



Fungal Symbionts Produce Prostaglandin E₂ to Promote Their Intestinal Colonization

Tze Guan Tan*, Ying Shiang Lim, Alrina Tan, Royston Leong[†] and Norman Pavelka*

Singapore Immunology Network (SigN), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore

Candida albicans is a ubiquitous fungal symbiont that resides on diverse human barrier surfaces. Both mammalian and fungal cells can convert arachidonic acid into the lipid mediator, prostaglandin E₂ (PGE₂), but the physiological significance of fungus-derived PGE₂ remains elusive. Here we report that a *C. albicans* mutant deficient in PGE₂ production suffered a loss of competitive fitness in the murine gastrointestinal (GI) tract and that PGE₂ supplementation mitigated this fitness defect. Impaired fungal PGE₂ production affected neither the *in vitro* fitness of *C. albicans* nor hyphal morphogenesis and virulence in either systemic or mucosal infection models. Instead, fungal production of PGE₂ was associated with enhanced fungal survival within phagocytes. Consequently, ablation of colonic phagocytes abrogated the intra-GI fitness boost conferred by fungal PGE₂. These observations suggest that *C. albicans* has evolved the capacity to produce PGE₂ from arachidonic acid, a host-derived precursor, to promote its own colonization of the host gut. Analogous mechanisms might undergird host-microbe interactions of other symbiont fungi.

Keywords: prostaglandin E₂, *Candida albicans*, symbiont, host-microbe interactions, phagocyte, virulence, arachidonic acid

OPEN ACCESS

Edited by:

Robert T. Wheeler,
University of Maine, United States

Reviewed by:

Ana Traven,
Monash University, Australia
Carolina Henritta Pohl,
University of the Free State,
South Africa

*Correspondence:

Norman Pavelka
normanpavelka@gmail.com
Tze Guan Tan
tan_tze_guan@immunol.a-star.edu.sg

[†]deceased

Specialty section:

This article was submitted to
Fungal Pathogenesis,
a section of the journal
Frontiers in Cellular and Infection
Microbiology

Received: 24 August 2019

Accepted: 03 October 2019

Published: 18 October 2019

Citation:

Tan TG, Lim YS, Tan A, Leong R and
Pavelka N (2019) Fungal Symbionts
Produce Prostaglandin E₂ to Promote
Their Intestinal Colonization.
Front. Cell. Infect. Microbiol. 9:359.
doi: 10.3389/fcimb.2019.00359

INTRODUCTION

Candida albicans is one of the most successful fungal symbionts in humans, colonizing 40–80% of individuals in industrialized nations and typically representing the predominant species within the fungal microbiota (Odds, 1987; MacCallum, 2010). In healthy people, *C. albicans* dwells on diverse barrier sites of the body, including the oral cavity, skin, female reproductive tract, and the intestines, where it does not cause symptomatic disease (Odds, 1987; MacCallum, 2010). However, *C. albicans* can turn pathogenic and result in mucosal or life-threatening invasive bloodstream infections under a variety of conditions that compromise host immunity, damage barrier surfaces, or disrupt the microbiota (Brown et al., 2012; Fan et al., 2015). Molecular profiling studies have identified *C. albicans* strains in the gastrointestinal (GI) tract as the provenance of hematogenous isolates during systemic infection (Nucci and Anaissie, 2001; Odds et al., 2006). Understanding the fungal and host factors that modulate the ability of *C. albicans* to thrive in different host niches as a pathogen or symbiont could therefore inform our development of new methods of combatting human fungal infections.

Screens of *C. albicans* mutants in mice have revealed several fungal pathways that mediate colonization of the host gut. Salient pathways include the regulation of the acquisition of iron and nutrients, in particular carbon and nitrogen, cell wall remodeling that augments fungal adhesion to intestinal mucins, and morphogenetic changes (White et al., 2007; Rosenbach et al., 2010; Chen et al., 2011; Pande et al., 2013; Perez et al., 2013; Sem et al., 2016; Bohm et al., 2017; Mamouei et al., 2017). Fungal colonization is, in turn, restricted by the host immune system.

Intestinal epithelial cells (IECs) produce antimicrobial peptides (AMPs) that kill *C. albicans* (Fan et al., 2015), and gut-resident CX₃CR1⁺ macrophages control the growth of *C. albicans* and other symbiont fungi in mice (Leonardi et al., 2018). Accordingly, some of the aforementioned genetic programs in *C. albicans* might also promote intestinal symbiosis by calibrating the host immune response toward the fungus or *vice versa*. For instance, cell wall remodeling can mask the exposure of β -glucan, an essential component of fungal cell walls, to host immunological sensing via dectin-1 (Ballou et al., 2016; Pradhan et al., 2018), and recognition of surface β -glucan by dectin-1 is strongly correlated with the competitive fitness of *Candida* species in the murine intestine (Sem et al., 2016). In addition, *C. albicans* upregulates the key morphogenetic regulator, *EFG1*, upon colonizing the gut, and *EFG1* is required for *C. albicans* to overcome host immunological pressures to persist in the GI tract long-term (Pierce and Kumamoto, 2012).

Prostaglandin E₂ (PGE₂) is a lipid mediator produced by mammalian cells that exerts pleiotropic effects on physiology, in particular the modulation of immunological responses (Kalinski, 2012). For instance, PGE₂ suppresses the maturation of dendritic cells and restrains the phagocytic activity of monocytes, macrophages, and neutrophils (Kalinski, 2012; Medeiros et al., 2012). Depending on the tissue context, PGE₂ can either promote (Chizzolini et al., 2008; Yao et al., 2009; Gagliardi et al., 2010; Smekens et al., 2010) or suppress (Betz and Fox, 1991; Valdez et al., 2012; Ma et al., 2016) pro-inflammatory CD4⁺ T helper (Th)-1 and -17 responses, which help rein in *C. albicans* infections in the bloodstream and at barrier surfaces, respectively (Kashem et al., 2015).

Of note, many fungal species, including *C. albicans*, also produce PGE₂ *in vitro* when supplied exogenously with the host-derived precursor, arachidonic acid (AA) (Noverr et al., 2002). *C. albicans* and most fungal species lack AA, which is derived from mammalian membrane phospholipids—but *C. albicans* can induce host cells to release AA (Castro et al., 1994). Moreover, fungus-derived PGE₂ exhibits immunomodulatory properties analogous to those of mammalian PGE₂ *in vitro* (Noverr et al., 2001) and *in vivo* (Kim et al., 2014). PGE₂ has also been reported to promote yeast-hyphae morphological switching (Kalo-Klein and Witkin, 1990; Noverr et al., 2001) and biofilm formation *in vitro* (Alem and Douglas, 2004)—traits commonly associated with fungal virulence. Given that the fungus is more typically associated with the host as a benign symbiont and inter-individual transmission often occurs in the absence of overt infection (Russell and Lay, 1973; Odds et al., 2006), it seems unlikely, however, that *C. albicans* has evolved the cellular machinery for synthesizing PGE₂ from an exogenous substrate for the sole purpose to increase its pathogenic potential or to cause immune-related disorders in the host. We thus posit that fungus-derived PGE₂ might mediate asymptomatic colonization by *C. albicans* of the mammalian intestinal environment. Indeed, we found that a *C. albicans* mutant with impaired PGE₂ production, while not exhibiting any defects in *in vitro* fitness or *in vivo* virulence, suffered a loss of competitive fitness relative to the wildtype (WT) strain in the murine intestine. We further report evidence suggesting that

fungal PGE₂ promotes intestinal symbiosis by *C. albicans* in part by reducing its killing by phagocytes.

MATERIALS AND METHODS

Fungal Strains

Fungal strains used in this study are listed in **Table S1**. All fungal strains were grown in yeast peptone dextrose (YPD) media [1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose] overnight at 30°C prior to use in experiments unless otherwise stated.

Construction of Fungal Strains

Fungal mutants were generated using the *SAT1*-flipping strategy as previously described (Sasse and Morschhauser, 2012; Sem et al., 2016). Briefly, the plasmid pSFS2 containing the *SAT1* flipper cassette was used to disrupt the open reading frame of the *OLE2*, *FET3*, and *FET31* genes of *C. albicans*. Sequences (500–600 bp) flanking the targeted genes were amplified using the primers listed in **Table S2**. Gene deletion was verified by quantitative polymerase chain reaction (qPCR) using the primers listed in **Table S2**.

Mice and Treatments

C57BL/6J and *Ccr2*-KO (B6.129S4-*Ccr2*^{tm1lf/j}) mice were maintained under specific-pathogen-free conditions at the A*STAR Biological Resource Centre (BRC) in Biopolis, Singapore. Adult mice (6–8 weeks of age) of either sex were used in all experiments. All animal experiments were approved by the BRC Institutional Animal Care and Use Committee (IACUC) in accordance with the guidelines of the Agri-Food & Veterinary Authority (AVA) and the National Advisory Committee for Laboratory Animal Research (NACLAR), Singapore. To deplete phagocytes, mice were injected intraperitoneally (i.p.) with control or clodronate-containing liposomes (Liposoma B.V.) every 2 days starting from 2 days prior to the inoculation of mice with fungi. To perform PGE₂ supplementation, mice were injected i.p. with 16.7 μ g/kg of 16,16-dimethyl-PGE₂ (Cayman Chemical) or vehicle [methyl acetate (Sigma)] daily, starting from day 0, throughout the course of fungal colonization.

Measurement of Fungal PGE₂ Production

Fungal PGE₂ production was measured by enzyme-linked immunosorbent assay (ELISA) as previously described (Yano and Fidel, 2011). Briefly, fungi were inoculated into fresh YPD to an optical density (OD) at 600 nm of 0.2 and grown at 37°C with shaking at 250 rpm for 2–2.5 h before peroxide-free arachidonic acid (Cayman Chemical) was added to a final concentration of 0.5 mM. Fungal growth was continued for another 48 h before supernatants were harvested. Levels of PGE₂ metabolite (PGEM), a stable derivative of PGE₂, in fungal supernatants were assayed using the PGEM EIA kit (Cayman Chemical) as per manufacturer instructions.

Quantification of Fungal Titers

Fungal titers for all experiments were determined by plating fungal cultures, tissue homogenates, or lavage fluids on YPD

agar containing 50 U/ml penicillin (Gibco) and 50 µg/ml streptomycin (Gibco). Plates were incubated at 37°C for 24–48 h and colony-forming-units (CFUs) enumerated.

Murine Infection With *C. albicans*

To elicit systemic candidiasis, mice were injected intravenously via the tail vein with a sublethal dose of *C. albicans* (2×10^5 cells). Oropharyngeal candidiasis (OPC) was established as previously described (Solis and Filler, 2012). Briefly, mice were injected subcutaneously (s.c.) with 100 mg/kg cortisone 21-acetate (Sigma) in 0.05% (v/v) Tween[®]-80 (Sigma)/phosphate buffered saline (PBS) 1 day prior to infection to induce transient immunosuppression. Mice were subsequently sedated with 150 mg/kg ketamine and 10 mg/kg xylazine before sublingual inoculation with a small cotton ball saturated with *C. albicans* cells (2×10^7 cells/ml) for 75 min. Vulvovaginal candidiasis (VVC) was established as previously described (Yano and Fidel, 2011). Briefly, female mice were treated s.c. with 0.1 mg of β-estradiol 17-valerate (Sigma) in sesame oil (Sigma) 3 days prior to infection. β-estradiol injections were maintained weekly thereafter. Mice were intravaginally inoculated with 5×10^4 *C. albicans* cells in 20 µl of PBS. Infected mice were monitored daily (systemic candidiasis and OPC) for survival and weight loss. Tongues from mice with OPC were homogenized by pressing the tissues through a 40 µm cell strainer. Homogenates were plated to assess fungal burden. Mice with VVC were intravaginally lavaged with 100 µl of PBS. Vaginal lavage fluid was plated to determine fungal burden or used for neutrophil quantification by flow cytometry as described below under “Antibodies and flow cytometry.”

Intestinal Colonization With *C. albicans*

Three to four days prior to inoculation, mice were placed on drinking water containing 1 g/l of penicillin G (Sigma) and 2 g/l of streptomycin (Sigma) (Sem et al., 2016). Antibiotics were changed twice weekly and maintained throughout the course of the experiment. Mice were gavaged with 10^7 *C. albicans* cells to establish intestinal colonization. To determine the intestinal fungal load, stools were collected, weighed, homogenized in PBS, and plated.

In vivo Intestinal Competition

Intestinal fungal competition experiments were performed as previously described (Sem et al., 2016). Briefly, mice were gavaged with 2×10^7 cells comprising the SC5314-dTomato strain and the test strain (WT or *ole2/ole2*) at a 1:1 ratio. The relative frequency of the test strain at each time point was determined by counting the number of fluorescent vs. non-fluorescent colonies under a fluorescence stereomicroscope (Olympus). The fitness coefficient of each test strain in each mouse was calculated as previously described (Sem et al., 2016). To control for inter-experimental variability, fitness coefficients were normalized to the mean of the coefficients for the WT strain within each treatment group (e.g., clodronate-treated group) for each experiment. Normalization was performed by calculating the difference

between each fitness coefficient value and the mean for the WT strain.

In vitro Competition

In vitro competition experiments were performed as previously described (Sem et al., 2016). Briefly, 1.25×10^6 cells comprising the SC5314-dTomato strain and the test strain (WT or *ole2/ole2*) at a 1:1 ratio were inoculated into 50 ml of media [YPD or yeast nitrogen base (YNB) (BD Difco) with amino acids] containing 0.5 mM of arachidonic acid or not and grown at 37°C. Every 8 or 16 h, fungal cultures were harvested, plated, and 1.25×10^6 cells re-inoculated into 50 ml of fresh media as described above. Relative proportions and fitness coefficients of each test strain were derived as described above.

Infection of Macrophages With *C. albicans*

Fungal resistance to macrophages at 24 h was determined as previously described (Sem et al., 2016). Briefly, confluent J774A.1 macrophages (ATCC) were infected with *C. albicans* cells at low multiplicities of infection (MOIs) ranging from 0.005 to 0.0003125 to ensure that essentially 100% of fungi were engulfed by the macrophages at 3 h post-infection. Infected macrophages were incubated at 37°C and fungal colonies enumerated after 24 h. Fungi were also seeded in wells without macrophages at varying densities to assess the number of CFUs plated in each experiment. The percentage of fungal cells that escaped killing by macrophages was calculated as previously described (Noble and Johnson, 2005). For short-term fungal killing assays, J774A.1 macrophages were infected at a MOI of 0.1. Extracellular fungi were removed 1 h post-infection by gently washing the macrophages with PBS. Macrophages were lysed with 0.02% Triton X-100 after an additional 2 h of incubation at 37°C and cell lysates plated on YPD to enumerate the intramacrophage fungal load. Where indicated, 100 nM of PGE₂ (Cayman Chemical) or the corresponding volume of vehicle (ethanol) was added to the cultures.

Filamentation Assays

Filamentation of *C. albicans* strains was assessed as previously described (Sem et al., 2016). Briefly, *C. albicans* cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS) at 37°C in 96-well plates for 24 h, or plated on Spider agar (1% nutrient broth, 1.35% agar, 0.4% potassium phosphate, 2% mannitol) at 37°C for 4 days before colonies were scored for their morphology, i.e., wrinkled vs. smooth, indicative of filamentous vs. non-filamentous cells, respectively. Arachidonic acid was added at 0.5 mM where indicated.

In vitro Cytotoxicity Assay

Confluent J774A.1 macrophages were infected with *C. albicans* cells at a MOI of 1 for 6 h. LDH released into the culture media was measured using the CytoTox 96[®] Non-Radioactive Cytotoxicity Assay (Promega) as per kit instructions and normalized to LDH levels in uninfected cells lysed with 1% Triton X-100 (Sigma).

Measurement of LL-37 Production From IECs

Confluent human colonic epithelial cell lines HT-29 and Caco-2 were infected with *C. albicans* cells at a MOI of 0.5 for 24 h. LL-37 secreted into the culture media was measured by the LL-37 ELISA kit (Cusabio) as per manufacturer instructions.

Assessment of Fungal Resistance to mCRAMP

Fungal resistance to mCRAMP was evaluated as previously described (Sem et al., 2016). Briefly, overnight cultures of *C. albicans* were inoculated into fresh YPD in the presence of 400 nM PGE₂ (Cayman Chemical) or vehicle (ethanol) for 3 h. Cells were washed, resuspended in the appropriate concentration of mCRAMP_{1–39} (Synpeptide), and incubated at 37°C for 2 h. Cells were washed extensively after mCRAMP exposure and stained with 50 μM of propidium iodide (PI) (Thermo Fisher Scientific) for 15 min. The proportion of live PI⁻ fungal cells was determined by flow cytometry.

Measurement of Transcript Levels by Quantitative PCR

RNA was extracted from a small segment of the distal colon harvested from *C. albicans*-colonized mice 7 days after inoculation. For *in vitro* infection experiments, J774A.1 macrophages were infected with fungi at the indicated MOIs and the macrophages lysed with TRIzol™ (Invitrogen) 6 h post-infection. Both colon and macrophage samples were homogenized in TRIzol™ using 0.5 mm glass beads (Sigma) and RNA extracted as per manufacturer instructions. cDNA was prepared from RNA using SuperScript™ III reverse transcriptase (Invitrogen) as per manufacturer instructions. Levels of transcripts of interest in cDNA were measured by qPCR using PerfeCTa SYBR® Green FastMix (Quantabio) and normalized to those of *Rpl13a*.

Isolation of Colonic Lamina Propria Cells

To remove the epithelial layer, colons were sliced open longitudinally to expose the luminal surface and shaken in DMEM containing 1 mM dithiothreitol, 5 mM EDTA, and 3% (v/v) FCS for 20 min at 37°C. Remaining tissue was washed in cold DMEM, minced, and digested in DMEM containing 1.5 mg/ml collagenase D (Roche), 50 μg/ml DNase I (Roche), and 2% (v/v) FCS for 45 min at 37 °C. Digested tissue was washed and filtered twice to obtain a single-cell suspension.

Antibodies and Flow Cytometry

Mammalian cells were stained with antibodies against CD3ε (145-2C11), CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), CD11c (N418), CD19 (6D5), CD45 (30F11), CD64 (X54-5/7.1), I-A/E (M5/114.15.2), Ly6G (1A8) from Biolegend and 4',6-diamidino-2-phenylindole (DAPI) (Thermo Fisher Scientific) to exclude dead cells. All immunocyte subsets were gated on live leukocytes (DAPI⁻CD45⁺). Macrophages, DCs, B cells, CD4⁺ T cells, and CD8⁺ T cells from the colonic lamina propria were defined as CD64⁺CD11b⁺CD11c⁺I-A/E⁺, CD64⁻CD11c⁺I-A/E⁺, CD3ε⁻CD19⁺, CD3ε⁺CD19⁻CD4⁺,

and CD3ε⁺CD19⁻CD8α⁺, respectively. Intravaginal neutrophils were defined as CD11b⁺Ly6G⁺ cells. Samples were acquired on the MACSQuant® VYB (Miltenyi Biotec) and analyzed using FlowJo v10.2 (Tree Star).

Statistics

Pairwise comparisons were performed using the Student's *t*-test or the paired Student's *t*-test, as appropriate, while multi-group comparisons were performed using the Two-way Analysis of Variance (ANOVA) with Sidak's multiple comparisons test (Prism 7; Graph-Pad), unless otherwise stated. *P*-values were deemed significant if <0.05. Data routinely presented as mean ± standard deviation (s.d.) unless otherwise stated.

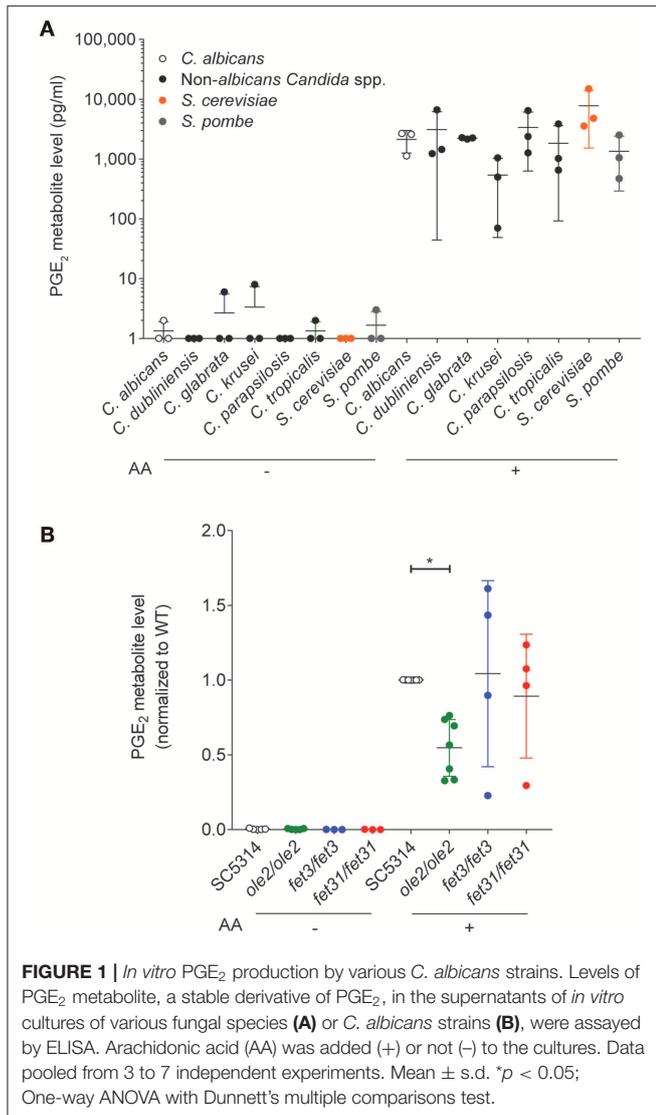
RESULTS

PGE₂-Defective *C. albicans* Mutant Exhibits No Fitness Defects *in vitro*

PGE₂ production has been described for *C. albicans* and other pathogenic fungi such as *Cryptococcus neoformans* and *Aspergillus* species (Noverr et al., 2002) as well as for some gut-dwelling symbiont *Candida* species (Shiraki et al., 2008; Ells et al., 2011; Mishra et al., 2014; Grozer et al., 2015). We measured *in vitro* PGE₂ production across an array of symbiont fungi and confirmed that all species tested, including *Saccharomyces cerevisiae*, secreted PGE₂ when supplied with exogenous AA (Figure 1A), implying a PGE₂ biosynthetic pathway conserved across the majority of fungal species.

We next sought a *C. albicans* mutant with altered PGE₂ production which we could use to study the physiological function of fungus-derived PGE₂. Although the PGE₂ biosynthetic pathway in *C. albicans* and other fungi remains largely uncharacterized, three genes, *OLE2*, *FET3*, and *FET31*, have been described to promote PGE₂ production *in vitro* (Erb-Downward and Noverr, 2007; Krause et al., 2015). We generated knockout (KO) mutants for each of these genes and assessed the mutants' capacities for PGE₂ secretion *in vitro* at 37°C. As expected, PGE₂ production by all fungal strains was strictly dependent on the provision of exogenous AA (Figure 1B). However, only the *ole2/ole2* mutant exhibited reproducibly diminished PGE₂ secretion. Concordant with a previous report (Erb-Downward and Noverr, 2007), PGE₂ production was not completely abrogated by *OLE2* ablation, implying the existence of alternative pathways to PGE₂ generation by *C. albicans*. We thus focused on the *ole2/ole2* mutant for all subsequent experiments.

To determine whether fungal PGE₂ modifies fungal fitness *in vitro*, we maintained either the WT or the *ole2/ole2* strain in competition with a fluorescently tagged WT strain (SC5314-dTomato) in serially passaged liquid batch cultures. The abundance of each strain relative to that of SC5314-dTomato was determined at regular intervals by plating the cultures on yeast peptone dextrose (YPD) agar followed by scoring of fluorescent and non-fluorescent colonies. The fitness of each strain can in turn be rigorously quantified by deriving a fitness coefficient from the rate of change of the relative abundance of each strain



(Sem et al., 2016). Even after 80 h [equivalent to ~44 and 63 generations for growth in YNB media and YPD, respectively] of serial subculturing, we detected no differences in competitive fitness between the *ole2/ole2* and WT strains in both the nutrient-rich YPD broth and the nutrient-poor YNB media, whether or not exogenous AA was supplied (Figure 2, Figure S1). Therefore, fungal PGE₂ does not appear to be important for fitness *in vitro*.

Fungal PGE₂ Is Not Required for Virulence

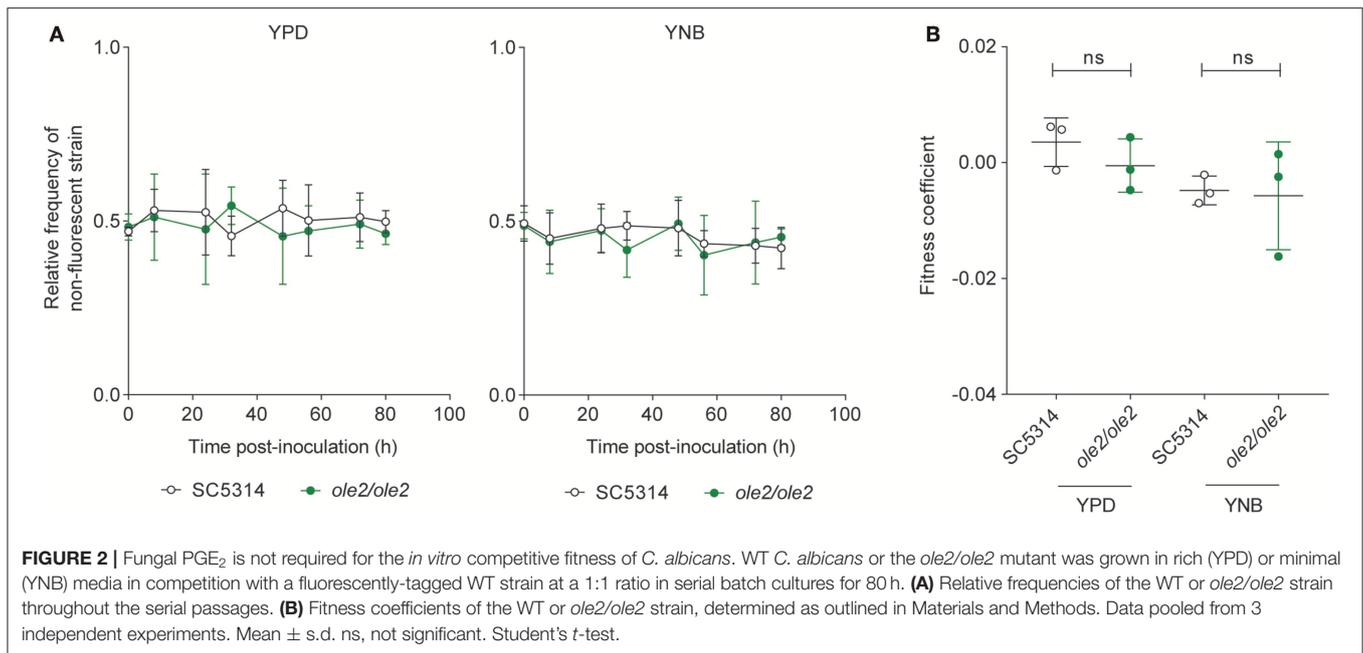
Since PGE₂ was reported to increase filamentation and biofilm formation *in vitro* (Noverr et al., 2001; Alem and Douglas, 2004; Kim et al., 2014), we asked whether defective PGE₂ synthesis by the *ole2/ole2* mutant influences its virulence. Surprisingly, the *ole2/ole2* mutant was fully capable of hyphal formation *in vitro*, both in response to fetal calf serum (FCS) and when plated on Spider agar, regardless of the presence of AA (Figures 3A,B).

Moreover, the ability of *C. albicans* to damage host cells—whose cell membranes are an abundant source of endogenous AA (Martin et al., 2016)—evidenced by the release of lactate dehydrogenase from infected murine macrophages, was not significantly altered by fungal PGE₂ (Figure 3C). Nonetheless, there exist occasional discrepancies between *in vivo* virulence and *in vitro* pathogenic traits (Noble et al., 2010). To clarify the role of fungus-derived PGE₂ during *in vivo* infections, we adopted murine models of hematogenously disseminated candidiasis and mucosal candidiasis of the oropharyngeal cavity and vaginal tract, all of which engage distinct anti-fungal immunological responses (Fidel, 2005; Conti et al., 2009; Kashem et al., 2015). The *ole2/ole2* mutant manifested comparable virulence as the WT strain in all tissues tested, across a variety of pathological parameters, including overall survival, fungal burden, weight loss, and neutrophil recruitment (Figures 3D–F, Figure S2). Our *in vitro* and *in vivo* infection data are hence not consistent with a role for fungal PGE₂ in mediating *C. albicans* virulence.

Fungal PGE₂ Promotes Intestinal Colonization

C. albicans is most commonly associated with the host as a symbiont in the gut. We therefore sought to evaluate whether fungal PGE₂ contributes to the fitness of *C. albicans* in the intestines. Mice are usually refractory to colonization by *C. albicans* but can be induced to harbor high titers of fungi for sustained durations if treated with antibiotics to deplete their indigenous microbiota (Koh et al., 2008; Fan et al., 2015). Colonization of the murine GI tract with *C. albicans* represents a *bona fide* model for fungal symbiosis with the mammalian host because it does not lead to symptomatic disease and invasive hyphae are rarely detected within the intestinal fungal population (White et al., 2007; Koh et al., 2008; Bohm et al., 2017).

To assess the intestinal fitness of the *ole2/ole2* mutant, we inoculated antibiotics-treated mice with either *ole2/ole2* or WT *C. albicans* in competition with SC5314-dTomato and tracked the relative frequency of each strain over time. The *ole2/ole2* mutant was significantly compromised in its competitive fitness relative to the WT strain, starting as early as 3 days post-inoculation, as evidenced by its lower relative frequency in the fecal fungal load and its lower fitness coefficient, determined as per our *in vitro* competitive fitness experiments (Figure 4A, Figure S3A). Although we were not able to test a reconstituted *ole2/ole2::OLE2* mutant, colonization of mice with another independently-derived *ole2/ole2* strain recapitulated the inferior competitive fitness of *C. albicans* in the GI tract in the absence of *OLE2* (Figures S3B,C). While we consistently observed a lower fitness coefficient for the *ole2/ole2* mutant relative to WT *C. albicans* across independent experiments, the fitness coefficient differential between the two strains varied in magnitude between experiments (Figure S3), possibly due to variation across different cohorts of mice. To facilitate a comparison of fungal fitness across pooled groups of mice, we normalized fitness coefficients to the mean coefficient of the WT strain within each experiment, thereby removing the batch



effects on fungal fitness. This analysis confirmed a significant impairment in the competitive fitness of the PGE₂-deficient *ole2/ole2* mutant in the murine gut (Figure 4B).

To determine if the loss of competitive fitness of the *ole2/ole2* strain in the gut is PGE₂-dependent, we supplemented *C. albicans*-colonized mice daily with 16,16-dimethyl-PGE₂ (dmPGE₂), a stable analog of PGE₂. Indeed, dmPGE₂ treatment partially restored the competitive fitness of the *ole2/ole2* mutant (Figures 4C,D, Figure S3D), in support of a role for fungal PGE₂ in mediating intestinal fitness of *C. albicans* under steady-state conditions. In contrast, dmPGE₂ provision did not further enhance the competitive fitness of the WT strain. Taken together, these data indicate that fungal PGE₂ exerts a specific function for *C. albicans*: promote its colonization of the host GI tract.

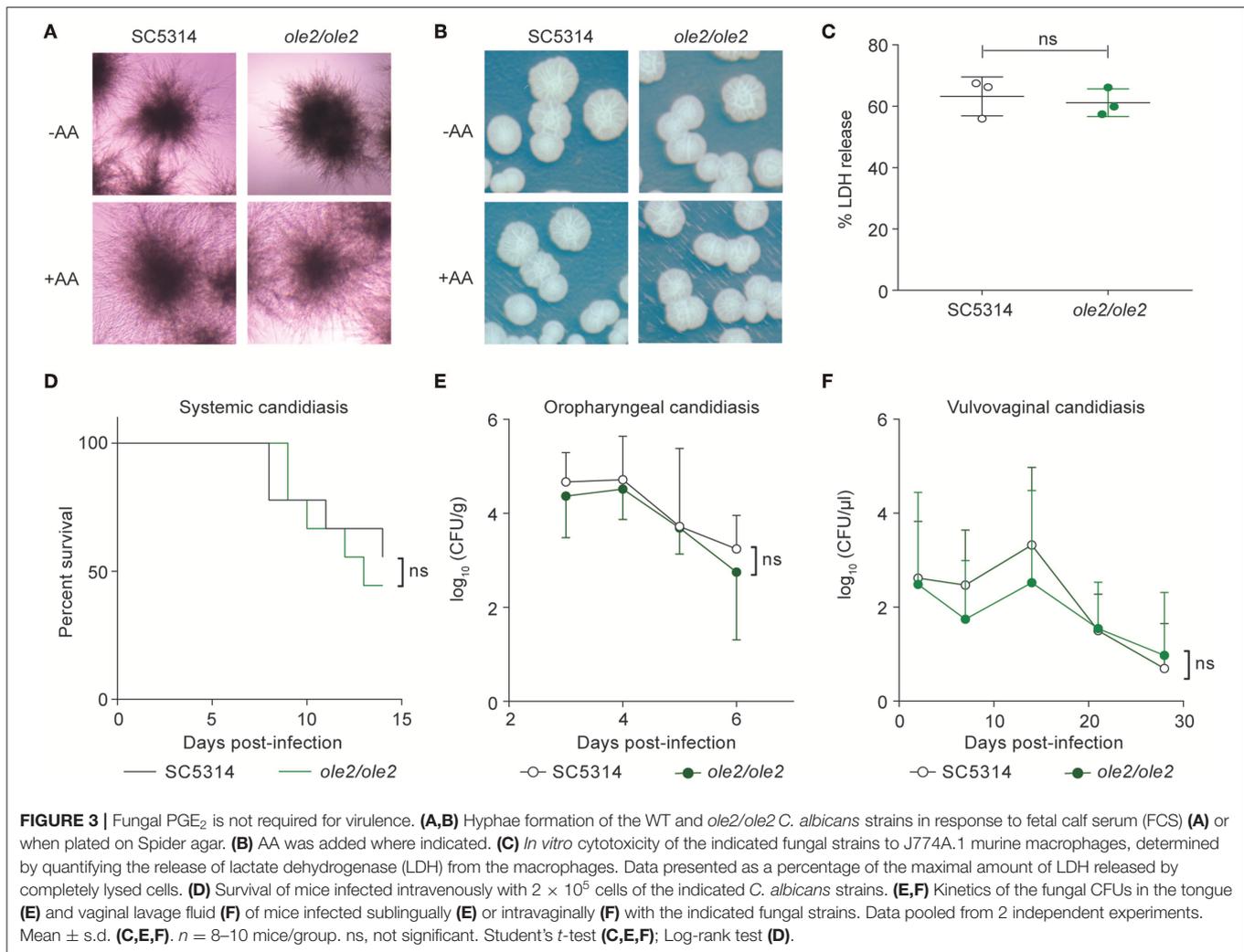
Fungal PGE₂ Does Not Affect AMP Production or Fungal Resistance to AMPs

AMPs such as mouse cathelicidin-related antimicrobial peptide (mCRAMP), encoded by *Camp*, have been demonstrated to mediate intestinal resistance to *C. albicans* colonization (Fan et al., 2015). We postulated that fungal PGE₂ might promote intestinal fitness of *C. albicans* by influencing AMP production from IECs or fungal resistance to AMPs. To address the former hypothesis, we stimulated human colonic IEC lines with WT *C. albicans* or the *ole2/ole2* mutant but observed no difference in the secretion of LL-37, the human ortholog of mCRAMP, by the IEC lines (Figure S4A). Indeed, infection of the IEC lines by fungi *in vitro* did not induce appreciable production of LL-37 above the background level. We also assessed *Camp* transcript levels in the colons of mice colonized with either WT or *ole2/ole2* *C. albicans* but found no difference in the quantities of *Camp* mRNA induced by the two fungal strains

in vivo (Figure S4B). Finally, we exposed fungi grown in the presence or absence of PGE₂ to mCRAMP *in vitro* to evaluate the effects of PGE₂ on fungal resistance to AMPs. Both the WT and *ole2/ole2* strains manifested comparable susceptibility to mCRAMP, and the addition of PGE₂ did not significantly alter fungal resistance to mCRAMP (Figure S4C). Hence the collective evidence does not support a role for fungus-derived PGE₂ in altering AMP production from IECs or fungal resistance to AMPs.

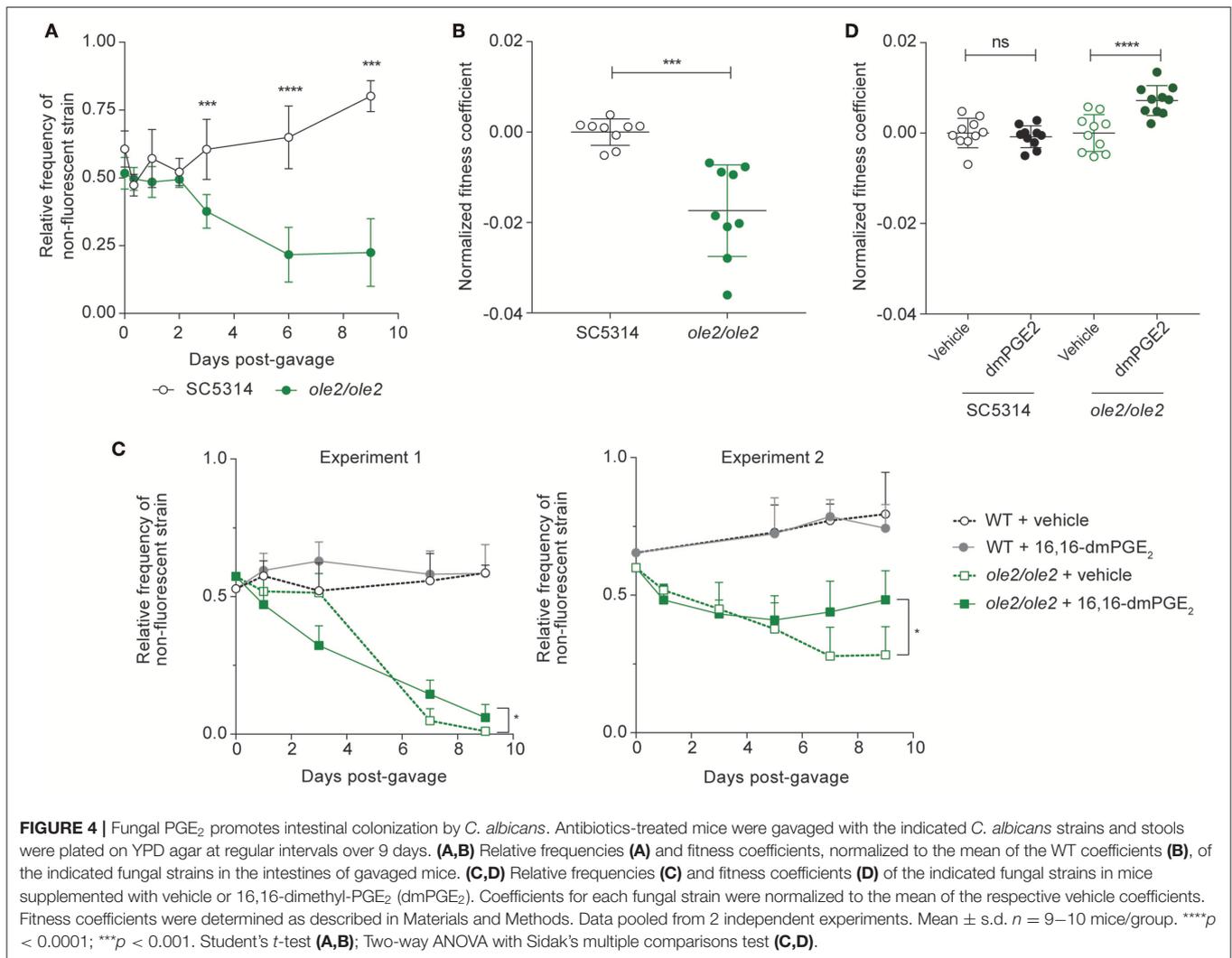
Fungus-Derived PGE₂ Reduces Killing of *C. albicans* by Intestinal Phagocytes

Since intestinal macrophages limit fungal colonization of the gut (Leonardi et al., 2018) and tissue-resident phagocytes are required for control of fungal infection in various tissues (Kashem et al., 2015; Dominguez-Andres et al., 2017; Sparber et al., 2018), we postulated that fungal PGE₂ might potentiate fungal fitness by acting on the fungi themselves and/or host tissue phagocytes to improve the ability of *C. albicans* to evade killing by phagocytes. To elucidate the relationship between fungal PGE₂, tissular phagocytes, and fungal fitness in the host, we assessed the competitive fitness of the WT and *ole2/ole2* strains in mice depleted of phagocytes. We first treated mice with clodronate-containing liposomes, which is toxic to a variety of phagocytic leukocytes, including macrophages and dendritic cells (DCs) (Weisser et al., 2012). In our experiments, clodronate treatment depleted colonic CD11b⁺ dendritic cells (DCs) but not macrophages, CD11b⁻ DCs, or non-phagocytic leukocytes (Figures S5A,B). The poor efficiency of macrophage ablation in clodronate-treated mice could possibly arise from differential accessibility and susceptibility of macrophages in the colon to intraperitoneally-administered clodronate relative to those from other tissue sites. Nonetheless, clodronate treatment was



sufficient to abrogate the fitness defect of the *ole2/ole2* mutant vis-à-vis the WT strain that was observed in control mice, thereby implicating CD11b⁺ DCs as another tissue-resident phagocytic population that restricts *C. albicans* in the gut (Figures 5A,B, Figure S6A). We further validated the preceding results by repeating the fungal competitive colonization experiment in a genetic model of phagocyte depletion. CCR2 is a chemokine receptor required for efficient reconstitution of the intestinal macrophage and CD11b⁺ DC compartments (Bain et al., 2014; Scott et al., 2015). Consequently, *Ccr2*-KO mice display a paucity of both macrophages and CD11b⁺ DCs but not CD11b⁻ DCs or lymphocytes in the colon (Figures S5C,D). In agreement with our findings in clodronate-treated mice, *Ccr2* deficiency abolished the fitness difference between the WT and *ole2/ole2* strains observed in WT mice (Figures 5C,D, Figure S6B). Our results collectively support a role for fungal PGE₂ in reducing the eradication of *C. albicans* by host intestinal phagocytes, including macrophages and CD11b⁺ DCs. We thus sought to compare the *ex vivo* fungicidal activity of host phagocytes toward WT and *ole2/ole2* *C. albicans*. Owing to difficulties in isolating

sufficient numbers of primary macrophages or DCs from the murine gut for this purpose, we turned to a well-described murine macrophage line J774A.1 and measured fungal killing by J774A.1 cells upon *C. albicans* infection *in vitro* using a previously reported method (Sem et al., 2016). Indeed, the *ole2/ole2* mutant exhibited reduced resistance to macrophages relative to the WT strain at 24 h post-infection but not at 3 h (Figure 5E, Figures S7A,B), and this defect in resistance to host killing can be reversed by the provision of exogenous PGE₂ (Figure 5F). We reasoned that the effects of fungus-derived PGE₂ on fungal killing at 24 h post-infection might require the transcriptional reprogramming of macrophages, as previously described (Soram et al., 2013; Kim et al., 2014; Yun et al., 2016). However, we did not observe a difference in the levels of transcripts associated with pro-inflammatory (*Il1b*) and immunosuppressive (*Arg1*, *Chil3*, *Retnla*, *Il10*) macrophage subsets between macrophages infected with WT or *ole2/ole2* *C. albicans* (Figure S7C). Overall, these results suggest that fungal PGE₂ confers *C. albicans* with a competitive advantage in the mammalian GI tract by protecting the fungi from the microbicidal activity of intestinal phagocytes.

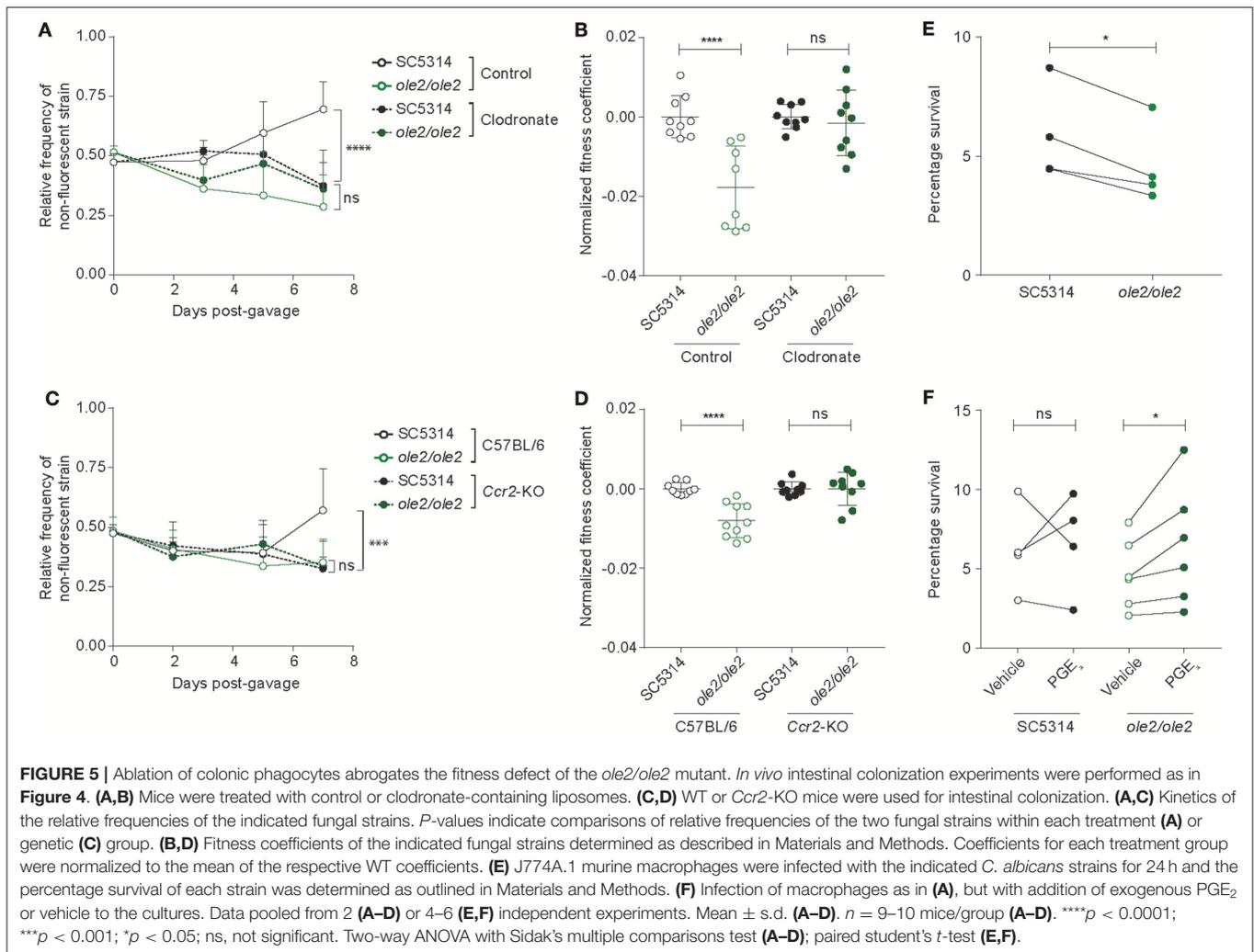


DISCUSSION

In this study, we sought to clarify the physiological function of PGE₂ production by *C. albicans*, a phenomenon long described (Noverr et al., 2001) but of unclear importance to either host or fungus. Using a PGE₂-deficient fungal mutant, we found no contribution of fungal PGE₂ to the *in vitro* and *in vivo* virulence of *C. albicans* across disparate murine models of candidiasis that affect different organs and engage distinct antifungal immunological responses. Instead, we uncovered a specific role for PGE₂ production by *C. albicans* in potentiating fungal colonization of the murine intestine. Interestingly, the effects of fungal PGE₂ appear to be linked to protection from killing by host phagocytes. Our results raise several questions that merit further elaboration.

How has *C. albicans* evolved the ability to synthesize PGE₂ from AA, a substrate that it lacks? No fungal orthologs of the mammalian biosynthetic enzymes for PGE₂—the cyclooxygenases, COX-1 and COX-2, and the PGE₂ synthase, PTGES—have been identified to date (Erb-Downward and

Noverr, 2007; Erb-Downward et al., 2008). Even using a sensitive method based on profile hidden Markov models (HMMER v.3) failed to identify distant homologs of the mammalian PGE₂ biosynthetic enzymes (F. L. Sirota & S. Maurer-Stroh, unpublished observation). In addition, the three genes identified to contribute to PGE₂ synthesis in *C. albicans*—*OLE2*, *FET3*, and *FET31*—do not harbor enzymatic domains that are known to bind to or act on AA or downstream prostaglandins. Indeed, *FET3* and *FET31* are putative iron transporters in *C. albicans* and might instead modulate fungal PGE₂ production indirectly, possibly by acting as cofactors or regulating cellular levels of iron that in turn impact PGE₂ synthesis. Genetic deficiency of *OLE2* in *C. albicans* did not completely eliminate PGE₂ production *in vitro* (Erb-Downward and Noverr, 2007; **Figure 1**), implying the existence of additional components of the fungal PGE₂ synthetic pathway. It is possible that the impact of *OLE2* on fungal PGE₂ production in the gut might be more profound than that observed *in vitro*, though we have not been able to address this empirically due to difficulties in precisely measuring PGE₂ levels in intestinal contents, which contain high levels of



substances that interfere with ELISA data linearity (data not shown). Thus, *C. albicans* has convergently evolved a mechanism to generate PGE₂ from a host-derived precursor, presumably in response to evolutionary pressures exerted by its lifestyle as an obligate symbiont in warm-blooded organisms.

Why does *C. albicans* go through the effort of producing PGE₂ at all? Earlier studies have implicated fungus-derived PGE₂ in pathogenesis, since PGE₂ promotes virulence traits such as germ tube and biofilm formation *in vitro* (Noverr et al., 2001; Alem and Douglas, 2004). Moreover, fungal PGE₂ can suppress host immunity, which might in turn favor fungal growth. For instance, PGE₂ emanating from a fungal bloom in the gut induced an immunoregulatory M2 phenotype in alveolar macrophages, which in turn exacerbated allergic airway inflammation in mice (Kim et al., 2014), and exposure to fungal PGE₂ diminished the protective immune response generated in response to immunization with DCs pulsed with *C. albicans* yeasts (Kundu and Noverr, 2011). However, the preceding findings do not point to an overt benefit for *C. albicans*, for which symbiosis is the *status quo* and infection is adventitious and often

a byproduct of sustained immunodeficiency and/or microbial dysbiosis. Accordingly, we observed no role for fungal PGE₂ in mediating the virulence of *C. albicans* under both *in vitro* and *in vivo* settings. Conversely, fungal PGE₂ production appeared to be important for promoting intestinal colonization by *C. albicans*. *C. albicans* is located at high titers in the gut and acquisition of the fungus often occurs early during ontogeny via transmission of maternal intestinal strains to the infant (Russell and Lay, 1973; Odds et al., 2006). It would thus be evolutionarily advantageous for *C. albicans* to evolve an independent means of generating PGE₂ to facilitate its own entrenchment as a symbiont in the gut.

How does fungal PGE₂ mediate intestinal colonization by *C. albicans*? Here we provide evidence in support of gut phagocytes, in particular macrophages and CD11b⁺ DCs, as a target of fungal PGE₂, since depletion of colonic phagocytes by two orthogonal approaches abrogated the fitness defect of the *ole2/ole2* mutant vis-à-vis the WT strain. The *ole2/ole2* mutant also sustained increased killing by a murine macrophage line *in vitro*, a phenomenon that was reversed by the addition of exogenous PGE₂. PGE₂-dependent fungal resistance to phagocytes could

occur via the paracrine activity of fungal PGE₂ on proximal phagocytes, and/or an autocrine effect of fungal PGE₂ on the fungus itself. PGE₂ has wide-ranging effects on phagocytes, most of which converge on immunosuppression (Kalinski, 2012; Medeiros et al., 2012). For instance, PGE₂ reduces the phagocytosis of fungi by macrophages (Serezani et al., 2012) and polarizes both fungi-exposed macrophages and DCs toward an immunoregulatory phenotype, characterized by an increased expression of suppressive factors such as arginase-1 (Suram et al., 2013; Kim et al., 2014; Yun et al., 2016). Hence, PGE₂ secreted by *C. albicans* proximal to or engulfed by intestinal phagocytes might dampen their uptake of more fungi, phagosome maturation, and/or production of inflammatory, antifungal factors beyond the ones we had tested (Figure S7C). The effects of fungal PGE₂ can also radiate beyond those of phagocytes to T cell responses primed by fungi-experienced phagocytes (Gagliardi et al., 2010; Smeekens et al., 2010), which in turn control fungal growth in the gut. PGE₂ is labile and likely has short-range activity, possibly even at the level of individual fungi-containing phagosomes in phagocytes (Hoffmann et al., 2012). This might account for the apparent cell-autonomous effect of fungal PGE₂ on intestinal fitness of *C. albicans*.

In addition, PGE₂ might act directly on *C. albicans* itself to augment its resistance to host immunity. Mucosal microbial communities, including those of *Candida*, have been likened to biofilms (de Vos, 2015), and certain genes crucial for the intestinal fitness of *C. albicans* are also required for biofilm formation (Ganguly and Mitchell, 2011; Bohm et al., 2017; Lagree and Mitchell, 2017), indicating common mechanisms underlying fungal fitness in both biofilms and the gut. Fungus-derived PGE₂ might therefore elicit biofilm-like transcriptional programs in gut fungi that enhance fitness via various means, such as the induction of stress response pathways that improve fungal resistance to immunological assaults from the host.

Our study focused on the role of fungus-derived PGE₂ but does not rule out a role for host-derived PGE₂ or other AA-derived lipid mediators in modulating intestinal symbiosis by *C. albicans*. Host phagocytes secrete PGE₂ when stimulated with *C. albicans* (Smeekens et al., 2010; Suram et al., 2013; Yun et al., 2016), though it is plausible that host- and fungus-derived PGE₂ exert distinct activities in a tissue- and cell-type-specific fashion, especially in light of the differential accessibility of immunocyte populations to fungi and the instability of PGE₂. Nevertheless, we predict that the effects of fungal PGE₂ on the intestinal fitness of *C. albicans* would be amplified in the absence of host-derived PGE₂. Notably, *C. albicans*, as well as other fungi and parasites, produces immunomodulatory lipid mediators from AA aside from PGE₂ *in vitro*, including prostaglandin D2 and leukotrienes (Szkudlinski, 2000; Herve et al., 2003; Tsitsigiannis et al., 2005; Valdez et al., 2012), some of which have been implicated in

modulating microbial fitness during pathogenesis. It is tempting to speculate that non-PGE₂ lipids can, akin to PGE₂, modulate the fitness of *C. albicans* and other symbiont microbes in the intestines and other barrier surfaces.

We report here a novel mechanism used by *C. albicans* to colonize the gut that is distinct from previously delineated pathways, such as the suppression of filamentation and changes in nutrient metabolism and iron acquisition (Nguyen et al., 2009). Since fungal colonization is a prerequisite for disseminated or mucosal diseases, this work provides new potential targets for therapeutic control of intestinal fungal populations to prevent human fungal infections.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/Supplementary Files.

ETHICS STATEMENT

The animal study was reviewed and approved by A*STAR Biological Resource Centre (BRC) Institutional Animal Care and Use Committee (IACUC).

AUTHOR CONTRIBUTIONS

TT, YL, AT, and RL performed experiments and data analysis. TT wrote the manuscript. NP edited the manuscript and supervised the study.

FUNDING

This study was supported by A*STAR Investigatorship award no. JCO/1437a00117 and by SIGn core funding to NP.

ACKNOWLEDGMENTS

This manuscript has been released as a Pre-Print at BioRxiv (Tan et al., 2018). We thank G. T. T. Le for technical assistance; S. Maurer-Stroh and F. L. Sirota for sharing unpublished bioinformatics analyses; Singapore Immunology Network (SIGn) Mouse Core for providing mice. This paper is dedicated to the memory of RL.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2019.00359/full#supplementary-material>

REFERENCES

Alem, M. A., and Douglas, L. J. (2004). Effects of aspirin and other nonsteroidal anti-inflammatory drugs on biofilms and planktonic cells of *Candida albicans*. *Antimicrob. Agents Chemother.* 48, 41–47. doi: 10.1128/AAC.48.1.41-47.2004

Bain, C. C., Bravo-Blas, A., Scott, C. L., Perdiguero, E. G., Geissmann, F., Henri, S., et al. (2014). Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat. Immunol.* 15, 929–937. doi: 10.1038/ni.2967

- Ballou, E. R., Avelar, G. M., Childers, D. S., Mackie, J., Bain, J. M., Wagener, J., et al. (2016). Lactate signalling regulates fungal beta-glucan masking and immune evasion. *Nat. Microbiol.* 2:16238. doi: 10.1038/nmicrobiol.2016.238
- Betz, M., and Fox, B. S. (1991). Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.* 146, 108–113.
- Bohm, L., Torsin, S., Tint, S. H., Eckstein, M. T., Ludwig, T., and Perez, J. C. (2017). The yeast form of the fungus *Candida albicans* promotes persistence in the gut of gnotobiotic mice. *PLoS Pathog.* 13:e1006699. doi: 10.1371/journal.ppat.1006699
- Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G., and White, T. C. (2012). Hidden killers: human fungal infections. *Sci. Transl. Med.* 4:165rv113. doi: 10.1126/scitranslmed.3004404
- Castro, M., Ralston, N. V., Morgenthaler, T. I., Rohrbach, M. S., and Limper, A. H. (1994). *Candida albicans* stimulates arachidonic acid liberation from alveolar macrophages through alpha-mannan and beta-glucan cell wall components. *Infect. Immun.* 62, 3138–3145.
- Chen, C., Pande, K., French, S. D., Tuch, B. B., and Noble, S. M. (2011). An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. *Cell Host Microbe* 10, 118–135. doi: 10.1016/j.chom.2011.07.005
- Chizzolini, C., Chicheportiche, R., Alvarez, M., de Rham, C., Roux-Lombard, P., Ferrari-Lacraz, S., et al. (2008). Prostaglandin E2 synergistically with interleukin-23 favors human Th17 expansion. *Blood* 112, 3696–3703. doi: 10.1182/blood-2008-05-155408
- Conti, H. R., Shen, F., Nayyar, N., Stocum, E., Sun, J. N., Lindemann, M. J., et al. (2009). Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J. Exp. Med.* 206, 299–311. doi: 10.1084/jem.20081463
- de Vos, W. M. (2015). Microbial biofilms and the human intestinal microbiome. *NPJ Biofilms Microbiomes* 1:15005. doi: 10.1038/npjbiofilms.2015.5
- Dominguez-Andres, J., Feo-Lucas, L., Minguito de la Escalera, M., Gonzalez, L., Lopez-Bravo, M., and Ardavin, C. (2017). Inflammatory Ly6C^{high} monocytes protect against candidiasis through IL-15-driven NK cell/neutrophil activation. *Immunity* 46, 1059–1072 e1054. doi: 10.1016/j.immuni.2017.05.009
- Ells, R., Kock, J. L., Albertyn, J., Kemp, G., and Pohl, C. H. (2011). Effect of inhibitors of arachidonic acid metabolism on prostaglandin E(2) production by *Candida albicans* and *Candida dubliniensis* biofilms. *Med. Microbiol. Immunol.* 200, 23–28. doi: 10.1007/s00430-010-0169-7
- Erb-Downward, J. R., Noggle, R. M., Williamson, P. R., and Huffnagle, G. B. (2008). The role of laccase in prostaglandin production by *Cryptococcus neoformans*. *Mol. Microbiol.* 68, 1428–1437. doi: 10.1111/j.1365-2958.2008.06245.x
- Erb-Downward, J. R., and Noverr, M. C. (2007). Characterization of prostaglandin E2 production by *Candida albicans*. *Infect. Immun.* 75, 3498–3505. doi: 10.1128/IAI.00232-07
- Fan, D., Coughlin, L. A., Neubauer, M. M., Kim, J., Kim, M. S., Zhan, X., et al. (2015). Activation of HIF-1 α and LL-37 by commensal bacteria inhibits *Candida albicans* colonization. *Nat. Med.* 21, 808–814. doi: 10.1038/nm.3871
- Fidel, P. L. Jr. (2005). Immunity in vaginal candidiasis. *Curr. Opin. Infect. Dis.* 18, 107–111. doi: 10.1097/01.qco.0000160897.74492.a3
- Gagliardi, M. C., Teloni, R., Mariotti, S., Bromuro, C., Chiani, P., Romagnoli, G., et al. (2010). Endogenous PGE2 promotes the induction of human Th17 responses by fungal α -glucan. *J. Leukoc. Biol.* 88, 947–954. doi: 10.1189/jlb.0310139
- Ganguly, S., and Mitchell, A. P. (2011). Mucosal biofilms of *Candida albicans*. *Curr. Opin. Microbiol.* 14, 380–385. doi: 10.1016/j.mib.2011.06.001
- Grozer, Z., Toth, A., Toth, R., Kecskemeti, A., Vagvolgyi, C., Nosanchuk, J. D., et al. (2015). *Candida parapsilosis* produces prostaglandins from exogenous arachidonic acid and OLE2 is not required for their synthesis. *Virulence* 6, 85–92. doi: 10.4161/21505594.2014.988097
- Herve, M., Angeli, V., Pinzar, E., Wintjens, R., Faveeuw, C., Narumiya, S., et al. (2003). Pivotal roles of the parasite PGD2 synthase and of the host D prostanoid receptor 1 in schistosome immune evasion. *Eur. J. Immunol.* 33, 2764–2772. doi: 10.1002/eji.200324143
- Hoffmann, E., Kotsias, F., Visentin, G., Bruhns, P., Savina, A., and Amigorena, S. (2012). Autonomous phagosomal degradation and antigen presentation in dendritic cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 14556–14561. doi: 10.1073/pnas.1203912109
- Kalinski, P. (2012). Regulation of immune responses by prostaglandin E2. *J. Immunol.* 188, 21–28. doi: 10.4049/jimmunol.1101029
- Kalo-Klein, A., and Witkin, S. S. (1990). Prostaglandin E2 enhances and gamma interferon inhibits germ tube formation in *Candida albicans*. *Infect. Immun.* 58, 260–262.
- Kashem, S. W., Igyarto, B. Z., Gerami-Nejad, M., Kumamoto, Y., Mohammed, J. A., Jarrett, E., et al. (2015). *Candida albicans* morphology and dendritic cell subsets determine T helper cell differentiation. *Immunity* 42, 356–366. doi: 10.1016/j.immuni.2015.01.008
- Kim, Y. G., Udayanga, K. G., Totsuka, N., Weinberg, J. B., Nunez, G., and Shibuya, A. (2014). Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE(2). *Cell Host Microbe* 15, 95–102. doi: 10.1016/j.chom.2013.12.010
- Koh, A. Y., Kohler, J. R., Cogshall, K. T., Van Rooijen, N., and Pier, G. B. (2008). Mucosal damage and neutropenia are required for *Candida albicans* dissemination. *PLoS Pathog.* 4:e35. doi: 10.1371/journal.ppat.0040035
- Krause, J., Geginat, G., and Tammer, I. (2015). Prostaglandin E2 from *Candida albicans* stimulates the growth of *Staphylococcus aureus* in mixed biofilms. *PLoS ONE* 10:e0135404. doi: 10.1371/journal.pone.0135404
- Kundu, G., and Noverr, M. C. (2011). Exposure to host or fungal PGE(2) abrogates protection following immunization with *Candida*-pulsed dendritic cells. *Med. Mycol.* 49, 380–394. doi: 10.3109/13693786.2010.532514
- Lagree, K., and Mitchell, A. P. (2017). Fungal biofilms: inside out. *Microbiol. Spectr.* 5:FUNK-0024-2016. doi: 10.1128/microbiolspec.FUNK-0024-2016
- Leonardi, I., Li, X., Semon, A., Li, D., Doron, I., Putzel, G., et al. (2018). CX3CR1(+) mononuclear phagocytes control immunity to intestinal fungi. *Science* 359, 232–236. doi: 10.1126/science.aao1503
- Ma, H., Wan, S., and Xia, C. Q. (2016). Immunosuppressive CD11b+Ly6Chi monocytes in pristane-induced lupus mouse model. *J. Leukoc. Biol.* 99, 1121–1129. doi: 10.1189/jlb.3A0415-158R
- MacCallum, D. M. (2010). “*Candida* infections and modelling disease,” in *Pathogenic Yeasts*, eds R. Ashbee and E. M. Bignell (Berlin: Springer-Verlag), 41–67.
- Mamouei, Z., Zeng, G., Wang, Y. M., and Wang, Y. (2017). *Candida albicans* possess a highly versatile and dynamic high-affinity iron transport system important for its commensal-pathogenic lifestyle. *Mol. Microbiol.* 106, 986–998. doi: 10.1111/mmi.13864
- Martin, S. A., Brash, A. R., and Murphy, R. C. (2016). The discovery and early structural studies of arachidonic acid. *J. Lipid Res.* 57, 1126–1132. doi: 10.1194/jlr.R068072
- Medeiros, A., Peres-Buzalaf, C., Fortino Verdán, F., and Serezani, C. H. (2012). Prostaglandin E2 and the suppression of phagocyte innate immune responses in different organs. *Mediators Inflamm.* 2012:327568. doi: 10.1155/2012/327568
- Mishra, N. N., Ali, S., and Shukla, P. K. (2014). Arachidonic acid affects biofilm formation and PGE2 level in *Candida albicans* and non-*albicans* species in presence of subinhibitory concentration of fluconazole and terbinafine. *Braz. J. Infect. Dis.* 18, 287–293. doi: 10.1016/j.bjid.2013.09.006
- Nguyen, L. N., Trofa, D., and Nosanchuk, J. D. (2009). Fatty acid synthase impacts the pathobiology of *Candida parapsilosis* in vitro and during mammalian infection. *PLoS ONE* 4:e8421. doi: 10.1371/journal.pone.0008421
- Noble, S. M., French, S., Kohn, L. A., Chen, V., and Johnson, A. D. (2010). Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nat. Genet.* 42, 590–598. doi: 10.1038/ng.605
- Noble, S. M., and Johnson, A. D. (2005). Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryotic Cell* 4, 298–309. doi: 10.1128/EC.4.2.298-309.2005
- Noverr, M. C., Phare, S. M., Toews, G. B., Coffey, M. J., and Huffnagle, G. B. (2001). Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect. Immun.* 69, 2957–2963. doi: 10.1128/IAI.69.5.2957-2963.2001
- Noverr, M. C., Toews, G. B., and Huffnagle, G. B. (2002). Production of prostaglandins and leukotrienes by pathogenic fungi. *Infect. Immun.* 70, 400–402. doi: 10.1128/IAI.70.1.400-402.2002
- Nucci, M., and Anaissie, E. (2001). Revisiting the source of candidemia: skin or gut? *Clin. Infect. Dis.* 33, 1959–1967. doi: 10.1086/323759
- Odds, F. C. (1987). *Candida* infections: an overview. *Crit. Rev. Microbiol.* 15, 1–5. doi: 10.3109/10408418709104444

- Odds, F. C., Davidson, A. D., Jacobsen, M. D., Tavanti, A., Whyte, J. A., Kibbler, C. C., et al. (2006). *Candida albicans* strain maintenance, replacement, and microvariation demonstrated by multilocus sequence typing. *J. Clin. Microbiol.* 44, 3647–3658. doi: 10.1128/JCM.00934-06
- Pande, K., Chen, C., and Noble, S. M. (2013). Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism. *Nat. Genet.* 45, 1088–1091. doi: 10.1038/ng.2710
- Perez, J. C., Kumamoto, C. A., and Johnson, A. D. (2013). *Candida albicans* commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. *PLoS Biol.* 11:e1001510. doi: 10.1371/journal.pbio.1001510
- Pierce, J. V., and Kumamoto, C. A. (2012). Variation in *Candida albicans* EFG1 expression enables host-dependent changes in colonizing fungal populations. *MBio* 3:e00117-12. doi: 10.1128/mBio.00117-12
- Pradhan, A., Avelar, G. M., Bain, J. M., Childers, D. S., Larcombe, D. E., Netea, M. G., et al. (2018). Hypoxia promotes immune evasion by triggering β -glucan masking on the *Candida albicans* cell surface via mitochondrial and cAMP-protein kinase A signaling. *MBio* 9:e01318-18. doi: 10.1128/mBio.01318-18
- Rosenbach, A., Dignard, D., Pierce, J. V., Whiteway, M., and Kumamoto, C. A. (2010). Adaptations of *Candida albicans* for growth in the mammalian intestinal tract. *Eukaryotic Cell* 9, 1075–1086. doi: 10.1128/EC.00034-10
- Russell, C., and Lay, K. M. (1973). Natural history of *Candida* species and yeasts in the oral cavities of infants. *Arch. Oral Biol.* 18, 957–962. doi: 10.1016/0003-9969(73)90176-3
- Sasse, C., and Morschhauser, J. (2012). Gene deletion in *Candida albicans* wild-type strains using the SAT1-flipping strategy. *Methods Mol. Biol.* 845, 3–17. doi: 10.1007/978-1-61779-539-8_1
- Scott, C. L., Bain, C. C., Wright, P. B., Sichien, D., Kotarsky, K., Persson, E. K., et al. (2015). CCR2(+)/CD103(-) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol.* 8, 327–339. doi: 10.1038/mi.2014.70
- Sem, X., Le, G. T., Tan, A. S., Tso, G., Yurieva, M., Liao, W. W., et al. (2016). β -glucan exposure on the fungal cell wall tightly correlates with competitive fitness of *Candida* species in the mouse gastrointestinal tract. *Front. Cell. Infect. Microbiol.* 6:186. doi: 10.3389/fcimb.2016.00186
- Serezani, C. H., Kane, S., Medeiros, A. I., Cornett, A. M., Kim, S. H., Marques, M. M., et al. (2012). PTEN directly activates the actin depolymerization factor cofilin-1 during PGE₂-mediated inhibition of phagocytosis of fungi. *Sci. Signal* 5:ra12. doi: 10.1126/scisignal.2002448
- Shiraki, Y., Ishibashi, Y., Hiruma, M., Nishikawa, A., and Ikeda, S. (2008). *Candida albicans* abrogates the expression of interferon-gamma-inducible protein-10 in human keratinocytes. *FEMS Immunol. Med. Microbiol.* 54, 122–128. doi: 10.1111/j.1574-695X.2008.00457.x
- Smekens, S. P., van de Veerdonk, F. L., van der Meer, J. W., Kullberg, B. J., Joosten, L. A., and Netea, M. G. (2010). The *Candida* Th17 response is dependent on mannan- and beta-glucan-induced prostaglandin E₂. *Int. Immunol.* 22, 889–895. doi: 10.1093/intimm/dxq442
- Solis, N. V., and Filler, S. G. (2012). Mouse model of oropharyngeal candidiasis. *Nat. Protoc.* 7, 637–642. doi: 10.1038/nprot.2012.011
- Sparber, F., Dolowschiak, T., Mertens, S., Lauener, L., Clausen, B. E., Joller, N., et al. (2018). Langerin+ DCs regulate innate IL-17 production in the oral mucosa during *Candida albicans*-mediated infection. *PLoS Pathog.* 14:e1007069. doi: 10.1371/journal.ppat.1007069
- Suram, S., Silveira, L. J., Mahaffey, S., Brown, G. D., Bonventre, J. V., Williams, D. L., et al. (2013). Cytosolic phospholipase A₂(α) and eicosanoids regulate expression of genes in macrophages involved in host defense and inflammation. *PLoS ONE* 8:e69002. doi: 10.1371/journal.pone.0069002
- Szkudlinski, J. (2000). Occurrence of prostaglandins and other eicosanoids in parasites and their role in host-parasite interaction. *Wiad. Parazytol.* 46, 439–446.
- Tan, T. G., Lim, Y. S., Tan, A. S., Leong, R., and Pavelka, N. (2018). Fungal symbionts produce prostaglandin E₂ to promote their intestinal colonization. *bioRxiv [preprint]*. doi: 10.1101/477117
- Tsitsigiannis, D. I., Bok, J. W., Andes, D., Nielsen, K. F., Frisvad, J. C., and Keller, N. P. (2005). *Aspergillus* cyclooxygenase-like enzymes are associated with prostaglandin production and virulence. *Infect. Immun.* 73, 4548–4559. doi: 10.1128/IAI.73.8.4548-4559.2005
- Valdez, P. A., Vithayathil, P. J., Janelins, B. M., Shaffer, A. L., Williamson, P. R., and Datta, S. K. (2012). Prostaglandin E₂ suppresses antifungal immunity by inhibiting interferon regulatory factor 4 function and interleukin-17 expression in T cells. *Immunity* 36, 668–679. doi: 10.1016/j.immuni.2012.02.013
- Weisser, S. B., van Rooijen, N., and Sly, L. M. (2012). Depletion and reconstitution of macrophages in mice. *J. Vis. Exp.* e4105. doi: 10.3791/4105
- White, S. J., Rosenbach, A., Lephart, P., Nguyen, D., Benjamin, A., Tzipori, S., et al. (2007). Self-regulation of *Candida albicans* population size during GI colonization. *PLoS Pathog.* 3:e184. doi: 10.1371/journal.ppat.0030184
- Yano, J., and Fidel, P. L. Jr. (2011). Protocols for vaginal inoculation and sample collection in the experimental mouse model of *Candida vaginitis*. *J. Vis. Exp.* 8:3382. doi: 10.3791/3382
- Yao, C., Sakata, D., Esaki, Y., Li, Y., Matsuoka, T., Kuroiwa, K., et al. (2009). Prostaglandin E₂-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. *Nat. Med.* 15, 633–640. doi: 10.1038/nm.1968
- Yun, B., Lee, H., Jayaraja, S., Suram, S., Murphy, R. C., and Leslie, C. C. (2016). Prostaglandins from cytosolic phospholipase A₂(α)/cyclooxygenase-1 pathway and mitogen-activated protein kinases regulate gene expression in *Candida albicans*-infected macrophages. *J. Biol. Chem.* 291, 7070–7086. doi: 10.1074/jbc.M116.714873

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Tan, Lim, Tan, Leong and Pavelka. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.