with both pathogenic species *C. gattii* and *C. neoformans* responsible for causing a severe disease. Specifically, *C. gattii* causes the most severe cases in immunocompetent individuals. Generally, both species of fungus are found in plant material in different geographic regions, having increased the number of cases over the last few years, mainly of *C. gattii*, where, despite being less frequent than *C. neoformans*, a considerable increase in the number of *C. gattii* clinical samples has been found, quite possibly due to changes in global climate dynamics in new geographic regions of incidence, since this fungus is more frequent in warmer climates. One of the factors that establishes these fungal species as extremely harmful to the human host is the evolution to cryptococcal meningitis after the initial stages of pneumonia, which is characterized by its difficult treatment and associated with a considerable worsening of the prospects for recovery. During the infection, *C. gattii* interacts with immune cells, which are activated by its various virulence factors, engaging antifungal immunity; however, the most studied virulence factors, capsular polysaccharides, intrinsically have the potential to subvert the immune system. This happens due to the blockade of cell activation receptors, modification of the cytokine microenvironment profile and activation of cell death pathways. Nevertheless, these polysaccharides facilitate the spread of the disease due to their immunomodulatory properties and confer increases in yeast size, also leading to reduced cryptococcal clearance in the host. However, despite the structural characterization of these constituents being elucidated, the mechanisms that involve their synthesis during the infection are still vaguely clarified. Nevertheless, progressively, many studies aim to describe these aspects and some have successfully uncovered important features between *Cryptococcus* species that contribute to the understanding of the disease. Thus, the awareness of cryptococcosis immunopathology not only open doors for disease prevention and control strategies, but also provides the necessary knowledge to better understand the evolutionary and adaptive aspects that condition the major virulence of one of the most significant pathogens.

Author Contributions: Conceptualization, I.D.-L., D.D.-R., L.M.d.F. and C.G.F.-d.-L.; Writing, I.D.-L., D.D.-R. and C.G.F.-d.-L.; Supervision, C.G.F.-d.-L., L.M.-P. and D.D.-R.; Project Administration, C.G.F.-d.-L., A.M., D.O.N., L.F.-d.-L., J.O.P., J.C.G.-d.-O. and E.B.d.S.-J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by the Brazilian National Research Council (CNPq), Rio de Janeiro State Science Foundation (FAPERJ), Fundação Oswaldo Cruz (FIOCRUZ), and Programa Institutos Nacionais de Ciência e Tecnologia (INCT), CNPq, Brazil. We thank Lindomar Miranda for helpful technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

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