Trends in Cell & Molecular Biology
Vol. 7, 2012

Mini-Review

Host sphingolipids and fungal infection

Asfia Qureshi[1] and Maurizio Del Poeta[1,2,3,4,*]

[1]Department of Biochemistry and Molecular Biology, [2]Microbiology and Immunology, [3]Craniofacial Biology and [4]Division of Infectious Diseases, Medical University of South Carolina, Charleston, SC 29425, USA

ABSTRACT

Fungal infections have dramatically increased during the last decade and new treatment strategies are required. Through a better understanding of the pathogenic processes leading to the killing of fungal microbes by host immune cells, new insights can be gained into the development of better therapeutic strategies. Recent studies have highlighted the importance of host sphingolipids in controlling the host-fungal relationship. This review aims to discuss new findings in this area.

KEYWORDS: fungal infection, host sphingolipids, *Cryptococcus neoformans*

INTRODUCTION

Nowadays, fungal infections are more common than ever before. The reasons for this are manifold. People have a longer life expectancy, and more and more of the world's population has compromised immune systems, a major risk factor for fungal infection. Likewise, the widespread use of antibiotics has contributed to the growing rate of fungal infections, as has the success in treating diseases like HIV/AIDS which has created a subgroup of the population susceptible to fungal infections [1]. In order to understand and find new treatments for fungal infections, especially those caused by opportunistic fungi, it is important to understand how sphingolipids are involved in the regulation of the delicate balance between fungus and host. Additionally, new emerging fungal pathogens such as *Cryptococcus gattii*, previously thought to be avirulent, have alarmingly been responsible for cryptococcosis outbreaks in almost exclusively immunocompetent people, predominantly in the Pacific Northwest of the United States [2-5]. Thus, perhaps, *Cryptococcus* should no longer be considered merely an opportunistic pathogen, but one that warrants continuing exploration and studies [6, 7].

*Corresponding author: Maurizio Del Poeta,
Department of Biochemistry and Molecular Biology,
Medical University of South Carolina,
173 Ashley Ave BSB 512A,
Charleston, SC 29425, USA.
delpoeta@musc.edu

Host sphingolipids

Sphingolipids are a class of lipids that serve not only as integral components of eukaryotic cell membranes [8, 9], but also act as signaling molecules in many cellular functions, playing critical roles in regulating patho-biological processes in several diseases including cancer, cardiovascular and neurodegenerative disorders, inflammation and infectious diseases. In mammalian cells, ceramide, sphingosine, sphingosine-1-phosphate, and glucosylceramide have been the most studied sphingolipids, and they regulate important processes, including the stress response, cell proliferation, apoptosis, angiogenesis, genetic diseases, and resistance to chemotherapy [10-15]. Microorganisms that do not produce sphingolipids, which includes most bacteria, are able to use host sphingolipids to promote their virulence. Therefore, in the context of bacterium-host interaction, the host is the source of

sphingolipids. However in the context of protozoan- and fungal-host interaction, both host and pathogen sphingolipids are involved.

Parasites and bacteria

In protozoa, the sphingolipid pathway is dependent on the organism and is not conserved among all members of the subkingdom [16]. For example, the etiologic agents of malaria, *Plasmodium* species, produce mammalian-like sphingolipids, while kinetoplastid protozoa such as *Giardia*, *Leishmania* and *Trypanosoma* species contain fungal-like sphingolipids. Glucosylceramide (GlcCer) plays a vital role in regulating cell cycle progression, membrane trafficking and stage differentiation in *G. lamblia* [17]. Indeed, current data on sphingolipids in pathogenic protozoans illustrates the roles these lipids play in multiple aspects of cellular life including differentiation, replication, trafficking and the synthesis of virulence factors [17-19]. In the intracellular protozoan *Plasmodium falciparum*, which resides in red blood cells and is the most virulent of the four Plasmodium species infecting humans, both sphinogomyelin synthase (SMS) [20-23] and glycosylceramide synthase (GCS) [24, 25] activities have been observed, suggesting that *Plasmodium* has a conserved mammalian-like sphingolipid biosynthetic pathway. Inhibition of these two enzymes leads to increased intracellular concentrations of ceramide and results in growth inhibition of the protozoan, suggesting that both these enzymes are crucial to the survival and virulence of *Plasmodium* [26]. This is important because exogenously added ceramide and sphingomyelinase cause a dose- and time-dependent inhibition of *P. falciparum* growth [27], suggesting that this approach could be exploited as potential alternative therapeutic strategies.

The kinetoplastid protozoan *Leishmania* live extracellularly within the sand fly midgut, but within an acidified, fusogenic phagolysosome of the macrophage within the mammalian host [19]. The predominant sphingolipids reported in *L. donovani*, the species associated with fatal visceral infections, (and eventually among all the *Leishmania* species), are the inositol phosphoceramides (IPCs), more typical to those found in fungi than in mammalian hosts [28-30]. Among the protozoan surface membrane glycolipids, which include lipophosphoglycan and glycosylinositolphospholipids, IPCs have been found to be the most abundant class [31, 32]. *Leishmania* parasites possess a potent neutral sphingomyelinase (SMase) called inositol phosphosphingolipid phospholipase C-like (ISCL), which is responsible for the degradation of both host-derived sphingomyelin and parasite-derived IPC [33]. This degradation of host-derived sphingomyelin (and not parasite-synthesized IPC) is essential for *Leishmania* virulence [33, 34] in mammals.

In host cells, the acid sphingomyelinase-ceramide system plays an important role in controlling the infection by bacterial pathogens such as *Neisseria gonorrhoeae*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* [35]. In the case of *P. aeruginosa*, formation of plasma membrane ceramide-enriched platforms (lipid rafts) enables internalization of the bacterium, which triggers apoptosis of the host cell and ultimately kills the pathogen. Infection by *P. aeruginosa* is often associated with cystic fibrosis, with mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Localization of CFTR protein to lipid rafts is necessary for uptake of *P. aeruginosa* by host cells. In CF patients, the mutated form of CFTR protein does not localize to rafts, altering the microbe internalization and increasing the extracellular proliferation and severity of the disease [35, 36].

Fungi

Apart from work on *Cryptococcus neoformans*, not a great deal is known about the role of host sphingolipids on fungal growth and virulence in other species, based on Pubmed and Google searches. Hence we will focus our attention solely on *C. neoformans*.

Cryptococcus neoformans

Cryptococcus neoformans is an encapsulated fungal pathogen that primarily affects immunocompromised patients (for example, HIV/AIDS patients, transplant patients and patients on long term steroid treatments). Alarmingly, *Cryptococcus gattii*,

previously thought to be avirulent, has recently emerged as a pathogen for immunocompetent individuals. Thus *Cryptococcus* can no longer be considered merely an opportunistic pathogen [2, 6, 7]. This environmental yeast is inhaled to the lungs, where it can live extracellularly or intracellularly within the phagolysosomes of alveolar macrophages. Infection with this organism is known as cryptococcosis. Current anticryptococcal treatments are still unable to completely eradicate the infection, forcing patients to remain on life-long antifungal therapy. *Cryptococcus* holds the notable distinction as being the only eukaryotic pathogen that produces a polysaccharide capsule, which consists of glucuronoxylomannan (GXM) and galactoxylomannan (GalXM), as well as mannoprotein [37]. These components all occupy spatially separate and discrete regions in the capsule of *C. neoformans* [38]. This antiphagocytic polysaccharide capsule is the major virulence factor of the fungus, with melanin being the second most important virulence component, and the ability to grow at 37°C a third virulence factor. Melanin has been shown to interfere with numerous host defense mechanisms, and it is well known that melanized *C. neoformans* cells are less susceptible to the toxic effects of microbicidal peptides than non-melanized cells [39]. In addition, melanization protects *C. neoformans* against injury secondary to nitrogen or oxygen derived radical attack [40, 41]. The regulation of these three virulence factors has been reviewed recently in several excellent works [42-47].

In *C. neoformans*, a novel regulatory pathway initiated by the sphingolipid enzyme inositol phosphoryl ceramide (IPC) synthase (Ipc1) has been identified [48, 49]. The effects of Ipc1 on melanin production was found to be potentially propagated through the production of diacylglycerol (DAG) and subsequent activation of protein kinase C1 (Pkc1) through its C1 domain. This putative Ipc1-DAG-Pkc1 pathway was the first evidence that the sphingolipid pathway is critical to the regulation of fungal virulence and established a key role for DAG generated from sphingolipid metabolism [49]. In addition to Ipc1, other enzymes of the sphingolipid pathway have also been identified as essential regulators of *C. neoformans* growth in the intracellular environment. Inositol phosphosphingolipid-phospholipase C1 (Isc1), an enzyme that metabolizes fungal inositol sphingolipids into phytoceramide, is essential for intracellular growth, as it provides protection from acidic, oxidative and nitrosative stresses of the phagolysosome microenvironment through Pma1-dependent mechanism(s) [50, 51]. A mathematical model of sphingolipid metabolism in *Cryptococcus neoformans* has been developed and the predictions on its biochemical sphingolipid adaptation to a shift from an alkaline to an acidic pH, mimicking the internalization of the fungus by phagocytic cells was very reliable and agreed well with experimental studies that identified inositol phosphorylceramide synthase (Ipc1), inositol phosphosphingolipid phospholipase C (Isc1), and phytoceramides of different lengths as important contributors to the response of *Cn* to H^+ATPase pump (Pma1) and ATP-mediated shifts in pH [50].

While Isc1 is essential for *C. neoformans* survival within the host phagolysosome, glycosylceramide synthase (GCS) is essential for extracellular growth [52, 53]. GCS is involved in controlling cell-cycle progression and fungal growth in environments characterized by a neutral/alkaline pH and physiological concentrations of CO_2, such as the lung alveolar spaces and bloodstream. The ΔGCS mutant strain is contained within lung granulomas and therefore does not disseminate to other organs. Several excellent reviews have been published that cover these areas in more detail [16, 54-59].

As components of the sphingolipid biosynthesis pathway in mammalian cells, sphingosine kinases 1 (SK1) and 2 (SK2) catalyse the phosphorylation of sphingosine to produce the bioactive lysophospholipid sphingosine-1-phosphate (S1P), which is well documented to regulate numerous facets of the immune system [60], and has been called the “Swiss army knife of sphingolipid signaling” [61]. The SK1/ S1P pathway is particularly important in macrophage function [62] and greatly affects the immune response in the lungs [63, 64]. It has been shown that SK1 has a key role in the formation of a granulomatous inflammation in response to pulmonary cryptococcosis [65]. In particular, SK1 is essential for preventing the dissemination of *C. neoformans*

when the host and/or *C. neoformans* factors promote intracellular parasitism, thereby indicating (indirectly) that the product of SK1 activity, S1P, affects the phagocytosis of *C. neoformans* by alveolar macrophages (AMs). McQuiston *et al.* have examined the effects of extracellular S1P on the AMs-*C. neoformans* interaction and investigated which S1P receptor is involved in this interaction [66]. Extracellular S1P increases the phagocytosis of *C. neoformans* by AMs through S1P receptor 2 (S1PR2). Using S1PR2-knockout mice the authors observed that AMs from these mice had decreased expression of Fcγ receptors and, thus, they ingested fewer *C. neoformans* compared with wild-type AMs. This suggests that the S1P-S1PR2 interaction and the consequent Fcγ regulation are important for favoring phagocytosis of *C. neoformans*.

Another intriguing sphingolipid metabolizing activity in host cells is carried out by sphingomyelin synthase (SMS) which is encoded by two genes: SMS1 and SMS2 [67-69]. SMS transfers a choline phosphate moiety from phosphatidylcholine (PC) to ceramide, thereby producing sphingomyelin (SM) and DAG [50, 68, 70]. This enzyme is particularly important because not only does it produce SM, a key component of cellular membranes, but also because it regulates the level of two bioactive lipid molecules such as ceramide and DAG. Since (a) ceramide can regulate transcription factors, such as NF-kB involved in cytokine production [71], (b) DAG controls antifungal activity by neutrophils through reactive oxygen species (ROS) production [72], and (c) SM has been implicated in controlling the host immune response in phagocytic cells [73], SMS plays an important role in the host.

Recently it has been identified that the host sphingolipid pathway in host immune cells, particularly neutrophils, is required to exert their killing activity on *C. neoformans* [74]. In particular, the inhibition of SMS activity profoundly impaired the killing ability of neutrophils by preventing the extracellular release of an anticryptococcal factor(s). Indeed, inhibition of protein kinase D1 (Pkd1), which controls vesicular sorting and secretion, and which is regulated by DAG produced by SMS, also totally blocked the extracellular killing activity. The expression of SMS genes, SMS activity and the levels of the lipids regulated by SMS (namely SM and DAG) as measured by electrospray ionization-mass spectrometry (ESI-MS), were up-regulated during neutrophil differentiation (using the HL-60 cell model system) [74]. Therefore it is hypothesized that secretion of anti-cryptococcal factors, such as myeloperoxidase, defensins, and others, may be under the control of the SMS-DAG-Pkd1 pathway. The use of matrix-assisted laser desorption-ionization mass spectrometric imaging (MALDI-MSI) of CBA/J mouse lung tissue infected with *C. neoformans* revealed that specific SM species (SM16:0) were associated with infiltration of neutrophils at the infection site. This study established a key role for SMS in the regulation of the killing activity of neutrophils against *C. neoformans* through a DAG-PKD1 dependent mechanism, and provided new insights into the protective role of host sphingolipids against a cryptococcal infection.

A more recent study focusing on further understanding the cryptococcal-neutrophil relationship and secreted anti-cryptocccal factor(s) has shown that unlike *Candida albicans*, neither the presence nor the capsule size of *C. neoformans* cells have any effect on neutrophil viability [75]. Interestingly, melanized *C. neoformans* cells totally abrogated the killing activity of neutrophils. It was found that pre-incubation with live but not “heat-killed” fungal cells significantly inhibits further killing activity of the medium (obtained from neutrophils) that contained anti-cryptococcal factors. MALDI imaging was again used to visualize the spatial distribution of lipids within thin sections of lungs from infected immunocompromised mice lacking T and natural killer (NK) cells (Tgε26 mice). These mice still have fully functional neutrophils, but since *C. neoformans* occurs in patients with low T cell counts (for example, HIV patients) [76, 77], the authors wished to explore whether SMS activity would still have a role against the fungus under conditions of T cell deficiency [75]. It was found that SM16:0 is observed in the immunocompromised mouse lung similarly as in the immunocompetent lung, suggesting that NK and/or T cells are not necessary to produce a basal level of the sphingolipid. When the lungs were infected with *C. neoformans* though, it was observed that SM16:0 was elevated only at day 12

of the infection and its overall distribution was much less organized in the immunocompromised compared to the immunocompetent host, suggesting that T and/or NK cells may have a role in activating SMS in the lung, and coordinating the SMS response of neutrophils against *C. neoformans*, especially at later stages of the *C. neoformans* infection. Taken together, these data suggest that *C. neoformans* may negatively regulate the killing activity of neutrophils.

Future work

Given the antifungal activity of SK1 and SMS in host immune cells, it is envisioned that an up-regulation of SK1 and/or SMS activity (for example, by lentiviral expression) in the HL-60 cell model system will enhance their killing activity against *Cryptococcus* and perhaps also against other microorganisms. This strategy could be employed to replenish the killing activity of neutrophils or macrophages, especially in conditions of immunodeficiency in which the antimicrobial activity of resident neutrophils and/or macrophages is impaired. In addition, further studies will reveal new regulatory mechanisms involved in the killing of *C. neoformans* by neutrophils and will assist in identifying the *nexus* between SK1, SMS, PKD and infection.

A cell based immunotherapy that can recapitulate neutrophil functions in neutropenic individuals afflicted with candidiasis is under development by stably transfecting HL-60 cells with a suicide trap to enable purging of the cells when desired, as well as a bioluminescence marker in order to track cells *in vivo* in mice [78]. The results of these studies lay the groundwork for continued translational development of these promising new technologies for the treatment of those infections that are resistant to current medications in the neutropenic host.

In conclusion, this is only the beginning of our understanding of host-fungal interactions in terms of lipid involvement. The use of host and fungal lipid metabolomics may add a new dimension of the fungus-host interaction that will help to integrate the understanding of such a complex and intriguing relationship.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grant AI56168, AI71142 and AI87541 (to M. D. P) and was conducted in a facility constructed with support from the National Institutes of Health, Grant Number C06 RR015455 from the Extramural Research Facilities Program of the National Center for Research Resources. Maurizio Del Poeta is a Burroughs Welcome New Investigator in Pathogenesis of Infectious Diseases.

REFERENCES

1. Nola, I., Kostovic, K., Oremovic, L., Soldo-Belic, A., and Lugovic, L. 2003, Acta Dermatovenerol. Croat., 11, 171.
2. Bartlett, K., Byrnes, E., Duncan, C., Fyfe, M., Galanis, E., Heitman, J., Hoang, L., Kidd, S., MacDougall, L., Mak, S., Marr, K., Morshed, M., West, S., and Kronstad, J. 2011, The emergence of *Cryptococcus gattii* infections on Vancouver Island and expansion in the Pacific Northwest in Cryptococcus: From Human Pathogen to Model Yeast, Heitman, J., Kozel, T. R., Kwon-Chung, K. J., Perfect, J. R., and Casadevall, A. (Eds.), ASM Press, Washington, DC, 313.
3. Byrnes, E. J., Li, W., Lewit, Y., Ma, H., Voelz, K., Ren, P., Carter, D. A., Chaturvedi, V., Bildfell, R. J., May, R. C., and Heitman, J. 2010, PLoS Pathog., 6, e1000850.
4. Datta, K., Bartlett, K. H., Baer, R., Byrnes, E., Galanis, E., Heitman, J., Hoang, L., Leslie, M. J., MacDougall, L., Magill, S. S., Morshed, M. G., and Marr, K. A. 2009, Emerg. Infect. Dis., 15, 1185.
5. Datta, K., Bartlett, K. H., and Marr, K. A. 2009, Interdiscip. Perspect. Infect. Dis., 2009, 176532.
6. Del Poeta, M. and Casadevall, A. 2012, Mycopathologia, 173, 303.
7. Kronstad, J., Saikia, S., Nielson, E. D., Kretschmer, M., Jung, W., Hu, G., Geddes, J. M., Griffiths, E. J., Choi, J., Cadieux, B., Caza, M., and Attarian, R. 2012, Eukaryot. Cell, 11, 109.

8. Dickson, R. C. and Lester, R. L. 1999, Biochim. Biophys. Acta, 1438, 305.
9. Hannun, Y. A. and Luberto, C. 2000, Trends Cell Biol., 10, 73.
10. Bell, R., Burns, D., Okazaki, T., and Hannun, Y. 1992, Adv. Exp. Med. Biol., 318, 275.
11. Hannun, Y. A., Luberto, C., and Argraves, K. M. 2001, Biochemistry, 40, 4893.
12. Luberto, C. and Hannun, Y. A. 1999, Lipids, 34, Suppl S5.
13. Obeid, L. M., Linardic, C. M., Karolak, L. A., and Hannun, Y. A. 1993, Science, 259, 1769.
14. Ogretmen, B. and Hannun, Y. A. 2001, Drug Resist. Updat., 4, 368.
15. Spiegel, S. and Milstien, S. 2003, Nat. Rev. Mol. Cell Biol., 4, 397.
16. Heung, L. J., Luberto, C., and Del Poeta, M. 2006, Infect. Immun., 74, 28.
17. Stefanic, S., Spycher, C., Morf, L., Fabrias, G., Casas, J., Schraner, E., Wild, P., Hehl, A. B., and Sonda, S. 2010, J. Lipid Res., 51, 2527.
18. Richmond, G. S., Gibellini, F., Young, S. A., Major, L., Denton, H., Lilley, A., and Smith, T. K. 2010, Parasitology, 137, 1357.
19. Zhang, K., Bangs, J. D., and Beverley, S. M. 2010, Adv. Exp. Med. Biol., 688, 238.
20. Haldar, K., Uyetake, L., Ghori, N., Elmendorf, H. G., and Li, W. L. 1991, Mol. Biochem. Parasitol., 49, 143.
21. Elmendorf, H. G. and Haldar, K. 1993, Embo J., 12, 4763.
22. Elmendorf, H. G. and Haldar, K. 1994, J. Cell Biol., 124, 449.
23. Ansorge, I., Jeckel, D., Wieland, F., and Lingelbach, K. 1995, Biochem J., 308, 335.
24. Couto, A. S., Caffaro, C., Uhrig, M. L., Kimura, E., Peres, V. J., Merino, E. F., Katzin, A. M., Nishioka, M., Nonami, H., and Erra-Balsells, R. 2004, Eur. J. Biochem., 271, 2204.
25. Gerold, P. and Schwarz, R. T. 2001, Mol. Biochem. Parasitol., 112, 29.
26. Pankova-Kholmyansky, I. and Flescher, E. 2006, Chemotherapy, 52, 205.
27. Pankova-Kholmyansky, I., Dagan, A., Gold, D., Zaslavsky, Z., Skutelsky, E., Gatt, S., and Flescher, E. 2003, Cell Mol. Life Sci., 60, 577.
28. Hsu, F. F., Turk, J., Zhang, K., and Beverley, S. M. 2007, J. Am. Soc. Mass Spectrom., 18, 1591.
29. Wassef, M. K., Fioretti, T. B., and Dwyer, D. M. 1985, Lipids, 20, 108.
30. Kaneshiro, E. S., Jayasimhulu, K., and Lester, R. L. 1986, J. Lipid Res., 27, 1294.
31. Denny, P. W., Field, M. C., and Smith, D. F. 2001, FEBS Lett., 491, 148.
32. Ralton, J. E., Mullin, K. A., and McConville, M. J. 2002, Biochem. J., 363, 365.
33. Zhang, O., Wilson, M. C., Xu, W., Hsu, F. F., Turk, J., Kuhlmann, F. M., Wang, Y., Soong, L., Key, P., Beverley, S. M., and Zhang, K. 2009, PLoS Pathog., 5, e1000692.
34. Xu, W., Xin, L., Soong, L., and Zhang, K. 2011, Infect. Immun., 79, 3377.
35. Grassme, H., Becker, K. A., Zhang, Y., and Gulbins, E. 2008, Biol. Chem., 389, 1371.
36. Kowalski, M. P. and Pier, G. B. 2004, J. Immunol., 172, 418.
37. Bose, I., Reese, A. J., Ory, J. J., Janbon, G., and Doering, T. L. 2003, Eukaryot. Cell., 2, 655.
38. Jesus, M. D., Nicola, A. M., Chow, S. K., Lee, I. R., Nong, S., Specht, C. A., Levitz, S. M., and Casadevall, A. 2010, Virulence, 1, 500.
39. Doering, T. L., Nosanchuk, J. D., Roberts, W. K., and Casadevall, A. 1999, Med. Mycol., 37, 175.
40. Nosanchuk, J. D. and Casadevall, A. 2003, Cell Microbiol., 5, 203.
41. Nosanchuk, J. D. and Casadevall, A. 2006, Antimicrob. Agents Chemother., 50, 3519.
42. Li, S. S. and Mody, C. H. 2010, Proc. Am. Thorac. Soc., 7, 186.
43. Alspaugh, J. A., Nichols, C. B., Xue, C., Shen, W. C., and Wang, P. 2011, G-Protein signaling pathways: regulating morphogenesis and virulence of *Cryptococcus* in Cryptococcus: From Human Pathogen to Model Yeast, Heitman, J., Kozel, T. R., Kwon-Chung, K. J., Perfect, J. R., and Casadevall, A. (Eds.), ASM Press, Washington, DC, 153.

44. Nielsen, K. and Kwon-Chung, K. J. 2011, A role for mating in Cryptococcal virulence in Cryptococcus: From Human Pathogen to Model Yeast, Heitman, J., Kozel, T. R., Kwon-Chung, K. J., Perfect, J. R., and Casadevall, A. (Eds.), ASM Press, Washington, DC, 167.
45. Idnurm, A., Bahn, Y. S., Shen, W. C., Rutherford, J. C., and Muhlschlegel, F. A. 2011, Sensing extracellular signals in *Cryptococcus neoformans* in Cryptococcus: From Human Pathogen to Model Yeast, Heitman, J., Kozel, T. R., Kwon-Chung, K. J., Perfect, J. R., and Casadevall, A. (Eds.), ASM Press, Washington, DC, 175.
46. Pfaller, M. A., Lodge, J. K., and Ghannoum, M. A. 2011, Drug resistance in *Cryptococcus*: epidemiology and molecular mechanisms in Cryptococcus: From Human Pathogen to Model Yeast, Heitman, J., Kozel, T. R., Kwon-Chung, K. J., Perfect, J. R., and Casadevall, A. (Eds.), ASM Press, Washington, DC, 203.
47. Fox, D. S., Djordjevic, J. T., and Sorrell, T. 2011, Signaling cascades and enzymes as *Cryptococcus* virulence factors in Cryptococcus: From Human Pathogen to Model Yeast, Heitman, J., Kozel, T. R., Kwon-Chung, K. J., Perfect, J. R., and Casadevall, A. (Eds.), ASM Press, Washington, DC, 217.
48. Heung, L. J., Luberto, C., Plowden, A., Hannun, Y. A., and Del Poeta, M. 2004, J. Biol. Chem., 279, 21144.
49. Heung, L. J., Kaiser, A. E., Luberto, C., and Del Poeta, M. 2005, J. Biol. Chem., 280, 28547.
50. Garcia, J., Shea, J., Alvarez-Vasquez, F., Qureshi, A., Luberto, C., Voit, E. O., and Del Poeta, M. 2008, Mol. Sys. Biol., 4, 183.
51. Shea, J. M., Kechichian, T. B., Luberto, C., and Del Poeta, M. 2006, Infect. Immun., 74, 5977.
52. Kechichian, T. B., Shea, J., and Del Poeta, M. 2007, Infect. Immun., 75, 4792.
53. Rittershaus, P. C., Kechichian, T. B., Allegood, J. C., Merrill, A. H. Jr., Hennig, M., Luberto, C., and Del Poeta, M. 2006, J. Clin. Invest., 116, 1651.
54. Singh, A. and Del Poeta, M. 2011, Cell Microbiol., 13, 177.
55. Rhome, R. and Del Poeta, M. 2009, Annu. Rev. Microbiol., 63, 119.
56. Rhome, R. and Del Poeta, M. 2010, Adv. Exp. Med. Biol., 688, 232.
57. Shea, J. M. and Del Poeta, M. 2006, Curr. Opin. Microbiol., 9, 352.
58. McQuiston, T. J., Haller, C., and Del Poeta, M. 2006, Mini Rev. Med. Chem., 6, 671.
59. Del Poeta, M. 2004, Eukaryot. Cell, 3, 1067.
60. Rivera, J., Proia, R. L., and Olivera, A. 2008, Nat. Rev. Immunol., 8, 753.
61. Maceyka, M., Milstien, S., and Spiegel, S. 2009, J. Lipid Res., 50, Suppl S272.
62. Weigert, A., Weis, N., and Brune, B. 2009, Immunobiology, 214, 748.
63. Jolly, P. S., Rosenfeldt, H. M., Milstien, S., and Spiegel, S. 2002, Mol. Immunol., 38, 1239.
64. Garg, S. K., Volpe, E., Palmieri, G., Mattei, M., Galati, D., Martino, A., Piccioni, M. S., Valente, E., Bonanno, E., De Vito, P., Baldini, P. M., Spagnoli, L. G., Colizzi, V., and Fraziano, M. 2004, J. Infect. Dis., 189, 2129.
65. McQuiston, T., Luberto, C., and Del Poeta, M. 2010, Infect. Immun., 78, 2342.
66. McQuiston, T., Luberto, C., and Del Poeta, M. 2011, Microbiology, 157, 1416.
67. Huitema, K., van den Dikkenberg, J., Brouwers, J. F., and Holthuis, J. C. 2004, Embo J., 23, 33.
68. Tafesse, F. G., Huitema, K., Hermansson, M., van der Poel, S., van den Dikkenberg, J., Uphoff, A., Somerharju, P., and Holthuis, J. C. 2007, J. Biol. Chem., 282, 17537.
69. Tafesse, F. G., Ternes, P., and Holthuis, J. C. 2006, J. Biol. Chem., 281, 29421.
70. Villani, M., Subathra, M., Im, Y. B., Choi, Y., Signorelli, P., Del Poeta, M., and Luberto, C. 2008, Biochem. J., 414, 31.
71. Miskolci, V., Castro-Alcaraz, S., Nguyen, P., Vancura, A., Davidson, D., and Vancurova, I. 2003, Arch. Biochem. Biophys., 417, 44.
72. Graham, D. B., Robertson, C. M., Bautista, J., Mascarenhas, F., Diacovo, M. J., Montgrain, V., Lam, S. K., Cremasco, V., Dunne, W. M., Faccio, R., Coopersmith, C. M., and Swat, W. 2007, J. Clin. Invest., 117, 3445.

73. Gutierrez, M. G., Gonzalez, A. P., Anes, E., and Griffiths, G. 2009, Cell Microbiol., 11, 406.
74. Qureshi, A., Subathra, M., Grey, A., Schey, K., Del Poeta, M., and Luberto, C. 2010, PLoS One, 5, e15587.
75. Qureshi, A., Grey, A., Rose, K. L., Schey, K. L., and Del Poeta, M. 2011, Front. Microbiol., 2, 193.
76. Kovacs, J. A., Kovacs, A. A., Polis, M., Wright, W. C., Gill, V. J., Tuazon, C. U., Gelmann, E. P., Lane, H. C., Longfield, R., Overturf, G., Macher, A. M., Fauci, A. S., Parrillo, J. E., Bennett, J. E., and Masur, H. 1985, Ann. Intern. Med., 103, 533.
77. King, J. W. and DeWitt, M. L. 2010, Cryptococcosis. *eMedicine* (http://emedicine.medscape.com/article/215354-overview).
78. Lin, L., Ibrahim, A. S., Baquir, B., Fu, Y., Applebaum, D., Schwartz, J., Wang, A., Avanesian, V., and Spellberg, B. 2010, J. Infect. Dis., 201, 1708.