Allergy and Immunology

AIMS Allergy and Immunology, 6(2): 71–89.
DOI: 10.3934/Allergy.2022008
Received: 14 March 2022
Revised: 21 May 2022
Accepted: 15 June 2022
Published: 23 June 2022

http://www.aimspress.com/journal/Allergy

Review

Glucuronoxylomannan: the salient polysaccharide in cryptococcal immunity

Mansur Aliyu[1,2], Ali Akbar Saboor-Yaraghi[1,*], Sadegh Khodavaisy[3], Behrouz Robat-Jazi[4] and Muhammad Ibrahim Getso[2]

[1] Department of Immunology, School of Public Health, Tehran University of Medical Sciences, International Campus, TUMS-IC, Tehran, Iran
[2] Department of Medical Microbiology and Parasitology, Faculty of Clinical Science, College of Health Sciences, Bayero University, Kano, Nigeria
[3] Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

* **Correspondence:** Email: asaboor@tums.ac.ir.

Abstract: Cryptococcal meningitis (CM) is a dominant cause of morbidity and mortality among patients with human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS) caused by *Cryptococcus neoformans* and *Cryptococcus gattii* species complex. The complex is composed of closely related members, yet with diverse epidemiology, pathogenesis, and drug-resistant pattern. Cell-mediated immunity is the strongest pillar in immunity to cryptococcosis, further worsening HIV/AIDS patients' scenario. Antifungal resistance and immune evasion again tilt the host-parasite balance in favor of the fungal pathogen. In this regard, researchers are actively challenged to discover immunotherapy and vaccine for CM, to produce specific treatment and prevention that will address CM conventional therapeutics failure. As the major capsular polysaccharide of the *Cryptococcus*, which is tightly linked to pathogenicity, immunogenicity, and immune evasion, the glucuronoxylomannan (GXM) is cardinally targeted for vaccine and immunotherapy development. Further, the amount of GXM shed in body fluids correlates with the disease severity. Herein, we reviewed the literature with the journey so far in line with GXM as the salient immunological target on cryptococcosis.

Keywords: cryptococcal meningitis; glucuronoxylomannan; diagnosis; immunotherapy; vaccine

Abbreviations: 188Re: rhenium-188; 213Bi: bismuth-213; CALAS: cryptococcal antigen latex agglutination system; C-IRIS: cryptococcosis-associated immune reconstitution inflammatory syndrome; CM: cryptococcal meningitis; CNPS: *C. neoformans* polysaccharide capsule; CNS: central nervous system; CrAg: cryptococcal capsular antigen; CTBA: cetyltrimethylammonium bromide; DCs: dendritic cells; EIA: enzyme immunoassay; GalXM: galactoxylomannan; GXM: glucuronoxylomannan; GXMGal: glucuronoxylomanogalactan; GXMR-CAR: GXM-specific chimeric antigen receptor; GXM-TT: GXM conjugated to tetanus toxoid; HIV/AIDs: human immunodeficiency virus/acquired immune deficiency syndrome; HLA: human leukocyte antigen; ICP: intracranial pressure; IFN-γ: interferon-gamma; IL: interleukin; IMMY: immuno-mycologics; LFA: lateral flow assay; MHC: major histocompatibility complex; MPL: monophosphoryl lipid A; NETS: neutrophil extracellular traps; NLRP3: nod-like receptors (NLR) family pyrin domain containing 3; P13-BSA: P13 conjugated to bovine serum albumin; P13-TT: P13 conjugated to tetanus toxoid; pAPC: professional antigen presenting cells; RANTES: regulated upon activation, normal T cell expressed and secreted; ROS: reactive oxygen species; SCID: severe combined immunodeficiency; SRBCs: sheep red blood cells; T11TS: T11 Target structure; TLRs: toll-like receptors; TNF-α: tumour necrosis factor-alpha; TT: tetanus toxoid

1. Cryptococcal meningitis

The *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes are the common causative agents of life-threatening cryptococcal meningitis (CM) among the immunocompromised, especially human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS) patients, other immunosuppressed patients and to a small extent immunocompetent individuals [1,2]. If available, timely diagnosis, specific treatment (immunotherapy), and vaccine can help convert the fatality recorded against CM in human populations. After many molecular studies, the *C. neoformans*/*C. gattii* species complex was divided into seven species haploids and four inter-species diploid/aneuploid hybrids. The species exhibit varying epidemiology, pathogenicity, and chemotherapeutic susceptibility, but all *C. neoformans*/*C. gattii* species complex members cause pneumonia and meningoencephalitis [3]. Generally, in this review, we use *C. neoformans* in the write-up and figures to refer to *C. neoformans*/*C. gattii* species complex.

Classification of the *C. neoformans*/*C. gattii* species complex summarized in Table 1 below were as follows, under *C. neoformans* we have *C. neoformans* sensu stricto formally serotype A (VNI/AFLP1 and VNII/VNB/AFLP1A, VNII/AFLP1B) and *C. deneoformans* (VNIV/AFLP2) previously serotype D. While the *C. gattii* had five species under it namely, *C. gattii* sensu lato (VGI/AFLP4: serotype B), *C. bacillisporus* (VGIII/AFLP5: serotype B & C), *C. deuterogattii* (VGII/AFLP6: serotype B), *C. tetragattii* (VGIV/AFLP7: serotype C), and *C. decagattii* (VGIV and VGIIIc/AFLP10: serotype B). Further, the diploid/aneuploid species hybrids between *C. neoformans* and *C. gattii* are *C. neoformans* × *C. deneoformans* hybrid (AFLP3/VNIII:serotype AD), *C. deneoformans* × *C. gattii* hybrid (AFLP8:serotype DB), *C. neoformans* × *C. gattii* hybrid (AFLP9:serotype AB) and *C. neoformans* × *C. deuterogattii* hybrid (AFLP11:serotype AB) [3–5].

Table 1. Classification of the *C. neoformans*/*C. gattii* species complex.

Parent specie(s)	Species	Serotype(s)
C. neoformans	*C. neoformans* sensu stricto (VNI/AFLP1, VNII/VNB/AFLP1A, VNII/AFLP1B)	A
	C. deneoformans (VNIV/AFLP2)	D
C. gattii	*C. gattii* sensu lato (VGI/AFLP4)	B
	C. bacillisporus (VGIII/AFLP5)	B and C
	C. deuterogattii (VGII/AFLP6)	B
	C. tetragattii (VGIV/AFLP7)	C
	C. decagattii (VGIV and VGIIIc/AFLP10)	B
Hybrid (diploid/aneuploidy)	*C. neoformans* × *C. deneoformans* hybrid (AFLP3/VNIII)	AD
C. neoformans and *C. gattii*	*C. deneoformans* × *C. gattii* hybrid (AFLP8)	DB
	C. neoformans × *C. gattii* hybrid (AFLP9)	AB
	C. neoformans × *C. deuterogattii* hybrid (AFLP11)	AB

The respiratory tract is the primary route of entry for *Cryptococcus*; the lungs and central nervous system (CNS) are the common sites of the invasion, causing a spectrum of infections from simple colonization to systemic/invasive fungemia and meningitis [6]. Figure 1 below outlines the pathogenesis of CM. Cryptococcal screening is recommended in HIV patients with a CD4 count of 100 cells/μL or less [7]. Conventionally, cryptococcosis is diagnosed in the laboratory using Indian ink staining, this procedure is retarded by reduced sensitivity. The fungal culture is considered the gold standard, however, a definitive diagnosis can be reached in one to two weeks. Serological detection of fungal capsular polysaccharides plays a significant role in CM diagnosis [8]. Enzyme immunoassay (EIA) and latex agglutination assays have been employed successfully in targeting the cryptococcal capsular antigen in body fluids to diagnose cryptococcosis [8,9].

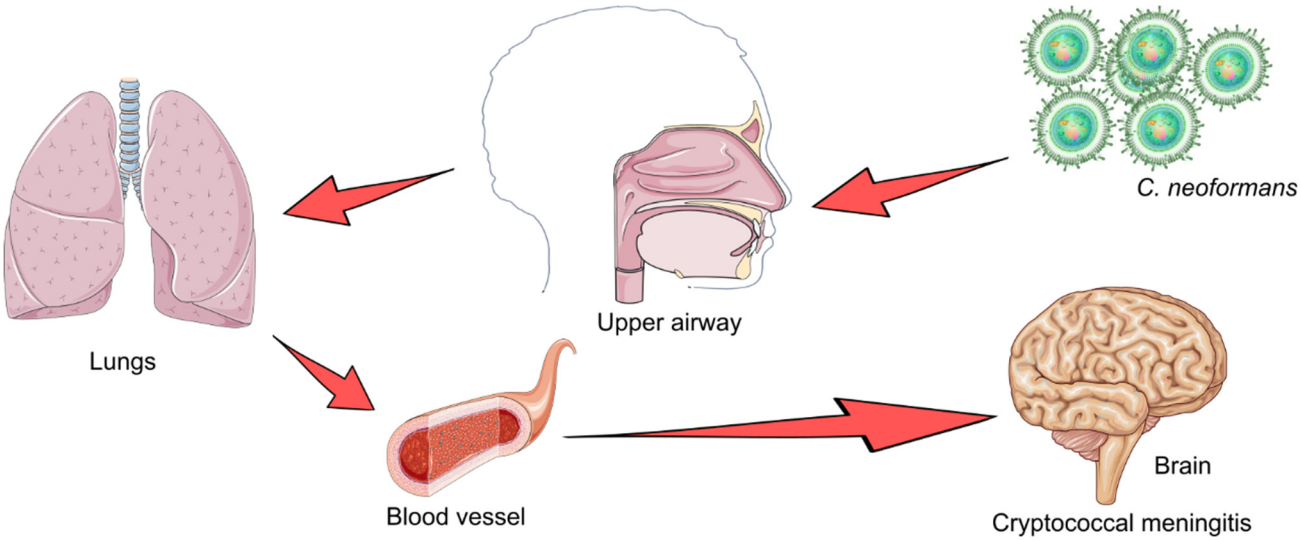

Figure 1. Pathogenesis of cryptococcal meningitis. A schematic representation of the pathogenesis of *C. neoformans* from transmission to the evolution of cryptococcal meningitis.

The immunochromatographic dipstick assay detects the cryptococcal antigen, principally glucuronoxylomannan (GXM), in serum by the fourth week of infection before meningism [10]. The cryptococcal capsular antigen (CrAg) lateral flow assay (LFA) being more sensitive than the CrAg latex detects 1 ng of CrAg/mL, while the latter detects 19 ng of CrAg/mL of the sample. The CrAg LFA employs simple technology that is convenient for mass production at affordable cost, for rapid testing, and for easy interpretation on a portable device for testing many parameters in a sample. Contrastingly, CrAg LFA has some setbacks as the chemical reporters can fail and the test is inappropriate for assays requiring high analytical sensitivity and reproducibility [11]. A study reported a false-negative serum CrAg LFA in a patient with disseminated cryptococcosis and positive *C. neoformans* blood culture result [12].

The cryptococcal antigen latex agglutination system (CALAS) is a quantitative/semiquantitative assay that detects cryptococcal polysaccharide capsular antigens. Despite CALAS accuracy in the diagnosis of CM using CSF, it had been substantially replaced by CrAg LFA due to the former's unfavorable requirement of laboratory expertise, long incubation, and interpretation dexterity. The FDA-approved Immuno-Mycologics (IMMY) CrAg LFA is considered superior among CrAg LFA platforms, however, needle prick-sample proved less sensitive compared to blood collected with a pipette and read after 20 minutes [13].

Other CrAg LFA available in the market include Dynamiker [14], CryptoPS (Biosynex) [15], StrongStep (Liming Bio) [16], and FungiXpert cryptococcal capsular polysaccharide K-Set (Genobio) [17]. Scientists have reported extensively on the properties of these CrAg LFA tests and their comparison with IMMY or CALAS [15,16,18–20]. Recently, researchers compared the four CrAg LFA certified in Europe and reported that IMMY and FungiXpert LFAs were able to detect all the species in the *C. neoformans*/*gattii* complex, however, Dynamiker and CryptoPS failed to diagnose *C. tetragattii* and *C. bacillisporus,* also *C. deuterogattii* is a blind spot on CrptoPS. Importantly *C. tetragattii* is prevalent in Subsaharan Africa and the Indo-Pakistani subcontinent; accounting for one-fifth of CM among HIV/AIDs patients [21].

2. The GXM

The *C. neoformans* polysaccharide capsule (CNPS); principal virulent factor, salient serological target, and critical immune evasion factor that is shed in culture and body fluids is composed of GXM (90%), galactoxylomannan (GalXM)—recently reviewed as GXMGal, and mannoproteins [22]. The intact capsular and the secreted GXM are both utilized for cryptococcal studies [23]. However, the duo possesses distinct chemical, physical, and antigenic properties [24]. The GXM is common to all the species in the *Cryptococcus neoformans/gattii* complex [25].

In the early 1990s, advancement was recorded in the identification of the GXM part of CNPS, which is specific for *C. neoformans* as such murine monoclonal antibodies were produced and characterized, which showed the upper hand in the diagnosis. Moreover, studies to uncover the role of anti-GXM for possible immunotherapy of *C. neoformans* commenced in *pari-passu* [26]. The cryptococcal GXM, unlike proteinaceous antigens, provoked an inadequate immune response. Previous studies coupled GXM to carrier proteins and use adjuvants to boost humoral immune response [27,28].

2.1. GXM structure

The component carbohydrate residues of GXM are mannose, xylose, and glucuronic acid in decreasing order. The mannan moieties provide GXM backbone through their lineal linkage to each other with α-(1→3) bond, while two xylose molecules occupy positions β-(1→2) and β-(1→4), and a glucuronic acid moiety takes up position β-(1→2) of the mannose molecule forming the octasaccharide structure of GXM as shown in Figure 2 below [29]. The GXM mannose backbone is substantially acylated at position 6 [30]. The GXM polysaccharide expressed on *C. neoformans* exercises six motifs triad, present in different motif combinations in any given GXM; a complexity superior to bacterial single oligosaccharide motif [31].

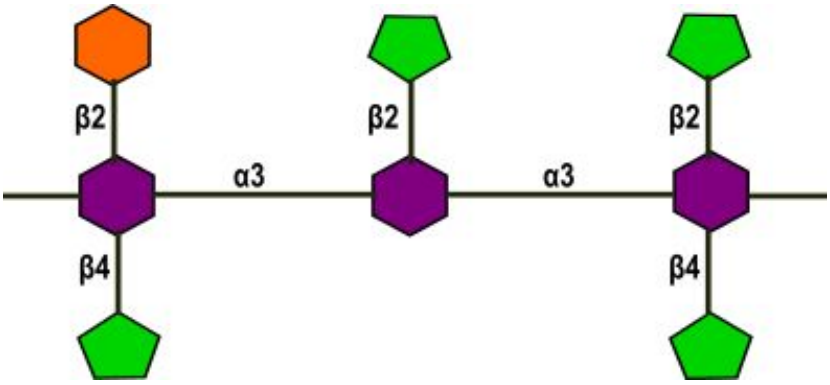

Figure 2. A schematic diagram of GXM: Mannose (purple hexagons) form the backbone structure of GXM linked to each other by α-(1→3) bond also linked to xylose (green pentagon) through β-(1→2) and β-(1→4) or glucuronic acid (orange hexagon) through β-(1→2) bonding. GXM: Glucuronoxylomannan.

The *C. neoformans* sensu stricto (serotype A) dominant triad has a six-residue repeat unit bearing two xylose side chains at β-(1→2) [32]. Glucuronic acid position confers structural variability of GXM having different motifs within the same isogenic *C. neoformans* phenotypes, yielding considerable structural and antigenic variations [33]. GXM provides the framework for the serotype classification of *Cryptococcus*, based on structural diversification of the GXM molecule. As each GXM possesses six differing triads (motifs) of oligosaccharides that account for its diversity making the molecule highly heterogeneous [32].

2.2. Immune response to GXM

The cryptococcal GXM is tolerogenic, poorly immunogenic, and provoked T cell-independent humoral immune response in humans and experimental animals [28,34]. Only 40% of CM patients produce detectable antibodies against cryptococcal polysaccharide capsular antigens [35]. The dendritic cells (DCs) recognize the *C. neoformans* GXM using toll-like receptors (TLR)-2 and TLR4, leading to the fungal agent's phagocytosis with no role in cytotoxicity. Bedsides, the protective immune response follows recognition of GXM from ingested fungus by nod-like receptors (NLR) family pyrin domain containing 3 (NLRP3) that promote expression of IL-1β [36], as depicted in

Figure 3b below. TLR4 is essential for GXM recognition and subsequent activation of macrophages [37].

Other pattern recognition receptors utilized by monocyte-derived macrophages to recognize GXM for subsequent uptake include CD14, CD18, and FcγRII, in addition to TLR2 and TLR4. Exposure of these macrophages to GXM demonstrates the immune system's downregulation through decreased expression of MHC I and MHC II and increased secretion of IL-10 which is counterproductive to cryptococcal immunity, as shown in Figure 3a below [38]. Phagocytic cells: macrophages and neutrophils form the first-line innate defense against cryptococcosis [39].

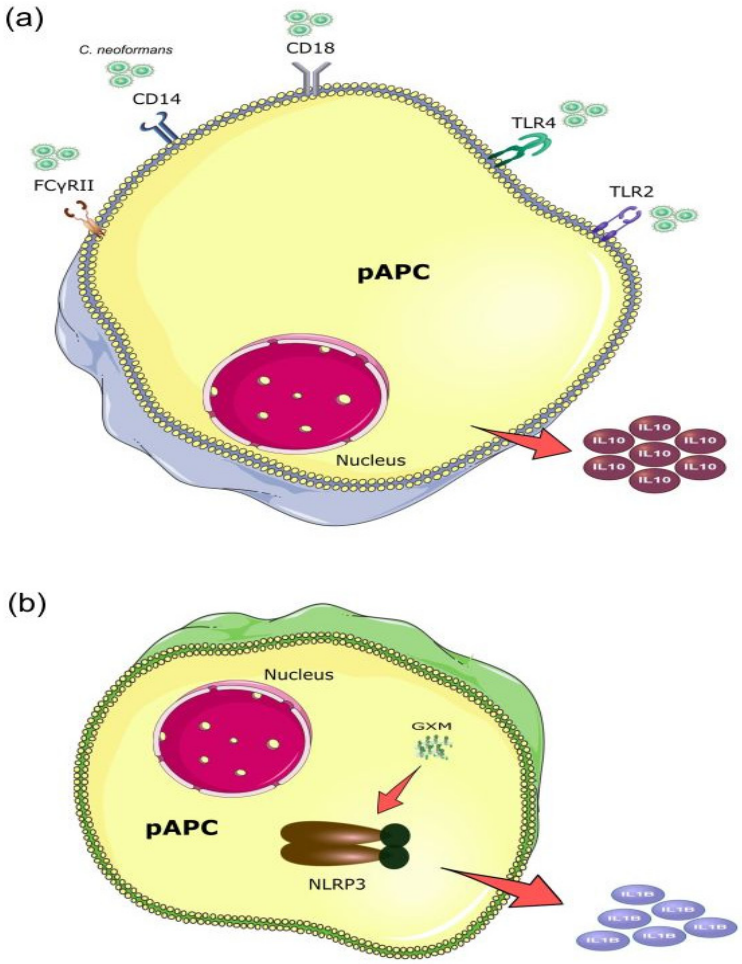

Figure 3. The double edge sword immune response to GXM. (a) Immunity response to *C. neoformans* through PAMPs like CD14, CD18, FCγII (CD32), TLR2 and TLR4 on pAPC usually resulted in an immune evasive response, where the pAPC will secrete IL-10, an anti-inflammatory cytokine that favors cryptococcal growth. (b) While the proinflammatory response from the pAPC usually results from the recognition of fungal antigen by NLPR3 within the cytosol and subsequent secretion of IL-1β, a proinflammatory cytokine that favors cryptococcal clearance. Interleukin (IL), nod-like receptor (NLR) family pyrin domain containing 3 (NLRP3), pathogen-associated molecular pattern (PAMPs), professional antigen-presenting cell (pAPC), toll-like receptor (TLR).

The proinflammatory Th1 subtypes of $CD4^+$ T cells that produce IFNγ as its signature cytokine was shown to be a key player in immunity to cryptococcosis, through its ability to polarize macrophages to M1 phenotype: fungicidal [40,41]. This fact was further supported by a higher prevalence of CM among HIV/AIDs patients with severely low CD4 cell count [41,42], a similar phenomenon was observed in idiopathic CD4 lymphocytopenia [43]. $CD4^+$ T cells are essential for cryptococcal clearance, a property that was shown to be transferred in the murine study, in addition to macrophages and neutrophils attraction [43]. In a murine study, it was reported that the presence of $CD4^+$ or $CD8^+$ T cells is indispensable for cryptococcal immunity [44]. Another study found IL-17A secreted by tissue-resident memory T cells to be a requisite towards immunity to cryptococcal infection in the study mice [34]. To further showcase the significance of Th1 and Th17 $CD4^+$ T cells, scientists reported consistently low levels of IFNγ and IL-17 secreting cells among HIV/AIDs patients with CM complicated by immune reconstitution inflammatory syndrome (IRIS) [45]. IFNγ, IL-17A and TNFα production by $CD4^+$ T cells function in immune cells chemotaxis and granuloma formation at the local site of cryptococcal infection; hence IL-$17A^{(-/-)}$ mice failed to form granuloma after dendritic cell vaccination for cryptococcal infection [34]. In sum, cell-mediated immunity through Th1 and M1 macrophages, in addition to humoral immunity with associated complement activation are salient towards cryptococcal elimination [46].

In an *in vitro* study, Rocha and coworkers reported that *C. neoformans* GXM blocks the release of neutrophil extracellular traps (NETs) and inhibits the generation of reactive oxygen species (ROS) needed for fungal killing by human neutrophils [47]. O-acetyl groups present in GXM are essential to immune evasion by the *Cryptococcus* [30]. Increased serum levels of GXM in patients with CM were found to be associated with monocyte anergy: reduced monocytes expression of HLA-DR and TNF-α, reduce Th1 response, increase expression of IL-6 and IP-10, and increase neutrophils count. Cumulatively, labeled as a mortal immune signature in CM patients [48]. Cell-mediated immunity through the expression of Th1 cytokines is a prerequisite to anti-cryptococcal immunity, while $CD8^+$ T cells provide anti-cryptococcal immunity in the absence of $CD4^+$ T cells [49]. GXM Immune evasion to phagocytes was outlined in Figure 4 below.

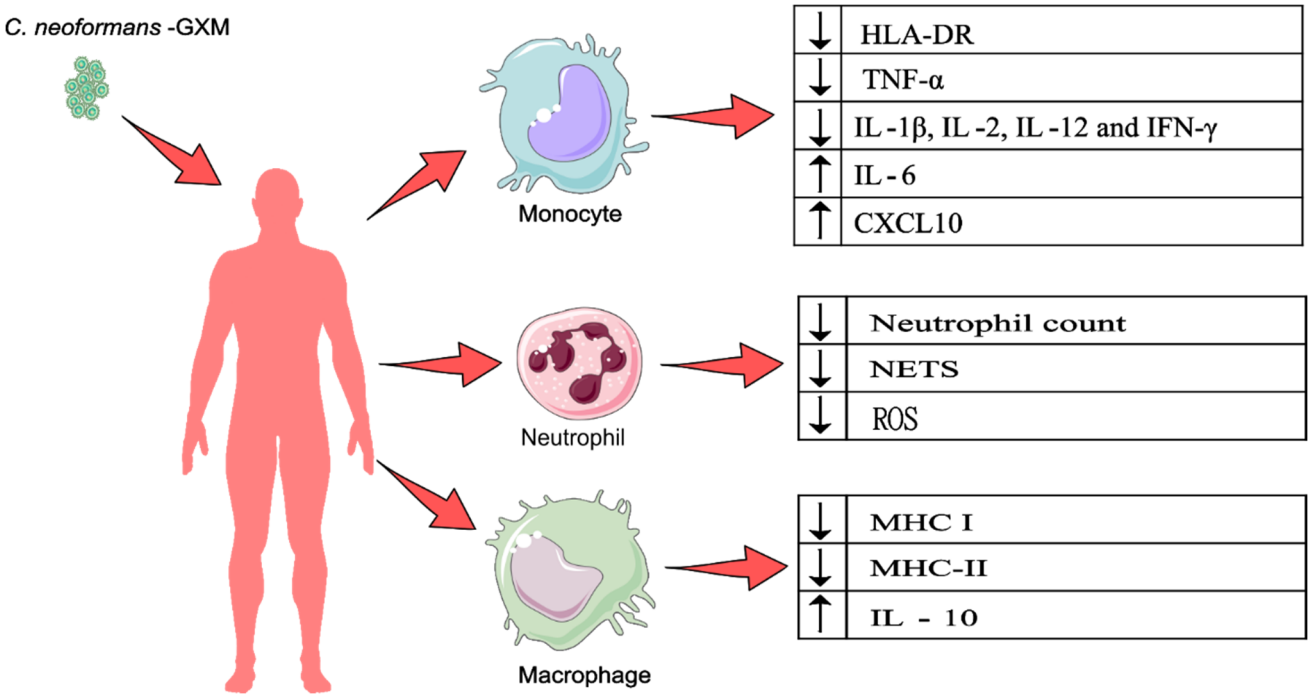

Figure 4. GXM Immune evasion. Phagocytes namely monocytes, neutrophils, and macrophages provide a defense to *C. neoformans* through engulfment of *C. neoformans*, but the microbe devises evasive strategies to counter lasting immunity from these cells through downregulation of proinflammatory responses, and influencing upregulation of anti-inflammatory response as shown for each cell type in the above diagram. DCs, not shown in the diagram, are superior to the above-mentioned cells in immunity to cryptococcosis. CXCL10: C-X-C motif chemokine ligand 10; DCs: dendritic cells; GXM: glucuronoxylomannan; HLA: human leukocyte antigen; IFN-γ: interferon-gamma; IL: interleukin; MHC: major histocompatibility complex; NETS: neutrophil extracellular traps; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-alpha.

Scientists found a direct association between a low serum GXM IgM and the development of cryptococcosis-associated immune reconstitution inflammatory syndrome (C-IRIS) among patients with CM. Further, GXM-specific IgM deficiency was shown to be associated with disseminated cryptococcosis among the study mice [50]. No association was found between C-IRIS and B cell populations [51]. Mice immunization with GXM conjugated to protein carrier yields different monoclonal antibodies (mAbs), some with protective properties, others with non-protective specificity [52]. GXM-specific IgM was shown to confer protective immunity to cryptococcosis [53].

The *C. neoformans* express GXM of varying molecular masses that were recognized by murine bone marrow-derived macrophages, but have a differing effect on the expression of IL-10, IL-6, TNF-α, and regulated upon activation, normal T cell expressed and secreted (RANTES:CCL5) [54]. The CSF secretion of GXM is positively correlated with levels of IL-6, IL-7, IL-8, and TNF-α expression among patients with CM [55]. Extracellular vesicles containing GXM and sterylglucosides were shown to protect against secondary cryptococcosis [56]. *C. neoformans* display varying cell morphology types during infection to evade the host's immune system [39].

A complex of GXM and GaLXM: glucuronoxylomanogalactan (GXMGal) released during infection evades the immune system through apoptosis induction on immune cells, thereby conferring fungal survival within the host [57,58]. Hence, the characteristic cytopenia observed in CM, further compounds immunosuppression among the patients [59]. GXM is antiphagocytic and resists respiratory burst stress [22,57]. Excessive production of GXM was directly related to decreasing survival of severe combined immunodeficiency (SCID) mice models infected with *C. neoformans* [60].

A clinical study reported a direct relationship between *C. neoformans* that produced large capsules *in vivo* with raised intracranial pressure (ICP), inadequate CNS immune response, reduced cryptococcal clearance, dwindle expression of pro-inflammatory cytokines, and low leukocyte count among patients with CM [61]. The GXM possesses several immunomodulatory effects during cryptococcal infections, which include increasing Fas Ligand expression, subduing LPS-induced signaling, blocking T cell proliferation, immune cells apoptosis induction, and interference of antigen presentation by professional antigen-presenting cells (pAPCs), cumulatively favoring cryptococcal survival [62]. Furthermore, GXM disrupts the healthy balance of pro-inflammatory to anti-inflammatory cytokines, facilitating anti-inflammatory cytokines expression in the presence of *Cryptococcus* pathogen [63].

2.3. GXM: diagnostic target

GXM is an essential component of *Cryptococcus* that is required for survival, pathogenicity, and immune evasion. GXM is continuously shed in the serum and CSF during infection. The body fluid level of GXM correlates directly with a fungal burden in CM [64]. As a formidable cryptococcal biomarker, scientists target GXM to diagnose cryptococcosis in the last four decades. Of note, low immunogenicity inherent to polysaccharide antigens seems to stand in the way of developing anti-GXM for immunoassays. So, Cryptococcal serological diagnostic immunoassays evolve in specificity from targeting the whole CNPS, narrowing it down to GXM [65]. Others developed polyclonal or monoclonal antibodies by coupling GXM to carrier proteins, to boost the polysaccharide's immune response [34,66,67].

Scientists describe a monoclonal antibody of IgG_1 subclass to CNPS after immunizing mice with extracted soluble capsular polysaccharide of *C. neoformans* serotype A, obtained through ultrafiltration of grown culture. The discovered IgG_1 cross-reacts mildly with other *C. neoformans* serotypes and *Trichosporon beigelii*. The anti-CNPS also successfully recognized the GXM of *C. neoformans* [68]. Devi and colleagues discover a superior monoclonal antibody response secondary to immunization of laboratory mice with a vaccine they synthesize from conjugation of +GXM to tetanus toxoid (TT) [28]. The same study's highest antibody response for both IgG and IgM in the mice was recorded when the initial immunization dose was followed by monophosphoryl lipid A (MPL) adjuvant injection on the second day [28]. In another study, the GXM was precipitated with cetyltrimethylammonium bromide (CTBA) and conjugated to TT using adipic acid as a spacer; the vaccine formed was then used for intraperitoneal immunization of Balb/c mice yielding monoclonal antibodies specific for all the four serotypes of *C. neoformans* [69].

2.4. GXM: a therapeutic target

The emergence of widespread antibiotic resistance has pushed the scientific world to search for alternative therapies for infectious diseases, which are affordable and effective, especially those that stimulate appropriate host responses [70]. The well-studied anti-GXM (18B7) was shown to hydrolyze the fungal oligosaccharide and increase GXM release from *Cryptococcus* [71], a therapeutic potential that needed to be elaborated. Interestingly, another study uncovered a mechanism by which GXM activates macrophages through TLR4 signaling to secrete pro-inflammatory cytokines providing protective immunity to the host [37].

Researchers reported promising preliminary studies of $CD8^{+}$T cells expressing GXM-specific chimeric antigen receptor (GXM-CAR) as a form of immunotherapeutic modality. Treatment of immunocompromised NSG mice infected with *C. neoformans* yielded a significantly lower fungal burden than the control [72]. The CD40L expressed by activated T cells interacts with CD40 molecules expressed by myeloid cells for successful co-stimulation that is tightly linked to NF-κβ activation: proinflammatory response. Hence, CD40L/CD40 immune checkpoint provides an interesting target for immunotherapy. [73]. The mutation of CD40L in humans and $CD40L^{(-/-)}$ among mice both leads to disseminated cryptococcosis, emphasizing the critical role of Th1 cells in cryptococcal immunity. Again combination therapy of CD40L and IL-2 in systemic murine cryptococcosis reveals extended survival of the study mice compared to the control [46]. Recombinant IFNγ therapy in a clinical study yielded a promising result, however, limited by side effects, and as such advocated as an adjuvant for systemic cryptococcal therapy [46,74].

Radiolabeled anti-GXM therapy shows promising results [75]. Radioimmunotherapy was conducted using 18B7 mAb labeled with alpha emitter bismuth-213 (^{213}Bi) or a powerful beta emitter rhenium-188 (^{188}Re) to target GXM in a mice model of *C. neoformans* infection. 18B7-^{213}Bi and 18B7-^{188}Re radiolabeled mAbs extend mice survival by 60% and 40% on the eleventh week of therapy, respectively [76]. Scytovirin, a lectin from cyanobacteria, has activity against mannose molecules found on microbial agents; has antiviral and antifungal effects. Scytovirin proves promising in inhibiting *C. neoformans* with lesser effect than *C. gattii* [77].

A glycopeptide isolated from sheep red blood cells (SRBCs) membrane termed T11TS was a CD2 receptor agonist, promoting T cell proliferation, activation, and survival [78]. In a rat model of CM, T11TS therapy facilitates fungal recognition through upregulated TLRs expression, increases antigen presentation by microglial cells, and promotes proinflammatory cytokines secretion, in sum promoting fungal immunity [79]. T11TS immunotherapy in a rodent model of *C. neoformans* infection was shown to induce nuclear retention of NFAT, a mechanism that causes upregulation of IL-2 expression [80]. Similarly, T11TS counters molecular mechanisms of *C. neoformans* immune evasion and facilitates immune recognition and effector immune response against the microbe, which cumulatively, favor *C. neoformans* clearance from the study rats [81]. Figure 5 summarizes various immunotherapeutics that target GXM discussed above.

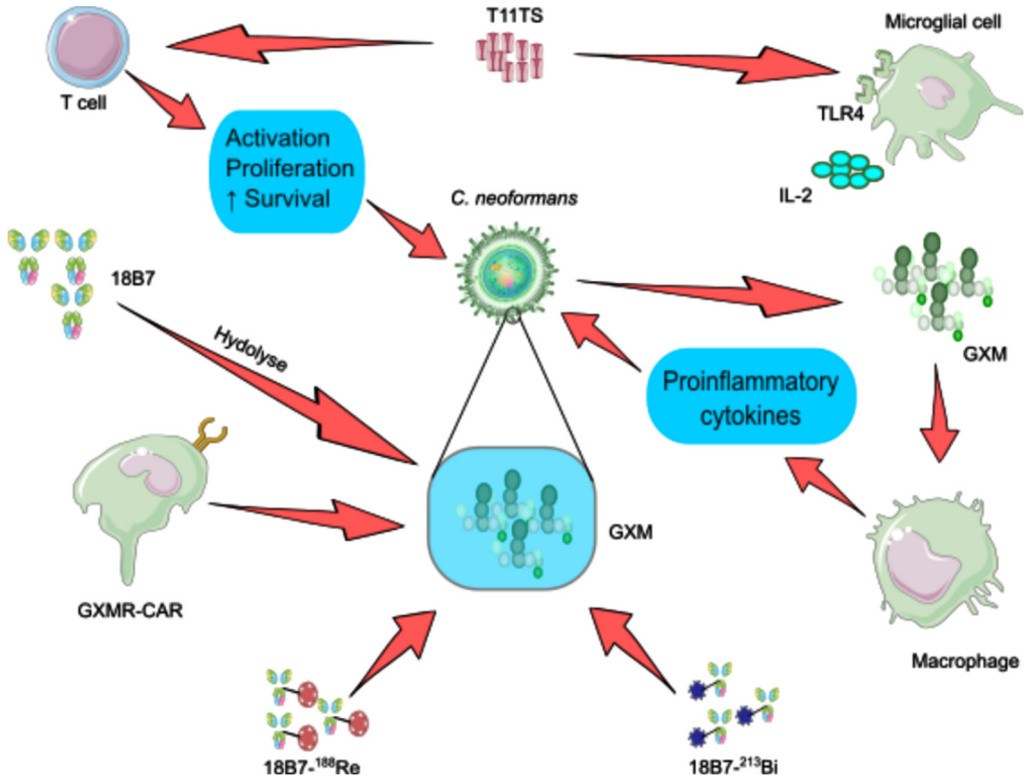

Figure 5. GXM as an immunotherapy target. GXM, the major polysaccharide in the *C. neoformans* capsule is a molecular target for immunotherapy. From the top, T11TS treatment upregulates TLR on microglial cells and serves as a growth factor on helper T cells facilitating a proinflammatory response to *C. neoformans*. A monoclonal antibody to GXM (18B7) facilitates the release of GXM from the *C. neoformans* capsule through hydrolysis of oligosaccharide constituent of GXM, on the left. The GXM as a polysaccharide activates macrophages by signaling through TLR4 that yields a proinflammatory response to target the *C. neoformans*, on the right. Cytotoxic T cells expressing GXMR-CAR specifically improve GXM recognition on *C. neoformans*. On the bottom of the diagram Radiolabeled anti-GXM: ^{213}Bi, beta emitter, and ^{188}Re, an alpha emitter both target GXM delivering the cytotoxic payload to the *C. neoformans*. GXM: glucuronoxylomannan; GXMR-CAR: GXM-specific chimeric antigen receptor; ^{213}Bi: bismuth-213; ^{188}Re: rhenium-188; T11TS: T11 target structure; TLR: toll-like receptor.

2.5. GXM: vaccine target

Researchers respond exponentially to the dire need to develop a vaccine for cryptococcosis, considering the morbidity and mortality associated with the systemic disease, which worsened with the advent of HIV infection. The cryptococcal vaccine is intended to protect individuals prone to severe cryptococcosis due to genetic predisposition or compromised immune system. Initial cryptococcal vaccines explore the immunization modality from the first principle perspective: using live attenuated and heat-killed *Cryptococcus* protocols [49]. A preclinical immunization study was conducted to compare the immune response between heat-killed *C. neoformans* and culture filtrate of

C. neoformans. The culture filtrate immunization gives way to the robust immune system that is $CD4^+$ T cell-dependent, associated with copious secretion of IFN-γ and IL-2, optimal fungal clearance, and increased mice survival. However, no anti-GXM was discovered in the studied mice sera. Of note, the heat-killed vaccine displayed an indifferent immune response [82]. Th17 polarization is a prerequisite to vaccine activity against cryptococcal pathogens [83]. Further, the lack of IFNγ, IL-17, and TNFα cytokines production in the study mice cumulatively lead to cryptococcal vaccine failure [34], which signifies the role of $CD4^+$ T cells in cryptococcal immunity.

GXM conjugated to tetanus toxoid (GXM-TT) used to immunize mice in the presence of monophosphoryl lipid A (MPL) as an adjuvant, recorded promising protection against systemic cryptococcosis through antibodies induction [84]. Researchers studied GXM mimotope's immunogenicity: P13 conjugated to either TT (P13-TT) or bovine serum albumin (P13-BSA) adjuvanted with aluminum hydroxide. Both vaccines yielded IgM and IgG to GXM and P13 that confer mice survival with decreased serum levels of GXM than controls [85]. Another study of P13 on chronically infected mice prolongs the study animals' survival through immunomodulation by IL-10 expression. However, the protective immune response recorded depends on the conjugate protein type, mice strain, and infection route [86].

Scientists cultured bone marrow DCs with a heat-killed clinical isolate of *C. gattii* species complex (*C. bacillisporus*:VGIII/AFLP5) and transferred them to mice, which were later infected with the clinical isolate. They reported prominent Th1 and Th17 cytokines expression that lowered the fungal load, decreased the lung GXM levels, improved the associated pathology, and increased mice survival [87]. Pulsed DC vaccines of *C. gattii* induce IL-17A secreting resident memory Th17 cells in the lung, which also potentiate the activity of neutrophils and giant immune cells in the study animals. The increased mice survival and the decreased fungal levels are deficient in IL-17A knock-out mice [88]. Other cryptococcal antigenic compounds (mannoprotein and MP98), synthetic cryptococcal proteins, GXM peptide mimetic, and fungal extract are being extensively studied to provide potent and safe vaccines [49] not covered in this review. Besides, with ongoing studies, it is hoped that there will be light at the end of the tunnel in the treatment and prevention of CM. Figure 6 below summarized the salient role played by GXM in immunity, pathogenicity, and vaccine development in cryptococcal infection.

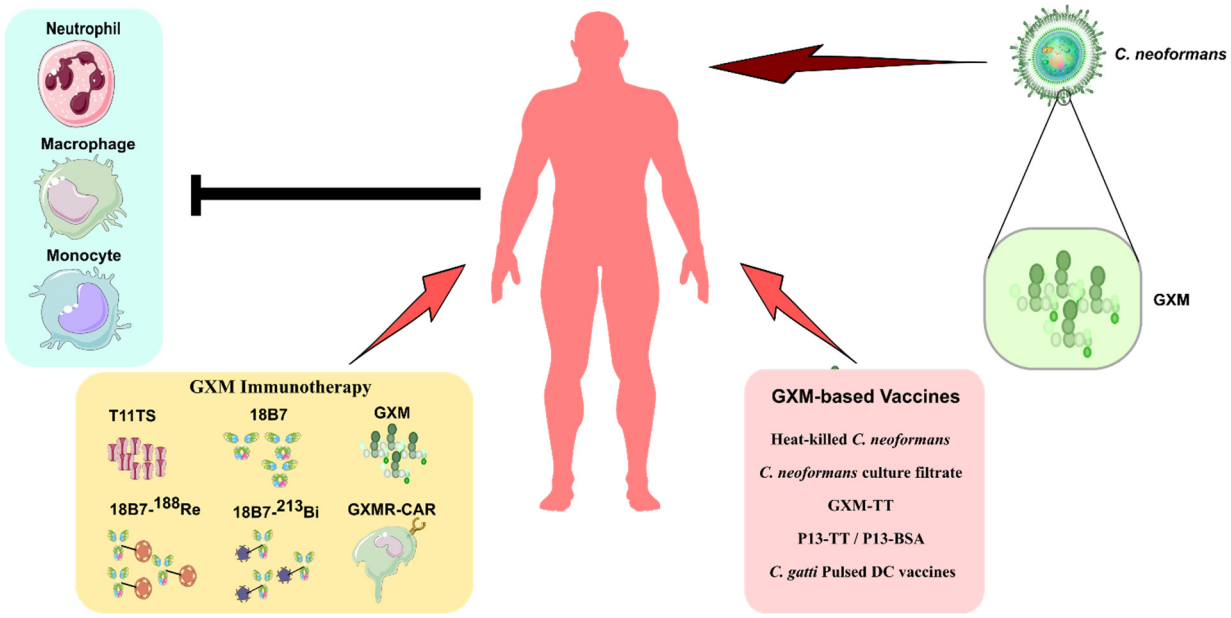

Figure 6. GXM is the cornerstone polysaccharide on the *C. neoformans* cell wall, responsible for downregulating the immune system by evading phagocytes, thereby inducing monocyte anergy, neutropenia, and facilitating M2 macrophages phenotype that are anti-inflammatory. Yet, the development of immunotherapy and vaccine both target GXM for a better outcome. GXM: glucoronoloxylomannan; GXMR-CAR: GXM-specific chimeric antigen receptor; ^{213}Bi: bismuth-213; ^{188}Re: rhenium-188; TT: tetanus toxoid; P13-TT: P13 conjugated to TT; P13-BSA: P13 conjugated to bovine serum albumin; DC: dendritic cell.

3. Conclusions

The global morbidity and mortality indices associated with CM, especially among HIV/AIDs patients, further complicated by widespread antifungal resistance, translate into the pressing need for immunotherapy and vaccine to curtail this severe infection's menace. Of note, *C. neoformans* evolves escape mechanisms to evade the immune system and persist in a strong or weak immune response. Both immunotherapy and vaccine development are still ongoing, and substantial literature targets GXM as a promising biomarker.

Authors' contributions

MA conceived and designed the manuscript outline, MA and AAS search and review the literature, and MA drew Figures 1–6. MA, AAS, SK, and MIG wrote the manuscript. BRJ and MIG critically revised the manuscript, and AAs and SK supervised all the processes. All authors reviewed and approved the final version of the manuscript for submission.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Cogliati M (2013) Global molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii*: an atlas of the molecular types. *Scientifica* 2013: 675213. https://doi.org/10.1155/2013/675213
2. Li Y, Zou M, Yin J, et al. (2020) Microbiological, epidemiological, and clinical characteristics of patients with cryptococcal meningitis at a tertiary hospital in China: a 6-year retrospective analysis. *Front Microbiol* 11: 1837. https://doi.org/10.3389/fmicb.2020.01837
3. Taha M, Tartor Y, Ibrahim S, et al. (2020) Molecular typing and susceptibility profile of *Cryptococcus neoformans* and *Cryptococcus gattii* species complex: an updated review. *J Anim Health Prod* 9: 17–26. https://doi.org/10.17582/journal.jahp/2020/9.s1.17.26
4. Francisco EC, de Jong AW, Hagen F (2021) Cryptococcosis and *Cryptococcus*. *Mycopathologia* 186: 729–731. https://doi.org/10.1007/s11046-021-00577-7
5. Kwon-Chung KJ, Bennett JE, Wickes BL, et al. (2017) The case for adopting the "species complex" nomenclature for the etiologic agents of Cryptococcosis. *mSphere* 2: e00357-16. https://doi.org/10.1128/mSphere.00357-16
6. Maziarz EK, Perfect JR (2016) Cryptococcosis. *Infect Dis Clin N Am* 30: 179–206. https://doi.org/10.1016/j.idc.2015.10.006
7. Cassim N, Schnippel K, Coetzee LM, et al. (2017) Establishing a cost-per-result of laboratory-based, reflex cryptococcal antigenaemia screening (CrAg) in HIV+ patients with CD4 counts less than 100 cells/μL using a Lateral Flow Assay (LFA) at a typical busy CD4 laboratory in South Africa. *PLoS One* 12: e0171675. https://doi.org/10.1371/journal.pone.0171675
8. Rivet-Danon D, Guitard J, Grenouillet F, et al. (2015) Rapid diagnosis of cryptococcosis using an antigen detection immunochromatographic test. *J Infection* 70: 499–503. https://doi.org/10.1016/j.jinf.2014.12.017
9. Illnait M, Vilaseca JC, Fernández CM, et al. (2001) Enzyme-linked immunosorbent assay for detection and quantification of *Cryptococcus neoformans* antigen. *Mem Inst Oswaldo Cruz* 96: 241–245. https://doi.org/10.1590/S0074-02762001000200018
10. Rajasingham R, Meya DB, Boulware DR (2012) Integrating cryptococcal antigen screening and pre-emptive treatment into routine HIV care. *J Acquir Immune Defic Syndr* 59: 85–91. https://doi.org/10.1097/QAI.0b013e31824c837e
11. Márquez M (2019) Lateral flow assay: a world of possibilities for the diagnostic. *Austin Clin Microbiol* 3: 1010.
12. Xu Y, Xia W, Ni F (2020) False-negative serum cryptococcal antigen lateral flow immunoassay result for a patient with disseminated cryptococcal disease. *Infect Drug Resist* 13: 2877–2881. https://doi.org/10.2147/IDR.S265784
13. Wake RM, Jarvis JN, Harrison TS, et al. (2018) Brief report: point of care cryptococcal antigen screening: pipetting finger-prick blood improves performance of immunomycologics lateral flow assay. *JAIDS J Acquired Immune Defic Syndr* 78: 574–578. https://doi.org/10.1097/QAI.0000000000001721
14. Kwizera R, Omali D, Tadeo K, et al. (2021) Evaluation of the dynamiker cryptococcal antigen lateral flow assay for the diagnosis of HIV-associated cryptococcosis. *J Clin Microbiol* 59: e02421-20. https://doi.org/10.1128/JCM.02421-20

15. Tenforde MW, Boyer-Chammard T, Muthoga C, et al. (2020) Diagnostic accuracy of the Biosynex CryptoPS cryptococcal antigen semiquantitative lateral flow assay in patients with advanced HIV disease. *J Clin Microbiol* 59: e02307–02320. https://doi.org/10.1128/JCM.02307-20
16. Mpoza E, Mukaremera L, Kundura DA, et al. (2018) Evaluation of a point-of-care immunoassay test kit "StrongStep" for cryptococcal antigen detection. *PLoS One* 13: e0190652. https://doi.org/10.1371/journal.pone.0190652
17. Rajasingham R, Wake RM, Beyene T, et al. (2019) Cryptococcal meningitis diagnostics and screening in the era of point-of-care laboratory testing. *J Clin Microbiol* 57: e01238-18. https://doi.org/10.1128/JCM.01238-18
18. Reagan KL, McHardy I, Thompson III GR, et al. (2019) Evaluation of the clinical performance of 2 point-of-care cryptococcal antigen tests in dogs and cats. *J Vet Intern Med* 33: 2082–2089. https://doi.org/10.1111/jvim.15599
19. Skipper C, Tadeo K, Martyn E, et al. (2020) Evaluation of serum cryptococcal antigen testing using two novel semiquantitative lateral flow assays in persons with cryptococcal antigenemia. *J Clin Microbiol* 58: e02046-19. https://doi.org/10.1128/JCM.02046-19
20. Noguera MC, Escandón P, Rodríguez J, et al. (2021) Comparison of two commercial tests (Immy vs. Dynamiker) for cryptococcal capsular antigen. *Rev Soc Bras Med Trop* 54: e0307-2021.
21. Shi D, Haas PJ, Boekhout T, et al. (2021) Neglecting genetic diversity hinders timely diagnosis of *Cryptococcus* infections. *J Clin Microbiol* 59: e02837-20. https://doi.org/10.1128/JCM.02837-20
22. Casadevall A, Coelho C, Cordero RJ, et al. (2019) The capsule of *Cryptococcus neoformans*. *Virulence* 10: 822–831. https://doi.org/10.1080/21505594.2018.1431087
23. Patil SA, Katyayani S, Arvind N (2012) Significance of antibody detection in the diagnosis of cryptococcal meningitis. *J Immunoassay Immunochem* 33: 140–148. https://doi.org/10.1080/15321819.2011.606862
24. Frases S, Nimrichter L, Viana NB, et al. (2008) *Cryptococcus neoformans* capsular polysaccharide and exopolysaccharide fractions manifest physical, chemical, and antigenic differences. *Eukaryotic Cell* 7: 319–327. https://doi.org/10.1128/EC.00378-07
25. Camacho E, Casadevall A (2018) Cryptococcal traits mediating adherence to biotic and abiotic surfaces. *J Fungi* 4: 88. https://doi.org/10.3390/jof4030088
26. Ulrich S, Ebel F (2020) Monoclonal antibodies as tools to combat fungal infections. *J Fungi* 6: 22. https://doi.org/10.3390/jof6010022
27. Guazzelli L, Crawford CJ, Ulc R, et al. (2020) A synthetic glycan array containing *Cryptococcus neoformans* glucuronoxylomannan capsular polysaccharide fragments allows the mapping of protective epitopes. *Chem Sci* 11: 9209–9217. https://doi.org/10.1039/D0SC01249A
28. Devi S, Schneerson R, Egan W, et al. (1991) *Cryptococcus neoformans* serotype A glucuronoxylomannan-protein conjugate vaccines: synthesis, characterization, and immunogenicity. *Infect Immun* 59: 3700–3707. https://doi.org/10.1128/iai.59.10.3700-3707.1991
29. Cherniak R, Reiss E, Slodki ME, et al. (1980) Structure and antigenic activity of the capsular polysaccharide of *Cryptococcus neoformans* serotype A. *Mol Immunol* 17: 1025–1032. https://doi.org/10.1016/0161-5890(80)90096-6
30. Urai M, Kaneko Y, Ueno K, et al. (2016) Evasion of innate immune responses by the highly virulent *Cryptococcus gattii* by altering capsule glucuronoxylomannan structure. *Front Cell Infect Microbiol* 5: 101. https://doi.org/10.3389/fcimb.2015.00101

31. Cherniak R, Valafar H, Morris LC, et al. (1998) *Cryptococcus neoformans* chemotyping by quantitative analysis of ^{1}H nuclear magnetic resonance spectra of glucuronoxylomannans with a computer-simulated artificial neural network. *Clin Diagn Lab Immunol* 5: 146–159. https://doi.org/10.1128/CDLI.5.2.146-159.1998
32. Kuttel MM, Casadevall A, Oscarson S (2020) *Cryptococcus neoformans* capsular GXM conformation and epitope presentation: a molecular modelling study. *Molecules* 25: 2651. https://doi.org/10.3390/molecules25112651
33. McFadden DC, Fries BC, Wang F, et al. (2007) Capsule structural heterogeneity and antigenic variation in *Cryptococcus neoformans*. *Eukaryot Cell* 6: 1464–1473. https://doi.org/10.1128/EC.00162-07
34. Ueno K, Yanagihara N, Shimizu K, et al. (2020) Vaccines and protective immune memory against cryptococcosis. *Biol Pharm Bull* 43: 230–239. https://doi.org/10.1248/bpb.b19-00841
35. Diamond RD, Bennett JE (1974) Prognostic factors in cryptococcal meningitis: a study in 111 cases. *Ann Intern Med* 80: 176–181. https://doi.org/10.7326/0003-4819-80-2-176
36. Sato K, Kawakami K (2017) Recognition of *Cryptococcus neoformans* by pattern recognition receptors and its role in host defense to this infection. *Med Mycol J* 58: J83–J90. https://doi.org/10.3314/mmj.17.011
37. Perera N, Yang FL, Chern J, et al. (2018) Carboxylic and O-acetyl moieties are essential for the immunostimulatory activity of glucuronoxylomannan: A novel TLR4 specific immunostimulator from *Auricularia auricula-judae*. *Chem Commun* 54: 6995–6998. https://doi.org/10.1039/C7CC09927D
38. Monari C, Bistoni F, Casadevall A, et al. (2005) Glucuronoxylomannan, a microbial compound, regulates expression of costimulatory molecules and production of cytokines in macrophages. *J Infect Dis* 191: 127–137. https://doi.org/10.1086/426511
39. de Oliveira HC, Trevijano-Contador N, Garcia-Rodas R (2019) Cryptococcal pathogenicity and morphogenesis. *Curr Fungal Infect Rep* 13: 67–76. https://doi.org/10.1007/s12281-019-00340-y
40. Elsegeiny W, Marr KA, Williamson PR (2018) Immunology of cryptococcal infections: developing a rational approach to patient therapy. *Front Immunol* 9: 651. https://doi.org/10.3389/fimmu.2018.00651
41. Mohamed SH, Nyazika TK, Ssebambulidde K, et al. (2022) Fungal CNS Infections in Africa: The neuroimmunology of cryptococcal meningitis. *Front Immunol* 13: 804674. https://doi.org/10.3389/fimmu.2022.804674
42. Hardison SE, Herrera G, Young ML, et al. (2012) Protective immunity against pulmonary cryptococcosis is associated with STAT1-mediated classical macrophage activation. *J Immunol* 189: 4060–4068. https://doi.org/10.4049/jimmunol.1103455
43. Rohatgi S, Pirofski L (2015) Host immunity to *Cryptococcus neoformans*. *Future Microbiol* 10: 565–581. https://doi.org/10.2217/fmb.14.132
44. Wozniak KL, Young ML, Wormley FL (2011) Protective immunity against experimental pulmonary cryptococcosis in T cell-depleted mice. *Clin Vaccine Immunol* 18: 717–723. https://doi.org/10.1128/CVI.00036-11
45. Meya DB, Okurut S, Zziwa G, et al. (2019) HIV-associated Cryptococcal immune reconstitution inflammatory syndrome is associated with aberrant t cell function and increased cytokine responses. *J Fungi* 5: 42. https://doi.org/10.3390/jof5020042

46. Zhou Q, Murphy WJ (2006) Immune response and immunotherapy to *Cryptococcus* infections. *Immunol Res* 35: 191–208. https://doi.org/10.1385/IR:35:3:191
47. Rocha JD, Nascimento MT, Decote-Ricardo D, et al. (2015) Capsular polysaccharides from *Cryptococcus neoformans* modulate production of neutrophil extracellular traps (NETs) by human neutrophils. *Sci Rep* 5: 8008. https://doi.org/10.1038/srep08008
48. Scriven JE, Graham LM, Schutz C, et al. (2016) A glucuronoxylomannan-associated immune signature, characterized by monocyte deactivation and an increased interleukin 10 level, is a predictor of death in cryptococcal meningitis. *J Infect Dis* 213: 1725–1734. https://doi.org/10.1093/infdis/jiw007
49. Van Dyke MCC, Wormley FL (2018) A call to arms: quest for a cryptococcal vaccine. *Trends Microbiol* 26: 436–446. https://doi.org/10.1016/j.tim.2017.10.002
50. Yoon HA, Kuniholm MH, Nakouzi A, et al. (2016) Association of decreased cryptococcal antibody levels with cryptococcosis-associated immune reconstitution inflammatory syndrome. *Open Forum Infect Dis* 3: 899. https://doi.org/10.1093/ofid/ofw194.64
51. Yoon HA, Nakouzi A, Chang CC, et al. (2019) Association between plasma antibody responses and risk for cryptococcus-associated immune reconstitution inflammatory syndrome. *J Infect Dis* 219: 420–428. https://doi.org/10.1093/infdis/jiy447
52. Maitta RW, Datta K, Chang Q, et al. (2004) Protective and nonprotective human immunoglobulin M monoclonal antibodies to *Cryptococcus neoformans* glucuronoxylomannan manifest different specificities and gene use profiles. *Infect Immun* 72: 4810–4818. https://doi.org/10.1128/IAI.72.8.4810-4818.2004
53. Maitta RW, Datta K, Pirofski LA (2004) Efficacy of immune sera from human immunoglobulin transgenic mice immunized with a peptide mimotope of *Cryptococcus neoformans* glucuronoxylomannan. *Vaccine* 22: 4062–4068. https://doi.org/10.1016/j.vaccine.2004.03.060
54. Albuquerque PC, Fonseca FL, Dutra FF, et al. (2014) *Cryptococcus neoformans* glucuronoxylomannan fractions of different molecular masses are functionally distinct. *Mycoses* 57: 53. https://doi.org/10.2217/fmb.13.163
55. Boulware DR, von Hohenberg M, Rolfes MA, et al. (2016) Human immune response varies by the degree of relative cryptococcal antigen shedding. *Open Forum Infect Dis* 3: ofv194. https://doi.org/10.1093/ofid/ofv194
56. Ana Caroline C, Rella A, Normile T, et al. (2019) *Cryptococcus neoformans* glucuronoxylomannan and sterylglucoside are required for host protection in an animal vaccination model. *Mbio* 10: e02909-18. https://doi.org/10.1128/mBio.02909-18
57. Decote-Ricardo D, LaRocque-de-Freitas IF, Rocha JDB, et al. (2019) Immunomodulatory role of capsular polysaccharides constituents of *Cryptococcus neoformans*. *Front Med* 6: 129. https://doi.org/10.3389/fmed.2019.00129
58. Vecchiarelli A, Pericolini E, Gabrielli E, et al. (2011) *Cryptococcus neoformans* galactoxylomannan is a potent negative immunomodulator, inspiring new approaches in anti-inflammatory immunotherapy. *Immunotherapy* 3: 997–1005. https://doi.org/10.2217/imt.11.86
59. Chuck SL, Sande MA (1989) Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. *N Engl J Med* 321: 794–799. https://doi.org/10.1056/NEJM198909213211205

60. Silva E, Silva M, Paula C, et al. (2016) Effect of GXM (glucuronoxylomannan) on the inflammatory response in lung infection caused by *Cryptococcus neoformans* (serotype A) in immunodeficient murine model (BALB/c-SCID). *J Med Microbiol Diagn* 5: 4–6.
61. Robertson EJ, Najjuka G, Rolfes MA, et al. (2014) *Cryptococcus neoformans* ex vivo capsule size is associated with intracranial pressure and host immune response in HIV-associated cryptococcal meningitis. *J Infect Dis* 209: 74–82. https://doi.org/10.1093/infdis/jit435
62. Vecchiarelli A, Pericolini E, Gabrielli E, et al. (2013) Elucidating the immunological function of the *Cryptococcus neoformans* capsule. *Future Microbiol* 8: 1107–1116. https://doi.org/10.2217/fmb.13.84
63. Denham ST, Verma S, Reynolds RC, et al. (2018) Regulated release of cryptococcal polysaccharide drives virulence and suppresses immune cell infiltration into the central nervous system. *Infect Immun* 86: e00662-17. https://doi.org/10.1128/IAI.00662-17
64. Gassiep I, Aye C, Armstrong M, et al. (2018) Correlation between serum cryptococcal antigen titre and meningitis in immunocompetent patients. *J Med Microbiol* 67: 1515–1518. https://doi.org/10.1099/jmm.0.000830
65. Nalintya E, Kiggundu R, Meya D (2016) Evolution of cryptococcal antigen testing: what is new? *Curr Fungal Infect Rep* 10: 62–67. https://doi.org/10.1007/s12281-016-0256-3
66. Casadevall A (2016) New Insights in to polysaccharide capsule structure and antibody-function from *Cryptococcus neoformans*. *Glycobiology* 26: 1386.
67. Nami S, Mohammadi R, Vakili M, et al. (2019) Fungal vaccines, mechanism of actions and immunology: A comprehensive review. *Biomed Pharmacother* 109: 333–344. https://doi.org/10.1016/j.biopha.2018.10.075
68. Dromer F, Salamero J, Contrepois A (1987) Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. *Infect Immun* 55: 742–748. https://doi.org/10.1128/iai.55.3.742-748.1987
69. Arturo Casadevall, Matthew Scharff, Mukherjee J (2002) Antibodies to polysaccharide of *C. neoformans*. U.S. Patent Application, US20030103977A1.
70. Hancock RE, Nijnik A, Philpott DJ (2012) Modulating immunity as a therapy for bacterial infections. *Nat Rev Microbiol* 10: 243–254. https://doi.org/10.1038/nrmicro2745
71. Bowen A, Wear MP, Cordero RJB, et al. (2017) A monoclonal antibody to *Cryptococcus neoformans* glucuronoxylomannan manifests hydrolytic activity for both peptides and polysaccharides. *J Biol Chem* 292: 417–434. https://doi.org/10.1074/jbc.M116.767582
72. Kumaresan P, Da Silva T, Laskowski T (2020) Glucuronoxylomannan in the *Cryptococcus* species capsule as a target for CAR^+ T-cell therapy. *J Immunol* 204: 231. https://doi.org/10.1101/715045
73. Tang T, Cheng X, Truong B, et al. (2021) Molecular basis and therapeutic implications of CD40/CD40L immune checkpoint. *Pharmacol Therapeut* 219: 107709. https://doi.org/10.1016/j.pharmthera.2020.107709
74. Antachopoulos C, Walsh TJ (2012) Immunotherapy of *Cryptococcus* infections. *Clin Microbiol Infec* 18: 126–133. https://doi.org/10.1111/j.1469-0691.2011.03741.x
75. Shah M, Garg G, Dadachova E (2015) Preclinical testing of radiopharmaceuticals for novel applications in HIV, bacterial and fungal infectious diseases. *Q J Nucl Med Mol Imaging* 59: 317–326.

76. Helal M, Dadachova E (2018) Radioimmunotherapy as a novel approach in HIV, bacterial, and fungal infectious diseases. *Cancer Biother Radio* 33: 330–335. https://doi.org/10.1089/cbr.2018.2481
77. Jones TH, McClelland EE, McFeeters H, et al. (2017) Novel antifungal activity for the lectin scytovirin: Inhibition of *Cryptococcus neoformans* and *Cryptococcus gattii*. *Front Microbiol* 8: 755. https://doi.org/10.3389/fmicb.2017.00755
78. Banerjee S, Khajanchi S, Chaudhuri S (2015) A mathematical model to elucidate brain tumor abrogation by immunotherapy with T11 target structure. *PLoS One* 10: e0123611. https://doi.org/10.1371/journal.pone.0123611
79. Hazra I, Sk Md OF, Datta A, et al. (2019) T11TS immunotherapy augments microglial and lymphocyte protective immune responses against *Cryptococcus neoformans* in the brain. *Scand J Immunol* 89: e12733. https://doi.org/10.1111/sji.12733
80. Omar F, Hazra I, Mondal S, et al. (2020) T11TS immunotherapy potentiates the repressed calcineurin-NFAT signalling pathway of T cells in *Cryptococcus neoformans* infected rats: a cue towards T-cell activation for antifungal immunity. *J Appl Microbiol* 129: 753–767. https://doi.org/10.1111/jam.14631
81. Omar F, Hazra I, Datta A, et al. (2020) Regulation of key molecules of immunological synapse by T11TS immunotherapy abrogates *Cryptococcus neoformans* infection in rats. *Mol Immunol* 122: 207–221. https://doi.org/10.1016/j.molimm.2020.04.021
82. Murphy JW, Schafer F, Casadevall A, et al. (1998) Antigen-induced protective and nonprotective cell-mediated immune components against *Cryptococcus neoformans. Infect Immun* 66: 2632–2639. https://doi.org/10.1128/IAI.66.6.2632-2639.1998
83. Rathore SS, Sathiyamoorthy J, Lalitha C, et al. (2022) A holistic review on *Cryptococcus neoformans*. *Microb Pathogenesis* 166: 105521. https://doi.org/10.1016/j.micpath.2022.105521
84. Devi SJ (1996) Preclinical efficacy of a glucuronoxylomannan-tetanus toxoid conjugate vaccine of *Cryptococcus neoformans* in a murine model. *Vaccine* 14: 841–844. https://doi.org/10.1016/0264-410X(95)00256-Z
85. Fleuridor R, Lees A, Pirofski L (2001) A cryptococcal capsular polysaccharide mimotope prolongs the survival of mice with *Cryptococcus neoformans* infection. *J Immunol* 166: 1087–1096. https://doi.org/10.4049/jimmunol.166.2.1087
86. Datta K, Lees A, Pirofski L (2008) Therapeutic efficacy of a conjugate vaccine containing a peptide mimotope of cryptococcal capsular polysaccharide glucuronoxylomannan. *Clin Vaccine Immunol* 15: 1176–1187. https://doi.org/10.1128/CVI.00130-08
87. Ueno K, Urai M, Ohkouchi K, et al. (2016) Dendritic cell-based vaccine against fungal infection. *Vaccine Des* 1403: 537–549. https://doi.org/10.1007/978-1-4939-3387-7_30
88. Ueno K, Urai M, Sadamoto S, et al. (2019) A dendritic cell-based systemic vaccine induces long-lived lung-resident memory Th17 cells and ameliorates pulmonary mycosis. *Mucosal Immunol* 12: 265–276. https://doi.org/10.1038/s41385-018-0094-4