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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 PGE<sub>2</sub> as the eicosanoid species responsible for this effect; however, we later found that a derivative of PGE<sub>2</sub>—15-keto-PGE<sub>2</sub>—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-PGE<sub>2</sub>, 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.<sup>1</sup> 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.<sup>2</sup> To kill *Cryptococcus*, macrophages must be activated by a Th1 CD4+ T-cell-mediated adaptive immune response (hence the prevalence in HIV/AIDS patients)<sup>3</sup>; 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.<sup>4</sup> Our hypothesis for this

study was that this manipulation might be mediated by eicosanoid species produced by the fungus.<sup>5</sup> *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.<sup>6</sup> 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.<sup>7,8</sup> 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*<sup>8</sup> and *LAC1*<sup>9</sup> 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<sub>2</sub> and its derivative 15-keto-PGE<sub>2</sub>, 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,<sup>10</sup> whereas laccase activity has been found to positively correlate with increased mortality in patients with HIV-associated cryptococcosis<sup>11</sup>—although how much this is due to PGE<sub>2</sub> 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<sub>2</sub>; 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<sub>2</sub> or 15-keto-PGE<sub>2</sub>.<sup>5</sup> 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<sub>2</sub> 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.<sup>12</sup>

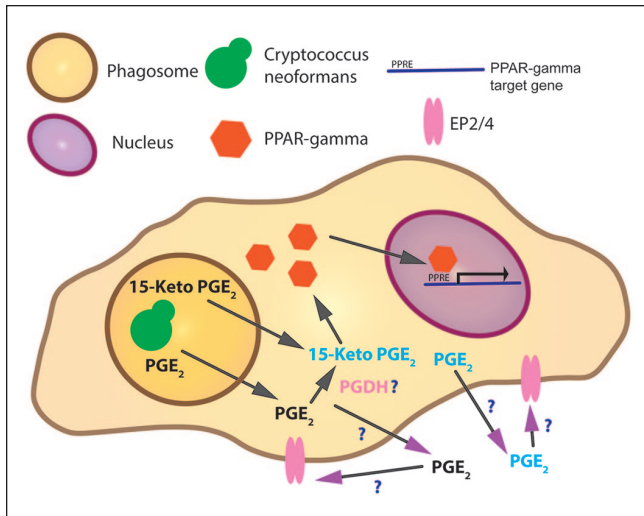
A major challenge we faced in our study was measuring eicosanoid levels during host-pathogen interactions and determining whether the eicosanoids measured were host or pathogen derived. A previous study by Shen and Liu<sup>13</sup> found that pulmonary levels of PGE<sub>2</sub> increased in mice infected 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<sub>2</sub> 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<sub>2</sub>; 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).<sup>5</sup> 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.<sup>14</sup> 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<sub>2</sub> 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<sub>2</sub>, we used a chemically altered version of PGE<sub>2</sub> called 16,16-dimethyl PGE<sub>2</sub> that cannot be metabolized.<sup>15</sup> To our surprise, the opposite outcome occurred—16,16-dimethyl PGE<sub>2</sub> could no longer rescue the growth of  $\Delta plb1$ . Under physiological conditions, PGE<sub>2</sub> can be further converted to 15-keto-PGE<sub>2</sub> by the enzyme 15-hydroxy prostaglandin dehydrogenase (PGDH; Figure 1).<sup>16</sup> We assumed that conversion from PGE<sub>2</sub> to 15-keto-PGE<sub>2</sub> could be a way for the host to mitigate the effects of *Cryptococcus*-derived (or exogenously added) PGE<sub>2</sub>. This was a eureka moment for our study because we realized that conversion of PGE<sub>2</sub> into 15-keto-PGE<sub>2</sub> was actually required for the growth of *Cryptococcus* and that if host eicosanoid signaling was being affected it was through a 15-keto-PGE<sub>2</sub> receptor rather than a PGE<sub>2</sub> receptor.

Our experiments had shown that *Cryptococcus*-derived 15-keto-PGE<sub>2</sub> 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<sub>2</sub> had been reported to be an agonist for peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ )—an intracellular receptor that is known to control inflammatory responses. PPAR $\gamma$  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 $\gamma$  and retinoid X receptor (RXR). Following heterodimer formation, the PPAR $\gamma$ /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.<sup>17</sup>



**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<sub>2</sub> or 15-keto-PGE<sub>2</sub> is produced by the fungus. Generation of 15-keto-PGE<sub>2</sub> is either by *Cryptococcus* or the host—or perhaps both. 15-Keto PGE<sub>2</sub> 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<sub>2</sub> 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 $\gamma$  reporter, we found that 15-keto-PGE<sub>2</sub> was unable to activate the PPAR $\gamma$  reporter itself; however, when 15-keto-PGE<sub>2</sub> was added in combination with a full PPAR $\gamma$  agonist troglitazone, the level of PPAR $\gamma$  activation was reduced compared with a troglitazone-only control. This indicated that 15-keto-PGE<sub>2</sub> could interact with PPAR $\gamma$  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<sub>2</sub> were reversed by a known PPAR $\gamma$  antagonist. From these data, we concluded that 15-keto-PGE<sub>2</sub> is a partial agonist to PPAR $\gamma$ , a finding that is supported by a previous study<sup>18</sup> (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 $\gamma$ .<sup>19,20</sup> The protein structure of PPAR $\gamma$  has evolved to provide different binding sites for full and partial agonists within the PPAR $\gamma$  ligand-binding domain (LBD)—full agonists bind to and stabilize the H12 alpha-helix of the LBD which produces a binding site for PPAR $\gamma$  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 $\gamma$  activation to

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

## Future Perspectives

1. Where is PGE<sub>2</sub> metabolized into 15-keto-PGE<sub>2</sub> during infection? PGE<sub>2</sub> is quickly metabolized into 15-keto-PGE<sub>2</sub> in living cells (Figure 1). In higher organisms, this reaction is performed by PGDH. It is therefore possible that PGE<sub>2</sub> produced by *Cryptococcus* is metabolized into 15-keto-PGE<sub>2</sub> by the host. 15-keto-PGE<sub>2</sub> 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 $\gamma$  activation by 15-keto-PGE<sub>2</sub> on host cells—specifically host macrophages? We have found that 15-keto-PGE<sub>2</sub> is a partial agonist to PPAR $\gamma$ ; this means that agonist binding only modulates a subset of PPAR $\gamma$ -controlled genes (Figure 1). Identifying this subset in host cells will be essential to understand how 15-keto-PGE<sub>2</sub> enables *Cryptococcus* to cause infection.
3. Do other *Cryptococcus*-derived eicosanoids promote virulence? Our study has focused on PGE<sub>2</sub>/15-keto-PGE<sub>2</sub> production by *Cryptococcus*. We also tested PGD<sub>2</sub> but found this had no effect on infection. *Cryptococcus* produces many more eicosanoids which could have synergistic effects to PGE<sub>2</sub>/15-keto-PGE<sub>2</sub> 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

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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