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PPAR-gamma Fun(gi) With Prostaglandin

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Abstract

In our recent publication, we show for the first time that the fungal pathogen *Cryptococcus neoformans* is able to manipulate host cells by producing eicosanoids that mimic those found in the host. Using complementary *in vivo* zebrafish and *in vitro* macrophage cell culture models of *Cryptococcus* infection, we found that these eicosanoids manipulate host innate immune cells by activating the host receptor PPAR-gamma which is an important regulator of macrophage inflammatory phenotypes. We initially identified PGE₂ as the eicosanoid species responsible for this effect; however, we later found that a derivative of PGE₂—15-keto-PGE₂—was ultimately responsible and that this eicosanoid acted as a partial agonist to PPAR-gamma. In this commentary, we will discuss some of the concepts and conclusions in our original publication and expand on their implications and future directions.

Keywords

Cryptococcus neoformans, fungal infection, host pathogen interactions, macrophages, zebrafish, eicosanoids, 15-keto-PGE₂, PPAR-gamma

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Cryptococcus neoformans is a pathogenic fungus that is ubiquitous in our everyday environment, but only those with severe or unusual immune deficiencies, such as HIV/AIDS, develop serious disease.¹ During infection *Cryptococcus* forms a close interaction with host macrophages—after phagocytosis by macrophages *Cryptococcus* is able to survive and replicate within the phagosome, subverting macrophage function and turning the macrophage into a niche for the establishment of infection.² To kill *Cryptococcus*, macrophages must be activated by a Th1 CD4+ T-cell-mediated adaptive immune response (hence the prevalence in HIV/AIDS patients)³; failure to control intracellular infection can lead to dissemination from the lungs into the central nervous system and the development of fatal fungal meningitis.

Cryptococcus can influence the activation state of infected macrophages, shifting them from protective Th1 activation states to a nonprotective Th2 state although the biological mechanisms behind this are unclear.⁴ Our hypothesis for this

study was that this manipulation might be mediated by eicosanoid species produced by the fungus.⁵ *Cryptococcus* can produce a number of eicosanoid species which closely resemble those found in the host but natural purpose of these lipids normally associated with cell to cell signaling in multicellular organisms is unknown.⁶ Macrophages and other innate immune cells are highly responsive to eicosanoid species such as prostaglandins and leukotrienes so we reasoned that eicosanoids produced by the fungus during intracellular infection could interfere with normal host signaling pathways.

Quantifying Eicosanoids During *Cryptococcus* Infection and Determining Their Source

Very little is known about eicosanoid synthesis pathways in *Cryptococcus*; only two *Cryptococcus* enzymes—phospholipase B1 (*PLB1*) and laccase (*LAC1*)—have been linked to eicosanoid synthesis in the fungus.^{7,8} The lack of

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homologs to eicosanoid synthesis enzymes found in higher organisms suggests that the pathway is distinct from anything previously described. Deletion mutants for *PLB1*⁸ and *LAC1*⁹ have been characterized in *Cryptococcus*. The *PLB1* mutant ($\Delta plb1$) shows a profound decrease in all eicosanoids produced by *C. neoformans* suggesting this enzyme is central to eicosanoid synthesis—possibly fulfilling a role analogous to phospholipase A_2 in mammalian cells. The *LAC1* mutant ($\Delta lac1$) is deficient in only PGE_2 and its derivative 15-keto- PGE_2 , suggesting this enzyme might fulfill a role analogous to prostaglandin E_2 synthase in mammalian cells. Both of these strains were used in our study to differentiate between host- and pathogen-derived eicosanoids; to aid the study of these strains in our zebrafish model, we produced green fluorescent protein-tagged versions of each strain. $\Delta plb1$ is known to have a growth defect in macrophages,¹⁰ whereas laccase activity has been found to positively correlate with increased mortality in patients with HIV-associated cryptococcosis¹¹—although how much this is due to PGE_2 synthesis as opposed to the role of laccase in the production of another cryptococcal virulence factor melanin. In our study, we were able to rescue the in vitro intracellular proliferation defect of $\Delta plb1$ with exogenous PGE_2 ; we also found that both $\Delta plb1$ and $\Delta lac1$ had reduced in vivo growth in our zebrafish larvae cryptococcosis model; however, only $\Delta plb1$ -infected fish responded to exogenous PGE_2 or 15-keto- PGE_2 .⁵ We attribute $\Delta lac1$'s unresponsiveness to exogenous prostaglandin treatment to the fact that laccase is also responsible for aforementioned melanin synthesis—thus, it is possible that for this strain both melanin and PGE_2 are required for wild-type levels of growth—or an unknown defect that was responsible for it being much more attenuated in animal infection than the $\Delta plb1$ mutant. We attempted to circumvent this difference by disrupting the macrophage immune response but found that any immunocompromise of this response was critical to survival and confounded any differences.¹²

A major challenge we faced in our study was measuring eicosanoid levels during host-pathogen interactions and determining whether the eicosanoids measured were host or pathogen derived. A previous study by Shen and Liu¹³ found that pulmonary levels of PGE_2 increased in mice infected with *C. neoformans*; however, they were unable to attribute this to host or pathogen production. In our study, we performed experiments to measure differences in PGE_2 content between wild-type H99 and $\Delta plb1$ -infected macrophages using 2 different methods—ELISA (enzyme-linked immunosorbent assay) and LC MS/MS (liquid chromatography-tandem mass spectrometry). We found that J774 macrophages did produce detectable levels of PGE_2 ; however, we did not see any significant difference between infected or uninfected macrophages or between H99, $\Delta plb1$ and $\Delta plb1:PLB1$ -infected cells (although the concentrations detected with ELISA and LC MS/MS were very similar).⁵ This suggested that *Cryptococcus*-derived eicosanoids present during infection were likely to be contained within the macrophage.

Measurement of these small, localized eicosanoid levels within infected macrophages proved very difficult with current analytical methods—to our knowledge, intracellular levels of pathogen-derived eicosanoids have never been quantitatively measured before. To overcome this difficulty, we took a different approach; we used a co-infection assay which has previously been used to investigate the interaction of different *Cryptococcus gattii* strains within the same macrophage.¹⁴ We predicted that the parental cryptococcal strain produced growth-promoting eicosanoids but $\Delta plb1$ could not; the $\Delta plb1$ strain should display improved intracellular replication when H99 is also present within the same macrophage. Indeed, we found that $\Delta plb1$ proliferated better when accompanied by 2 wild-type yeast cells in the same macrophage as opposed to when 2 $\Delta plb1$ yeast cells were accompanied by 1 wild-type yeast cell. These experiments confirmed to us that *Cryptococcus* produced eicosanoids during macrophage infection and suggested that they did remain contained within the macrophage—important because it indicated that any host receptor targeted was likely to be intracellular.

Identifying a Mechanism

Our initial experiments indicated that PGE_2 was the eicosanoid species required for *Cryptococcus* growth because exogenous addition of this species was sufficient to rescue the growth defects of $\Delta plb1$ in J774 macrophages and zebrafish larvae. Intending to boost the observed effects of PGE_2 , we used a chemically altered version of PGE_2 called 16,16-dimethyl PGE_2 that cannot be metabolized.¹⁵ To our surprise, the opposite outcome occurred—16,16-dimethyl PGE_2 could no longer rescue the growth of $\Delta plb1$. Under physiological conditions, PGE_2 can be further converted to 15-keto- PGE_2 by the enzyme 15-hydroxy prostaglandin dehydrogenase (PGDH; Figure 1).¹⁶ We assumed that conversion from PGE_2 to 15-keto- PGE_2 could be a way for the host to mitigate the effects of *Cryptococcus*-derived (or exogenously added) PGE_2 . This was a eureka moment for our study because we realized that conversion of PGE_2 into 15-keto- PGE_2 was actually required for the growth of *Cryptococcus* and that if host eicosanoid signaling was being affected it was through a 15-keto- PGE_2 receptor rather than a PGE_2 receptor.

Our experiments had shown that *Cryptococcus*-derived 15-keto- PGE_2 promoted cryptococcal growth and that any host receptors involved were likely to be intracellular (Figure 1). While searching for putative receptors, we found that 15-keto- PGE_2 had been reported to be an agonist for peroxisome proliferator-activated receptor gamma (PPAR γ)—an intracellular receptor that is known to control inflammatory responses. PPAR γ is a cytosolic receptor that has a variety of ligands including many lipid eicosanoids. Ligand binding leads to the formation of a heterodimer between PPAR γ and retinoid X receptor (RXR). Following heterodimer formation, the PPAR γ /RXR complex translocates to the nucleus and acts as a transcription factor controlling the expression of genes which possess a peroxisome proliferator hormone response element.¹⁷

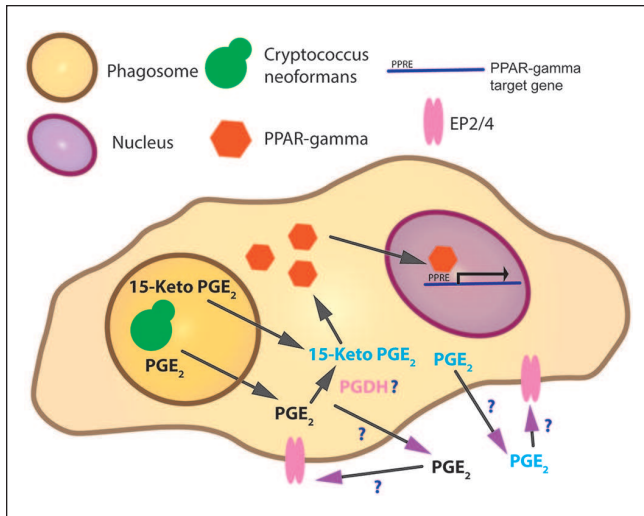


Figure 1. Evidenced and potential pathways of eicosanoid and PPAR-gamma interactions during *Cryptococcus* infection of macrophages.

Note. During infection, *Cryptococcus* resides within the phagosome. Prostaglandin E_2 or 15-keto- PGE_2 is produced by the fungus. Generation of 15-keto- PGE_2 is either by *Cryptococcus* or the host—or perhaps both. 15-Keto PGE_2 is a partial agonist to PPAR-gamma. PPAR-gamma is a cytosolic eicosanoid receptor; on ligand binding and activation, PPAR-gamma translocates to the nucleus where it binds to and activates genes with a PPRE target motif. In addition, prostaglandin E_2 may bind its extracellular receptors EP2/4 on macrophages or other cells. PPAR = peroxisome proliferator-activated receptor; PPRE = peroxisome proliferator hormone response elements; PGDH = 15-hydroxy prostaglandin dehydrogenase.

Through in vivo experiments with a transgenic zebrafish PPAR γ reporter, we found that 15-keto- PGE_2 was unable to activate the PPAR γ reporter itself; however, when 15-keto- PGE_2 was added in combination with a full PPAR γ agonist troglitazone, the level of PPAR γ activation was reduced compared with a troglitazone-only control. This indicated that 15-keto- PGE_2 could interact with PPAR γ in some capacity either as a partial agonist (a partial agonist is an agonist that binds to a receptor with a weak affinity and as a result does not fully activate the receptor) or an antagonist. To resolve this question, we proved that the effects of 15-keto- PGE_2 were reversed by a known PPAR γ antagonist. From these data, we concluded that 15-keto- PGE_2 is a partial agonist to PPAR γ , a finding that is supported by a previous study¹⁸ (Figure 1). Interestingly, we settled on this conclusion through interpretation of our data and it was only afterward that we became aware of other partial agonists against PPAR γ .^{19,20} The protein structure of PPAR γ has evolved to provide different binding sites for full and partial agonists within the PPAR γ ligand-binding domain (LBD)—full agonists bind to and stabilize the H12 alpha-helix of the LBD which produces a binding site for PPAR γ transactivators. In contrast, partial agonists do not interact with the H12 alpha-helix and as a result do not provide stabilization of this region but binding still produces PPAR γ activation to

varying magnitudes.²¹ Partial agonism is a mechanism that allows great flexibility in transcription factor function, rather than modulating the full gamut of PPAR γ -controlled genes, a partial agonist will only activate a subset of these genes. This means a receptor like PPAR γ can produce a variety of different transcriptional responses depending on the partial agonists present.

Future Perspectives

1. Where is PGE_2 metabolized into 15-keto- PGE_2 during infection? PGE_2 is quickly metabolized into 15-keto- PGE_2 in living cells (Figure 1). In higher organisms, this reaction is performed by PGDH. It is therefore possible that PGE_2 produced by *Cryptococcus* is metabolized into 15-keto- PGE_2 by the host. 15-keto- PGE_2 has been detected in the supernatant of *Cryptococcus* cultures so it is also likely that *Cryptococcus* possesses an enzyme similar in function to PGDH.
2. What is the effect of PPAR γ activation by 15-keto- PGE_2 on host cells—specifically host macrophages? We have found that 15-keto- PGE_2 is a partial agonist to PPAR γ ; this means that agonist binding only modulates a subset of PPAR γ -controlled genes (Figure 1). Identifying this subset in host cells will be essential to understand how 15-keto- PGE_2 enables *Cryptococcus* to cause infection.
3. Do other *Cryptococcus*-derived eicosanoids promote virulence? Our study has focused on PGE_2 /15-keto- PGE_2 production by *Cryptococcus*. We also tested PGD $_2$ but found this had no effect on infection. *Cryptococcus* produces many more eicosanoids which could have synergistic effects to PGE_2 /15-keto- PGE_2 or completely different effects. In view of our findings, future studies in this area should also consider metabolites which could be produced from *Cryptococcus* eicosanoids within the host.

Declaration of Conflicting Interests

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References

1. Gibson JF, Johnston SA. Immunity to *Cryptococcus neoformans* and *C. gattii* during cryptococcosis. *Fungal Genet Biol.* 2015;78:76-86.
2. Rudman J, Evans RJ, Johnston SA. Are macrophages the heroes or villains during cryptococcosis. *Fungal Genet Biol.* 2019;132:103261.
3. Voelz K, Lammas DA, May RC. Cytokine signaling regulates the outcome of intracellular macrophage parasitism by *Cryptococcus neoformans*. *Infect Immun.* 2009;77:3450-3457.
4. Wiesner DL, Specht CA, Lee CK, et al. Chitin recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcal infection. *PLoS Pathog.* 2015; 11(3):e1004701.
5. Evans RJ, Pline K, Loynes CA, et al. 15-keto-prostaglandin E2 activates host peroxisome proliferator-activated receptor gamma (PPAR-gamma) to promote *Cryptococcus neoformans* growth during infection. *PLoS Pathog.* 2019;15(3): e1007597.
6. Noverr MC, Erb-Downward JR, Huffnagle GB. Production of eicosanoids and other oxylipins by pathogenic eukaryotic microbes. *Clin Microbiol Rev.* 2003;16(3):517-533.
7. Erb-Downward JR, Noggle RM, Williamson PR, Huffnagle GB. The role of laccase in prostaglandin production by *Cryptococcus neoformans*. *Mol Microbiol.* 2008;68(6):1428-1437.
8. Cox GM, McDade HC, Chen SC, et al. Extracellular phospholipase activity is a virulence factor for *Cryptococcus neoformans*. *Mol Microbiol.* 2001;39(1):166-175.
9. Zhu X, Williamson PR. Role of laccase in the biology and virulence of *Cryptococcus neoformans*. *FEMS Yeast Res.* 2004;5(1):1-10.
10. Evans RJ, Li Z, Hughes WS, Djordjevic JT, Nielsen K, May RC. Cryptococcal phospholipase B1 is required for intracellular proliferation and control of titan cell morphology during macrophage infection. *Infect Immun.* 2015;83(4):1296-1304.
11. Sabiiti W, Robertson E, Beale MA, et al. Efficient phagocytosis and laccase activity affect the outcome of HIV-associated cryptococcosis. *J Clin Invest.* 2014;124:2000-2008.
12. Bojarczuk A, Miller KA, Hotham R, et al. *Cryptococcus neoformans* intracellular proliferation and capsule size determines early macrophage control of infection. *Sci Rep.* 2016;6:21489.
13. Shen L, Liu Y. Prostaglandin E2 blockade enhances the pulmonary anti-*Cryptococcus neoformans* immune reaction via the induction of TLR-4. *Int Immunopharmacol.* 2015;28(1):376-381.
14. Voelz K, Johnston SA, Smith LM, Hall RA, Idnurm A, May RC. "Division of labour" in response to host oxidative burst drives a fatal *Cryptococcus gattii* outbreak. *Nat Commun.* 2014;5:5194.
15. Ohno H, Morikawa Y, Hirata F. Studies on 15-hydroxyprostaglandin dehydrogenase with various prostaglandin analogues. *J Biochem.* 1978;84(6):1485-1494.
16. Tai HH, Ensor CM, Tong M, Zhou H, Yan F. Prostaglandin catabolizing enzymes. *Prostaglandins Other Lipid Mediat.* 2002;68-69:483-493.
17. Lemberger T, Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu Rev Cell Dev Biol.* 1996;12:335-363.
18. Chou WL, Chuang LM, Chou CC, et al. Identification of a novel prostaglandin reductase reveals the involvement of prostaglandin E2 catabolism in regulation of peroxisome proliferator-activated receptor gamma activation. *J Biol Chem.* 2007;282(25):18162-18172.
19. Atanasov AG, Wang JN, Gu SP, et al. Honokiol: a non-adipogenic PPARgamma agonist from nature. *Biochim Biophys Acta.* 2013;1830(10):4813-4819.
20. Bhalla K, Hwang BJ, Choi JH, et al. N-Acetylfarnesylcysteine is a novel class of peroxisome proliferator-activated receptor gamma ligand with partial and full agonist activity in vitro and in vivo. *J Biol Chem.* 2011;286(48):41626-41635.
21. Kroker AJ, Bruning JB. Review of the structural and dynamic mechanisms of PPARgamma partial agonism. *PPAR Res.* 2015;2015:816856.