Volume 4. Issue 1. 2021

ProClinS Pathology

Opinion

Open Access

"Is *Cryptococcus neoformans* a "Sleeping Giant" With Deadly Intentions?"

Author: Arpita Singh*

Molecular Biology Unit, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India

***Corresponding author:** Arpita Singh, Molecular Biology Unit, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India, E-mail: arpitamanu@gmail.com

Received: January 22, 2021; **Accepted:** February 02, 2021; **Published:** February 03, 2021

ABSTRACT

Natural calamities, global warming and a change in the overall habitat are directly related to the emergence of newer variety of pathogenic microbes. *Cryptococcus neoformans* is known more commonly as the causal organism of AIDs-defining illnesses since the last 2 decades. Cryptococcosis or cryptococcal meningitis caused by *C. neoformans* is usually seen in an immune-compromised mammalian host, causing large scale fatality due to the host's failure to respond to available therapeutics and drug resistance in the fungus. This pathogen with the ability to re-infect even after lying dormant or be clinically asymptomatic for several years seems to be evolving to a more complex type due to host-pathogenic interaction. More often than not, the seriousness of the infection results due to the resurgence of latent infections in the least –suspecting host. They modulate the host immune responses, manipulates it in their own favor in a manner that is still mostly unknown. Thus, remaining alert and suspicious of this opportunistic pathogen as well as continuing the search for next-generation therapeutics is the need of the hour.

KEYWORDS: *Cryptococcus neoformans*, *Cryptococcus neoformans* species complex, Pandemic, Latent, Immune modulation

OPINION

Human beings have in general the tendency to speculate and have lingering questions like "what if"?. Most of the time though it can trigger unnecessary thought processes, it is not altogether without any pros. Thus, contemplating *Cryptococcus neoformans* [1] as a sleeping giant can also be considered to be a mechanism to keep the human brain active and alert of a possible future event that may have catastrophic outcomes. Historically speaking, it's occurrences[2] have already been associated with one of the most severe pandemics of the 80s-90sand is still the causal organism in around one-third of AIDS-related death, numbering to a couple of millions in the present decade. According to the Centers for Diseases Control and Prevention (CDC) USA, the world sees around 220,000 active cases of Cryptococcal meningitis per year. Out of the global burden of Cryptococcal meningitis linked to HIV related cases, the highest is in Africa, followed by approximately 44000 in Asia and Pacific, with Europe, Middle East, and North Africa trailing far behind [3,4].

Fungi, to most of us, are organisms that cause various diseases, most commonly skin infections in the toenails, eyes, etc. It is incredible to note that out of an estimated 3.8-5.1 million fungal species, around 144000 are till-date identified through high thru-put sequencing techniques, which includes yeasts, rusts, smuts, mildews, molds, and mushrooms. Around 30 % of plant diseases are caused by fungi numbering over 8000 species [5]. Infections caused by *Cryptococcus, Aspergillus* (environmental), *Candida* (endogenous) and *Pneumocystis*, are primarily life-threatening in individuals with impaired immunity or other underlying conditions. They are deadliest in Africa, especially in the sub-Saharan region, being the 5th leading cause of fatalism in this part of the world. Recently there has been an upward surge of the overall fungal disease burden, associated probably with the present trend of the increasing emergence of host with an impaired immune system and the appearance of drug-resistant fungal pathogens. Effective treatment of cryptococcal infections is limited by hetero resistance [6], efficacy, toxicity, availability of current therapeutics[7,8,9], as is the case in infections caused by other? fungal pathogens. Fungal disease, in general, is a neglected scenario [10]. It is imperative to mention here that there are a

large number of fungal species that are currently non-pathogenic, but possesses all the potential to become a pathogen, given a change in thermal conditions of the earth arises [11,12]. This is because fungus usually thrives in cold and moist conditions and thus those which are non-pathogenic, especially to humans, are unable to survive the host thermal conditions. This may change with global warming, leading to increasing number of fungus adapting themselves to a raise in temperatures and hence increasing their chances of surviving inside a human host manifold. Understandably thus the ability to survive in 37°C, the mammalian body temperature, is considered an important virulence factor for *C. neoformans* [13] and also other pathogenic microbes. Hence, an onslaught by pathogenic fungal diseases in the coming decade, in spite of continuing discovery and identification of newer advanced chemotherapeutics, cannot be ruled out altogether. In fact, it seems to be a very real possibility and gives us all the more reason to think and plan ahead of a combative strategy, to deal with this futuristic emergence of a deadlier variety of a pandemic- creating fungus *C. neoformans*, especially as this has a tendency to remain hidden for an extensive period of time.

Taxonomically, there are 2606 strains with published genotypes under the genus *Cryptococcus* which includes two distinct species *C. neoformans* and *C. gattii* with an ever–increasing genetic, phenotypic diversity being discovered with more and more advanced screening techniques, resulting thereby in failure in coming to a consensus while naming them. *C. neoformans* had been held solely responsible for cryptococcosis since its identification. It was only much later, after many improved molecular diagnostic techniques started identifying several different strains that naming them became confusing as many were also the result of mating [14] and were hybrids [15]. However, the recently proposed term "species complex"[16], to be added to either *Cryptococcus neoformans* or *Cryptococcus gatti* is the best alternative solution as of now, for avoiding confusion. The most widely accepted method for taxonomic categorization is based on molecular typing techniques, Multi-Locus Sequence Typing (MLST), Multi-Locus Micro Satellite Typing, Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), PCR Finger Printing etc. All these techniques under the umbrella of Whole Genome Sequencing are resource –absorbing and hence a gap in the actual number of existing varieties will always remain, especially in resource-poor countries. The most commonly followed, easily understood, and commonly referred to categorization is *Cryptococcus neoformans var grubii*, more commonly known as strain wild-type H99, can be of either serotype A (molecular type VN I, VN II, VNB)or AD (VN III), while *Cryptococcus neoformans var neoformans* belongs to the serotype D and molecular type VN IV[17]. The possibility of mixed infection either with a single strain that may undergo several microevolutions and /or co-infection with multiple strains at different time points during a human infection thus always exists, complicating further the treatment regime and increasing the chance of therapy-failure often.

This organism is airborne, a fungus that resides in pigeon guano, decaying timber, soil, and hence has a global abundance. Cryptococcal infection is invasive, results from inhalation of either the fungus itself in desiccated form or its spore form and the severity of the attack are dependent on the host immune system and the predilection of this fungus for the brain and Central Nervous System. Actively growing cryptococcal cells, usually, in the size range 5-10 μm, are unable to penetrate deep into the host lung. Interestingly though, the existence of very small or micro cells (2-4 μm) [18] and very large "Titan" (>~ 12-100 μm) variants had been reported also [19,20]. Characteristics like polyploidy, highly–cross-linked capsules, thickened cell wall with increased levels of chitin in titan cells, favor in modulating both immune and genetic adaptations of this pathogen once inside host cells. Genetic material accumulates in the titan cells, not due to some kind of mitosis defects or lowered viability, implicating that the cryptococcal cells employ a distinct kind of cell cycle regulation for better survival by escaping the nitrosative or oxidative damages, especially once it becomes intracellular. The microcells also are better adapted for growth inside macrophages. It can adapt itself to incur better uptake and ironically also escape the lytic cycles of the macrophages somehow. Paradoxically though, it is reported that the more active uptake of the fungus by the macrophages, the more aggressively the disease progresses. They can stay hidden or dormant inside a human body for years as they are clever enough to create an ecological niche or haven for many years, be left there undetected, and hence is a case of a therapeutic challenge. Dormancy in general in crptococcal cells was characterized by a specific metabolic state. The microcells, which are metabolically inactive, thus may play a role here in keeping the pathogen latent extensively, though this field is still mostly unexplored yet. The ability to adapt and exist in both intra and extracellular environments had given this fungus a bonus point in matters related to escape predation. A latent infection also sees the presence of giant cells more commonly. Given their nature of infection as opportunistic, when the body's immune system gets weakened due to various reasons like an organ transplant, presence of HIV, etc they start taking over the body's defense system. However, dormancy or latency may or may not always end up in reactivation upon immune suppression. It is dependent not only on the host system alone but also on the crosstalk between host and pathogen [21] alongside the continuous microevolution of the pathogen inside the host cells after internalization. Presumably though what ultimately leads to a resurrection of an old infection is still a matter of intense research.

Cryptococcal cells are known to be immunologically inert compared to their fungal brothers as their virulence factors like GXM (glucuronoxylomannan), suppresses pro-inflammatory nuclear pathway: the fungal cells themselves blocks dendritic

maturation and the MHC Class II-dependent immune pathway and inhibits pro-inflammatory cytokines like IL-12& 23 [22].How they manage to pull off this kind of clever masking phenomenon and turn the table around for its benefits, altering the host immune responses by re-polarizing from a strong TH1 response to weak TH2 responses, is a real enigma to the scientific field. It manages to keep the host defense system low profile by evading all the inflammatory signals and thus stays hidden, apparently waiting for a favorable time for emergence. This reminds us of the "sleeping giant" of our childhood grandma's fairytale, who when awaken becomes ferocious and deadly. There arises thus, the need for prudent and cautious use of some chemotherapeutics [23] and identification of newer alternate next generation drugs, be it based on immune-modulating molecules [24], or the host-defense system itself or other chemotherapeutics presumably age-old but originating from modern chemical genetics [25] against *Cryptococcus neoformans* species complex.

The fatality by this yeast-like fungus is caused when the pathogen gets its entry into the "highly protected and sterile" brain after crossing the blood-brain barrier. It had been quite extensively proved that urease is one of the most important factor facilitating their traversal to the brain [26], [27], closely followed by Hyaluronic acid [28], MPR1[29], and the "Trojan Horse"[30] mechanism. Mammalian host favors the yeast form of these fungi, most probably by its temperature, while amoebic form favors the hyphal form. This selective favoritism has its origin in evolutionary biology where both the amoeba and macrophages share common properties like phagocytizing particles into the vacuoles and secretion of lysosomal enzymes that digest the particles. Accumulation of polysaccharide vesicles harmful to the macrophages, blebbing of the cell membrane are the strategies employed by them when it comes across an amoeba. Though this fungus seems to be evolutionarily trained through amoeba to deal or evade macrophages and come out harmless themselves, they originated as an environmental pathogen and not as a human pathogen. Over its pathogenic history, it may be speculated that human beings became its accidental host [31], perhaps in- during the search of a suitable ecological niche and its virulence thus became a function of the immune status of its host. It acquired many special traits, most importantly the ability to manipulate its cell size and morphology, like the formation of micro or titan cells, or oval budding cell for facilitating transcytosis, modification of their own membrane to affects its pathogenicity [32], and perhaps macrophage membrane for either yeast expulsion or transfer from cell to cell, etc. This reminds us of the Covid 19 [33] phenomenon which is still ravaging the earth. History had repeatedly shown us examples of seemingly harmless microbes becoming a deadly pathogen of the humans accidentally and then going –on to cause mind-boggling human casualties; now the onus lies on us to learn from it and be pro-active.

ACKNOWLEDGEMENT

The author is thankful to Prof. Sunit K Singh, Molecular Biology Unit, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005 for overall support and providing laboratory space. This work was funded by the Government of India, Ministry of Science and Technology, Department of Science and Technology, KIRAN DIVISION, Grant Number: SR/WOS-A/LS-182/2018 to A.S.

REFERENCES

1. Idnurm A, Bahn YS, Nielsen K, Lin X, Fraser JA, Heitman J. Deciphering the model pathogenic fungus Cryptococcus neoformans. Nat Rev Microbiol. 2005 Oct;3(10):753-64. doi: 10.1038/nrmicro1245. PMID: 16132036.
2. Chang CC, Chen SC. Colliding Epidemics and the Rise of Cryptococcosis. J Fungi (Basel). 2015 Dec 23;2(1):1. doi: 10.3390/jof2010001. PMID: 29376920; PMCID: PMC5753082.
3. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017 Aug;17(8):873-881. doi: 10.1016/S1473-3099(17)30243-8. Epub 2017 May 5. PMID: 28483415; PMCID: PMC5818156.
4. Chowdhary A, Rhandhawa HS, Prakash A, Meis JF. Environmental prevalence of Cryptococcus neoformans and Cryptococcus gattii in India: an update. Crit Rev Microbiol. 2012 Feb;38(1):1-16. doi: 10.3109/1040841X.2011.606426. Epub 2011 Dec 1. PMID: 22133016.
5. Davin-Regli A, Lavigne JP, Pagès JM. Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. Clin Microbiol Rev. 2019 Jul 17;32(4):e00002-19. doi: 10.1128/CMR.00002-19. PMID: 31315895; PMCID: PMC6750132.
6. Sionov E, Lee H, Chang YC, Kwon-Chung KJ. Cryptococcus neoformans overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. PLoSPathog. 2010 Apr 1;6(4):e1000848. doi: 10.1371/journal.ppat.1000848. PMID: 20368972; PMCID: PMC2848560.
7. Bicanic T, Harrison T, Niepieklo A, Dyakopu N, Meintjes G. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. Clin Infect Dis. 2006 Oct 15;43(8):1069-73. doi: 10.1086/507895. Epub 2006 Sep 7. PMID: 16983622.

8. Brouwer AE, Rajanuwong A, Chierakul W, Griffin GE, Larsen RA, White NJ, Harrison TS. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet. 2004 May 29;363(9423):1764-7. doi: 10.1016/S0140-6736(04)16301-0. PMID: 15172774.
9. Day JN, Chau TTH, Wolbers M, Mai PP, Dung NT, Mai NH, Phu NH, Nghia HD, Phong ND, Thai CQ, Thai LH, Chuong LV, Sinh DX, Duong VA, Hoang TN, Diep PT, Campbell JI, Sieu TPM, Baker SG, Chau NVV, Hien TT, Lalloo DG, Farrar JJ. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med. 2013 Apr 4;368(14):1291-1302. doi: 10.1056/NEJMoa1110404. PMID: 23550668; PMCID: PMC3978204.
10. Molloy SF, Chiller T, Greene GS, Burry J, Govender NP, Kanyama C, Mfinanga S, Lesikari S, Mapoure YN, Kouanfack C, Sini V, Temfack E, Boulware DR, Dromer F, Denning DW, Day J, Stone NRH, Bicanic T, Jarvis JN, Lortholary O, Harrison TS, Jaffar S, Loyse A. Cryptococcal meningitis: A neglected NTD? PLoSNegl Trop Dis. 2017 Jun 29;11(6):e0005575. doi: 10.1371/journal.pntd.0005575. PMID: 28662028; PMCID: PMC5490932.
11. Benedict K, Park BJ. Invasive fungal infections after natural disasters. Emerg Infect Dis. 2014 Mar;20(3):349-55. doi: 10.3201/eid2003.131230. PMID: 24565446; PMCID: PMC3944874.
12. Garcia-Solache MA, Casadevall A. Global warming will bring new fungal diseases for mammals. mBio. 2010 May 18;1(1):e00061-10. doi: 10.1128/mBio.00061-10. PMID: 20689745; PMCID: PMC2912667.
13. Perfect JR. Cryptococcus neoformans: the yeast that likes it hot. FEMS Yeast Res. 2006 Jun;6(4):463-8. doi: 10.1111/j.1567-1364.2006.00051.x. PMID: 16696642.
14. Lin X, Hull CM, Heitman J. Sexual reproduction between partners of the same mating type in Cryptococcus neoformans. Nature. 2005 Apr 21;434(7036):1017-21. doi: 10.1038/nature03448. PMID: 15846346.
15. Ni M, Feretzaki M, Li W, Floyd-Averette A, Mieczkowski P, Dietrich FS, Heitman J. Unisexual and heterosexual meiotic reproduction generate aneuploidy and phenotypic diversity de novo in the yeast Cryptococcus neoformans. PLoS Biol. 2013 Sep;11(9):e1001653. doi: 10.1371/journal.pbio.1001653. Epub 2013 Sep 10. PMID: 24058295; PMCID: PMC3769227.
16. Kwon-Chung KJ, Bennett JE, Wickes BL, Meyer W, Cuomo CA, Wollenburg KR, Bicanic TA, Castañeda E, Chang YC, Chen J, Cogliati M, Dromer F, Ellis D, Filler SG, Fisher MC, Harrison TS, Holland SM, Kohno S, Kronstad JW, Lazera M, Levitz SM, Lionakis MS, May RC, Ngamskulrongroj P, Pappas PG, Perfect JR, Rickerts V, Sorrell TC, Walsh TJ, Williamson PR, Xu J, Zelazny AM, Casadevall A. The Case for Adopting the "Species Complex" Nomenclature for the Etiologic Agents of Cryptococcosis. mSphere. 2017 Jan 11;2(1):e00357-16. doi: 10.1128/mSphere.00357-16. PMID: 28101535; PMCID: PMC5227069.
17. Lin X, Heitman J. The biology of the Cryptococcus neoformans species complex. Annu Rev Microbiol. 2006;60:69-105. doi: 10.1146/annurev.micro.60.080805.142102. PMID: 16704346.
18. Zhou X, Zafar H, Sephton-Clark P, Mohamed S, Chapuis A, Makarova M, et al. Host environmental conditions induce small fungal cell size and alter population heterogeneity in Cryptococcus neoformans. 2020.
19. Okagaki LH, Nielsen K. Titan cells confer protection from phagocytosis in Cryptococcus neoformans infections. Eukaryot Cell. 2012 Jun;11(6):820-6. doi: 10.1128/EC.00121-12. Epub 2012 Apr 27. PMID: 22544904; PMCID: PMC3370461.
20. Zaragoza O, García-Rodas R, Nosanchuk JD, Cuenca-Estrella M, Rodríguez-Tudela JL, Casadevall A. Fungal cell gigantism during mammalian infection. PLoSPathog. 2010 Jun 17;6(6):e1000945. doi: 10.1371/journal.ppat.1000945. Erratum in: PLoSPathog. 2010;6(6). doi: 10.1371/annotation/0675044c-d80f-456f-bb63-4f85fb1d0c33. PMID: 20585557; PMCID: PMC2887474.
21. Okagaki LH, Strain AK, Nielsen JN, Charlier C, Baltes NJ, Chrétien F, Heitman J, Dromer F, Nielsen K. Cryptococcal cell morphology affects host cell interactions and pathogenicity. PLoSPathog. 2010 Jun 17;6(6):e1000953. doi: 10.1371/journal.ppat.1000953. Erratum in: PLoSPathog. 2010;6(6). doi: 10.1371/annotation/1b59fd9e-9ac9-4ea8-a083-14c413c80b03. PMID: 20585559; PMCID: PMC2887476.
22. Szymczak WA, Sellers RS, Pirofski LA. IL-23 dampens the allergic response to Cryptococcus neoformans through IL-17-independent and -dependent mechanisms. Am J Pathol. 2012 Apr;180(4):1547-59. doi: 10.1016/j.ajpath.2011.12.038. Epub 2012 Feb 16. PMID: 22342846; PMCID: PMC3349902.
23. Bryan AM, You JK, McQuiston T, Lazzarini C, Qiu Z, Sheridan B, Nuesslein-Hildesheim B, Del Poeta M. FTY720 reactivates cryptococcal granulomas in mice through S1P receptor 3 on macrophages. J Clin Invest. 2020 Sep 1;130(9):4546-4560. doi: 10.1172/JCI136068. PMID: 32484801; PMCID: PMC7456236.
24. Jarvis JN, Meintjes G, Rebe K, Williams GN, Bicanic T, Williams A, Schutz C, Bekker LG, Wood R, Harrison TS. Adjunctive interferon-γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS. 2012 Jun 1;26(9):1105-13. doi: 10.1097/QAD.0b013e3283536a93. PMID: 22421244; PMCID: PMC3640254.

25. Brown JCS, Nelson J, VanderSluis B, Deshpande R, Butts A, Kagan S, Polacheck I, Krysan DJ, Myers CL, Madhani HD. Unraveling the biology of a fungal meningitis pathogen using chemical genetics. Cell. 2014 Nov 20;159(5):1168-1187. doi: 10.1016/j.cell.2014.10.044. PMID: 25416953; PMCID: PMC4243055.
26. Shi M, Li SS, Zheng C, Jones GJ, Kim KS, Zhou H, Kubes P, Mody CH. Real-time imaging of trapping and urease-dependent transmigration of Cryptococcus neoformans in mouse brain. J Clin Invest. 2010 May;120(5):1683-93. doi: 10.1172/JCI41963. Epub 2010 Apr 26. PMID: 20424328; PMCID: PMC2860939.
27. Singh A, Panting RJ, Varma A, Saijo T, Waldron KJ, Jong A, Ngamskulrungroj P, Chang YC, Rutherford JC, Kwon-Chung KJ. Factors required for activation of urease as a virulence determinant in Cryptococcus neoformans. mBio. 2013 May 7;4(3):e00220-13. doi: 10.1128/mBio.00220-13. PMID: 23653445; PMCID: PMC3663189.
28. Liu TB, Kim JC, Wang Y, Toffaletti DL, Eugenin E, Perfect JR, Kim KJ, Xue C. Brain inositol is a novel stimulator for promoting Cryptococcus penetration of the blood-brain barrier. PLoSPathog. 2013;9(4):e1003247. doi: 10.1371/journal.ppat.1003247. Epub 2013 Apr 4. PMID: 23592982; PMCID: PMC3617100.
29. Na Pombejra S, Jamklang M, Uhrig JP, Vu K, Gelli A. The structure-function analysis of the Mpr1 metalloprotease determinants of activity during migration of fungal cells across the blood-brain barrier. PLoS One. 2018 Aug 30;13(8):e0203020. doi: 10.1371/journal.pone.0203020. PMID: 30161190; PMCID: PMC6117016.
30. Sorrell TC, Juillard PG, Djordjevic JT, Kaufman-Francis K, Dietmann A, Milonig A, Combes V, Grau GE. Cryptococcal transmigration across a model brain blood-barrier: evidence of the Trojan horse mechanism and differences between Cryptococcus neoformans var. grubii strain H99 and Cryptococcus gattii strain R265. Microbes Infect. 2016 Jan;18(1):57-67. doi: 10.1016/j.micinf.2015.08.017. Epub 2015 Sep 11. PMID: 26369713.
31. Casadevall A. Evolution of intracellular pathogens. Annu Rev Microbiol. 2008;62:19-33. doi: 10.1146/annurev.micro.61.080706.093305. PMID: 18785836.
32. Singh A, Wang H, Silva LC, Na C, Prieto M, Futerman AH, Luberto C, Del Poeta M. Methylation of glycosylated sphingolipid modulates membrane lipid topography and pathogenicity of Cryptococcus neoformans. Cell Microbiol. 2012 Apr;14(4):500-16. doi: 10.1111/j.1462-5822.2011.01735.x.Epub 2012 Jan 9. PMID: 22151739; PMCID: PMC3302964.
33. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020 Apr;5(4):536-544. doi: 10.1038/s41564-020-0695-z. Epub 2020 Mar 2. PMID: 32123347; PMCID: PMC7095448.