Semin Immunopathol (2015) 37:123–130
DOI 10.1007/s00281-014-0461-5

REVIEW

Candida albicans phospholipomannan: a sweet spot for controlling host response/inflammation

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Received: 15 July 2014 / Accepted: 4 November 2014 / Published online: 14 November 2014

Abstract Fungal cell walls contain several types of glycans, which play important roles in the pathogenesis of fungal infection and host immune response. Among them, glycosphingolipids have attracted much attention lately since they contribute actively to the fungi development and fungal-induced pathogenesis. Although glycosphingolipids are present in pathogenic and non-pathogenic fungi, pathogenic strains exhibit distinct glycan structures on their sphingolipids, which contribute to the regulatory processes engaged in inflammatory response. In *Candida albicans*, phospholipomannan (PLM) represents a prototype of these sphingolipids. Through its glycan and lipid moieties, PLM induces activation of host signaling pathways involved in the initial recognition of fungi, causing immune system disorder and persistent fungal disease. In this review, first we describe the general aspects of *C. albicans* sphingolipids synthesis with a special emphasize on PLM synthesis and its insertion into the cell wall. Then, we discuss the role of PLM glycosylation in regulating immune system activation and its contribution to the chronic persistent inflammation found in *Candida* infections and chronic inflammatory diseases.

Keywords Glycosphingolipids · Inflammatory response · Regulation · Yeasts

Thierry Jouault and Emerson Soares Bernardes contributed equally to this work

In memorandum to Pierre-André Trinel who discovered and characterized phospholipomannan from *Candida albicans*

This article is a contribution to the special issue on Immunopathology of Fungal Diseases - Guest editor: Jean-Paul Latge

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Introduction

Sphingolipids play an important role in the regulation of the balance between the microbe and the host. Some microorganisms such as bacteria and viruses do not produce sphingolipids, but are able to utilize the host's sphingolipids to promote their virulence, the host being the source of the sphingolipids. In the context of the protozoan- and fungus-host interactions, both host and pathogen sphingolipids can be involved. The determination of the mechanisms by which sphingolipid(s) (host, microbe, or both) modulate the host-parasite interaction is thus particularly important not only because it may provide important insights into the development of new therapeutic strategies but also because the outcome of this sphingolipid interaction may either lead to commensalism or to the dysregulation of the immune homeostasis. A microbial sphingolipid may interfere with host intracellular signaling and thereby alters the removal and destruction of the microbial cell or it may elicit an autoimmune response through molecular mimicry [1].

Although *Candida albicans* is the prominent opportunistic fungal pathogen causing mucocutaneous and systemic infections, little information is known about its sphingolipids except that a glucosylceramide has been described and that inositol phosphorylceramides (IPC), mannosylinositol phosphorylceramide (MIPC), and mannosyldiinositol phosphorylceramide (M $(IP)_2C$) have been characterized in its hyphal form [1]. In the search for new glycosylated

components of the *C. albicans* cell wall, a peculiar molecule with structural features of lipopolysaccharide was identified [2]. First named according to its molecular weight (14–18 kDa antigen), the description of its primary structure revealed the presence of phosphorus, lipid, and a large part of glycans composed of β-1,2-linked mannoses leading to rename this component as phospholipomannan (PLM) [3]. Nowadays, PLM is known as a mannosylated sphingolipid, sharing a glycan moiety composed of a long chain of β-1,2-linked mannoses similar to that present in the mannan of *C. albicans* linked to the lipid part of the molecule. Our knowledge about PLM structure has improved considerably and, recently, two important β-mannosyl transferases (Bmts) responsible for the elongation of the mannoside chain were identified [4].

Even though the role of PLM in yeast biology is still unknown, it has been evidenced in this pathogenic yeast suggesting a possible role for this glycolipid in the pathogenesis of *C. albicans*. PLM has been considered as a virulence factor of *C. albicans* whose activity is, at least partly, attributed to the presence of β-mannose. Therefore, this review will summarize our current knowledge about the role of PLM in the host-yeast cell relationship and the importance of the glycan moiety and the lipid core in inducing its biological activities. The purpose of this review is to analyze how the interaction of microbial surface molecules with host cell receptors may affect the course of the infection and to raise questions for further investigation with a special interest on their possible role in different context such as inflammatory chronic diseases.

Sphingolipids biosynthesis

Recent studies have highlighted the role of glycolipids, including sphingolipids, in the development of fungi and fungal-induced pathogenesis [1, 5, 6]. Sphingolipids are primary structural components of cell membranes and are bioactive molecules that act as second messengers in growth regulation [7]. They mediate activities as diverse as responses to stress [8, 9] and cell wall integrity [10]. Metabolism and function of sphingolipids from the MIPC family have been characterized in the non-pathogenic yeast *Saccharomyces cerevisiae* [10, 11] and will not be the focus of this review.

Sphingolipids are biosynthesized in the endoplasmic reticulum (ER) [12]. Palmitoyl-CoA is first condensed with serine by the serine palmitoyltransferase complex in the ER and the synthesis ends with the formation of ceramides, more precisely phytoceramides (Fig. 1). Fungal ceramide moieties contain phytosphingosine instead of the sphingosine found in mammals. They also present longer fatty acids linked to the phytosphingosine via amide bonds (C24–C26 instead of C16–C18). Depending on fungal species and growth conditions, the nature of the fatty acid may vary. Further modifications of phytoceramides such as glycosylation occur in the Golgi apparatus. Phytoceramides are transported from the ER to the Golgi apparatus by two pathways: an anterograde transport involving a vesicle coat protein, COPII, and a non-vesicular transport through ER-Golgi membrane contacts [13, 14]. In the yeast *S. cerevisiae*, once in the medial Golgi compartment, ceramide is converted into IPC by addition of phosphoryl-inositol, which is catalyzed by the IPC synthase Aur1 [8, 15, 16]. IPC is then transported to the Golgi lumen where it undergoes a mannosylation step to form MIPC. This reaction is catalyzed by two redundant IPC mannosyltransferases, Csg1 and Csh1 [17] which complex with the Ca^{2+}-binding protein, ScCsg2, certainly regulating the enzymes activity [18]. In the Golgi lumen, another inositol-phosphoryl transferase, Ipt1, adds a second phosphorylinositol group to MIPC that leads to the generation of M $(IP)_2C$ [8]. M $(IP)_2C$ is the major glycosphingolipid (GSL) of *S. cerevisiae*. Mannosylated and non-mannosylated IPC are secreted either to the plasma membrane or to the vacuole [19]. MIPC and M $(IP)_2C$ accumulate to the plasma membrane whereas IPC accumulates to the vacuole. *C. albicans* can synthesize IPC, MIPC, and M $(IP)_2C$ but displays some differences with *S. cerevisiae*. *C. albicans* has homologues of Aur1 and Ipt1, which were given the same names and are involved in IPC and M $(IP)_2C$ biosynthesis, respectively [20, 21]. However, *C. albicans* has only one IPC mannosyltransferases, Mit1, the homologue of Csg1, involved in the mannosylation of MIPC (Fig. 1).

Biogenesis of PLM: a *Candida albicans* glycosphingolipid

With PLM, a fourth class of myo-inositol containing sphingolipids was discovered in *C. albicans*. PLM is a phosphomannosylated MIPC, which has undergone several β-1,2 mannosylation steps [22, 23]. Like M $(IP)_2C$, PLM derives from MIPC as *mit1*Δ mutant does not have PLM. For PLM biosynthesis, MIPC is phosphomannosylated rather than linked to phosphoryl-inositol (Fig. 1) [23]. The enzyme involved in MIPC phosphomannosylation has not been described yet. Biosynthesis of *C. albicans* PLM is achieved when phosphomannosylated MIPC is further β-1,2 mannosylated [23]. PLM hyper mannosylation (up to 20 mannose units) seems crucial to relocate PLM from the plasma membrane to the cell wall and for its secretion in the environment [24]. The mechanisms involved are unknown but a change in the hydrophobicity of the molecule might be involved. β-1,2 oligomannosides also belong to the vast oligomannose repertoire of *C. albicans* cell wall phosphopeptidomannan (PPM) and mannoproteins [25, 26]. By contrast with α-mannosyltransferases, Bmts have not been identified in *S. cerevisiae*, which does not present any β-1,2 mannosides. Instead, Bmts were identified in another non-

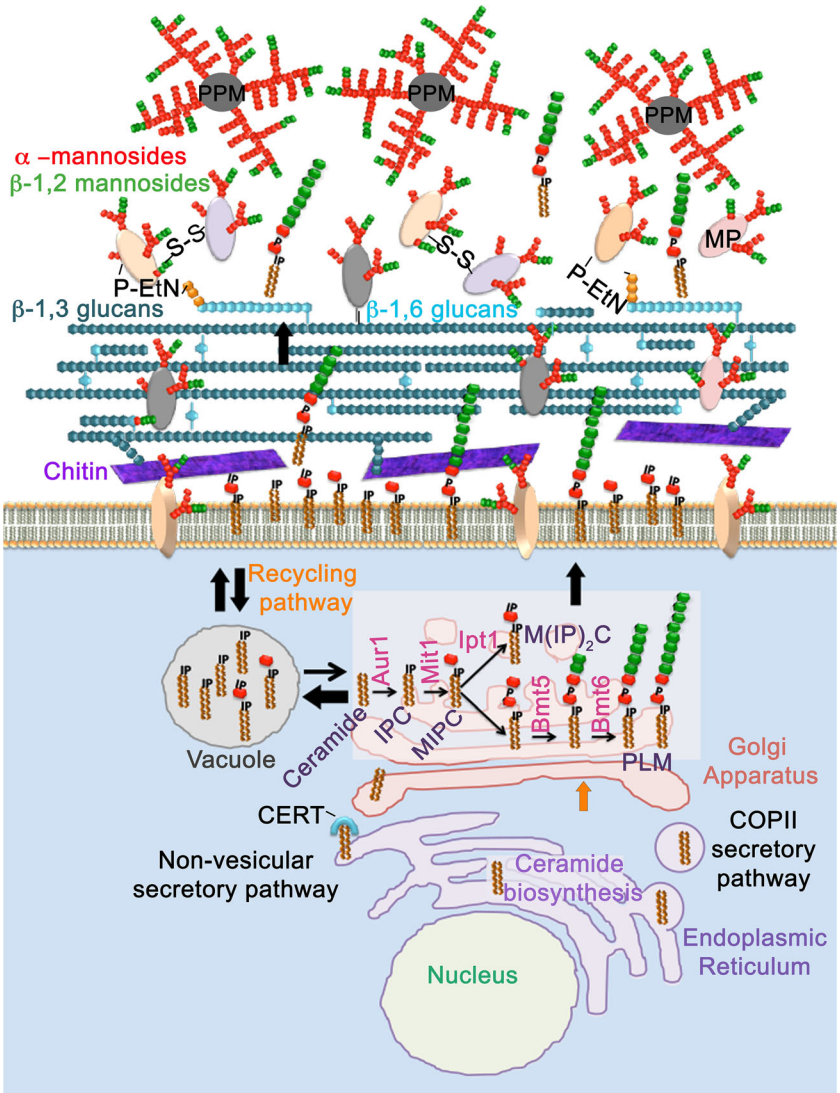

Fig. 1 *C. albicans* glycosphingolipids biosynthesis and transport from the endoplasmic reticulum to the plasma membrane and, for PLM, to the cell wall among other glycoconjugates. Ceramides are biosynthesized in the endoplasmic reticulum and are transported from the ER to the Golgi apparatus where they are converted into IPC by addition of phosphoryl-inositol. IPC is then transported through vesicular (COPII) or unvesicular pathway to the Golgi lumen where it undergoes a mannosylation step to form MIPC. A second phosphorylinositol group is transferred to MIPC leading to the generation of M $(IP)_2C$. IPC is mainly located in the vacuole whereas MIPC and M $(IP)_2C$ are accumulated in the plasma membrane. For PLM biosynthesis, MIPC is phosphomannosylated rather than linked to phosphoryl-inositol. Biosynthesis of PLM is achieved after β-1,2 mannosylation by Bmts5 and 6. PLM is then transferred from the Golgi to the cell membrane and to the yeast cell wall schematized on this figure. As PLM is shed to the culture medium, it is certainly distributed through the cell wall: both in the deep layer of the cell wall composed of chitin, β-1,6 and β-1,3 glucans, and mannoproteins and in the surface layer of the cell wall composed of phosphopeptidomannan and mannoproteins. PPM and mannoproteins carry α- and β-1,2 mannosides. However, additional studies are needed to investigate the exact location of PLM in the cell wall and to investigate the contribution of PPM, PLM, and mannoproteins to surface expression of β-1,2 mannosides

pathogenic yeast, *Pichia pastoris*, and then in *C. albicans* [27]. Bmts form a family of nine members that display a strong substrate and step of β-mannosylation specificities. Bmts1-4 are specific for PPM whereas Bmts5 and 6 β-1,2 mannosylate PLM (Fig. 2a) [4, 27]. Bmt1, Bmt2, and Bmt5 add the first β-mannose, Bmt 3 adds the second β-mannose, and Bmt4 and Bmt6 the third β-mannose and the following ones. It is not clear yet which Bmt adds the second β-mannose on PLM but it is assumed that Bmt5 can add both the first and the second β-mannose to PLM glycan such as Mnt1 and Mnt2 that are able to add both the first and the second α-mannose to mannoprotein O-glycans [28]. Bmt1 can, as already known, add the first β-mannose on its synthetic substrate but also, to a lesser extent, the second one [29]. Gene tree analysis of the nine identified Bmts highlights the proximity of enzymes involved in similar function such as Bmt2 and 5, which add the first mannose on phosphomannose and Bmt4 and 6, which are involved in the elongation of the phosphomannoside

chains (Fig. 2b). Regulation of the different Bmts activities at a transcriptional (*BMTs* expression) or posttranscriptional level contributes to heterogeneous expression of β-1,2 mannosides at the cell surface that depends on *C. albicans* strains and environmental factors [3, 25, 30]. Oddly enough, β-1,2 mannosylation of PPM and PLM are distinctly regulated [3, 30]. Expression of *BMTs* gene-encoding enzymes initiating PPM acid stable (Bmt1) and acid labile (Bmt2) fractions and PLM (Bmt5) was dependent on growth conditions and strains. Altogether, even if not constitutively expressed, expression of *BMT5* was less dependent on growth conditions compared to *BMT1* and *BMT2* (Fig. 2c). PLM β-1,2 mannosylation is certainly less disrupted by changes of environmental conditions than PPM.

PLM in fungal recognition

Glycosylation plays a major role in fungal-host cell interactions. Therefore, it is not surprising that the great majority fungus cell wall components are carbohydrates, displayed in diverse forms as represented in the Fig. 1: (1) phosphopeptidomannan (O-linked and N-linked α/β-mannosides); (2) β-glucans (β-1,3 glucan and β-1,6 glucan); (3) chitin, a β1,4-linked homopolymer of N-acetylglucosamine crosslinked to β-1,3 glucan; and (4) phospholipomannan (PLM), a glycosphingolipid composed of β-1,2-linked mannoses.

The recognition of such microbial glycan structures is achieved by a variety of receptors on the surfaces of epithelia and myeloid cells. These include toll-like receptors (TLRs), C-type lectins (CTLs), and Nod-like receptors (NLRs), which bind to specific (glycan) epitopes on the surface of pathogens [31, 32]. These so-called pathogen recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs) now form the basis of our understanding of innate immune recognition. For example, TLR2, TLR4, dectin-2, Mincle, DC-SIGN, and galectin-3 have major roles in the recognition of fungal mannans [33–37], and dectin-1 possibly associated to galectin-3, and complement receptor 3 (CR3) are the major PRRs involved in the detection of β-glucans [38–40].

Despite the well-known role of β-1,2-oligomannosides as adhesin and the pathogenicity factor of *C. albicans*, so far the role of PLM as a *C. albicans* adhesion molecule has not been established. In contrast, to other complex sphingolipids, such as IPC, MIPC, and M $(IP)_2C$, which are anchored to the plasma membrane, PLM can be shed through the cell wall. The glycan moiety of PLM confers the molecule a hydrophilic character that allows its localization in the cell wall. The contribution of PLM to the surface expression of β-1,2-oligomannosides needs nevertheless to be further investigated.

Galectin-3, a glycan-binding protein, is considered a macrophage receptor for β-1,2-oligomannosides. Indeed, galectin-3 is able to discriminate between *S. cerevisiae* and *C. albicans* by recognition of β-1,2-oligomannosides that are specifically and prominently expressed by *C. albicans* but not *S. cerevisiae* [41]. This property of galectin-3 is dependent on the association with TLR2, which is required for efficient uptake and endocytosis of both *C. albicans* and *S. cerevisiae*. However, since macrophage activation but not *C. albicans* endocytosis was affected by deletion of galectin-3, the interaction between PLM and galectin-3 may not account for the fungal adhesion [41]. Further experiments are in progress to address whether phagocytosis of *bmt6*Δ mutant of *C. albicans* is altered, which may shed light on the possible role of PLM in fungal adhesion to the host cells.

PLM β-1,2-mannosides/galectin-3 interaction and its role in macrophage activation

The role of PLM on macrophage activation has been consistently demonstrated over the last two decades. Indeed, PLM is a strong inflammatory stimulus, which is able to trigger NFκB activation and the production of pro-inflammatory cytokines in macrophages [42] and other cells [43]. However, only recently was demonstrated that PLM β-1,2-mannosides play a crucial role in macrophage activation [44]. On this regard, PLM isolated from *C. albicans* serotype A (whose mannosylated arm is composed of long chains of oligomannosides with up to 19 β-1,2-linked mannosides) induces a strong pro-inflammatory cytokine production. However, PLM from *C. albicans* serotype B (whose glycan moiety is composed of up to 10 mannosides with predominant trimannosides) did not stimulate the cells to produce TNF-α. In addition, PLM from *bmt6*Δ mutant of *C. albicans* (which presents only short chains of oligomannosides with up to three mannosides) did not stimulate the cells to produce TNF-α. Therefore, it is tempting to speculate that PLM activates macrophages through the specific recognition of β-1,2-mannosides by TLR2/galectin-3 complexes as it is the case for isolated β-1,2-mannosides [33, 45]. Surprisingly, galectin-3 enhanced PLM-induced macrophage stimulation possibly due to its capacity to form oligomeric complexes with PLM, favoring the interaction with cell surface receptors. However, this was only true in the case of PLM presenting long chain of β-1,2-mannosides, which are the target of the lectin. So far, it is not known whether the formation of complexes between PLM and galectin-3 would enhance secondary binding to TLR2 or to other glycosylated receptors at the cell surface. It could also be possible that galectin-3/PLM complexes bind to dectin-1 and TLR2, thus enhancing cell activation (Fig. 3). Interestingly, similar to PLM, exogenous galectin-3 was also shown to promote the attachment of

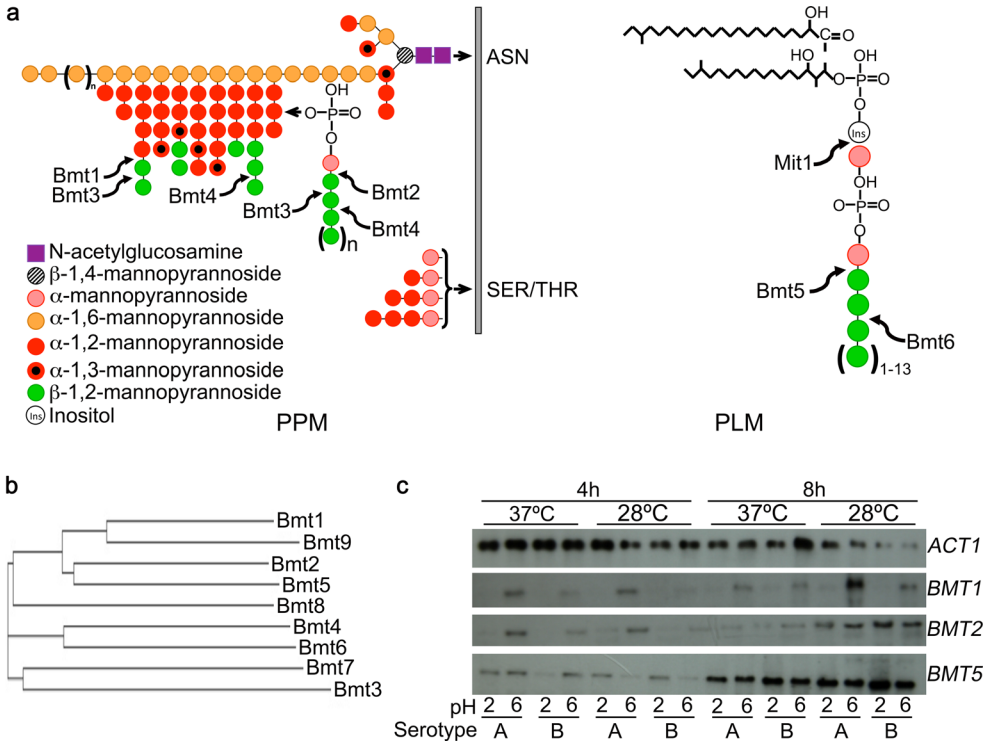

Fig. 2 Features of *C. albicans* β-1,2 mannosyltransferases (Bmts). **a** Six of the nine Bmts are involved in mannosylation of either phosphopeptidomannan (PPM) and phospholipomannan (PLM). Bmt1, Bmt2, and Bmt5 initiate the β-1,2 mannosylation steps on PPM acid stable or labile fractions or on PLM, respectively. Bmt3 adds the second β-mannose on PPM substrates whereas Bmt4 and Bmt6 add the third β-mannose and the following. **b** Neighbor-joining phylogenetic tree of the nine identified Bmts. This tree, based on the entire enzyme sequences, illustrates the relatedness of enzymes involved in similar function such as Bmt2 and 5, which add the first mannose on phosphomannose and Bmt4 and 6, which are involved in the elongation of the phosphomannose chains. **c** Regulation of the different β-mannosyltransferases gene transcription according to environmental conditions (temperature and pH) and the serotype of *C. albicans*, evidencing the absence of modulation of β-mannosyltransferase involved in mannosylation of PLM

bacterial lipopolysaccharide (LPS) to the cell membrane and to enhance the LPS-mediated neutrophil activation in vitro [46].

PLM glycan and immune system regulation

The interaction between microbial and host glycosphingolipids could explain many aspects of the immune system. As signaling molecules, sphingolipids can trigger actions and responses that would either prevent host damage from the microbe (or from the host itself) without the establishment of disease or favor the initiation of damage signals that could promote a pathological process. There have been recent successes in demonstrating that inhibition of microbe-utilized sphingolipids limits the pathogenicity of these organisms. Thus, a better understanding of sphingolipid-mediated microbial pathogenesis and how sphingolipid interactions can be modified to benefit the host may forge inroads into new therapeutic strategies and provide alternatives to traditional chemotherapeutic approaches to infectious disease.

PLM is able to modulate the immune system to favor the pathogen. Indeed, PLM has been shown to deregulate signaling pathways involved in yeast phagocytosis through its activity on the mitogen-activated protein kinase-1 (MKP-1), a specific phosphatase that modulates extracellular signal-regulated kinases (ERK) phosphorylation (Fig. 3). Through this process, PLM dephosphorylates ERK, which is activated during phagocytosis of yeast cells by macrophages [47] ending to the apoptosis of the macrophages that have engulfed the yeast and to the survival of engulfed yeast within the vesicles [48]. This process involves mainly the lipid part of the molecule since β-1,2-mannosides by themselves do not activate MKP-1 whereas isolated lipid moiety of PLM regulates ERK-phosphorylation (Ibatta-Ombeta, unpublished data). Another role in host cell regulation was recently demonstrated. PLM serves as a second stimulus for activation of inflammasome. Both mannosylated PLM and PLM bearing short chains of oligomannosides are able to activate caspase-1 in macrophages and to stimulate IL1β production by these cells (Fig. 3). PLM presenting long chain of β-mannosides is nevertheless a stronger activator of caspase-1 than PLM purified from *C. albicans* serotype B or from the *bmt6*Δ mutant. Thus, PLM-induced caspase-1 activation is not directly related to its glycosylation pattern and might be linked to the lipid core, which is similar in all PLMs.

Since PLM can be shed by yeast cells upon contact with macrophages [24] and patients with *Candida* sepsis display

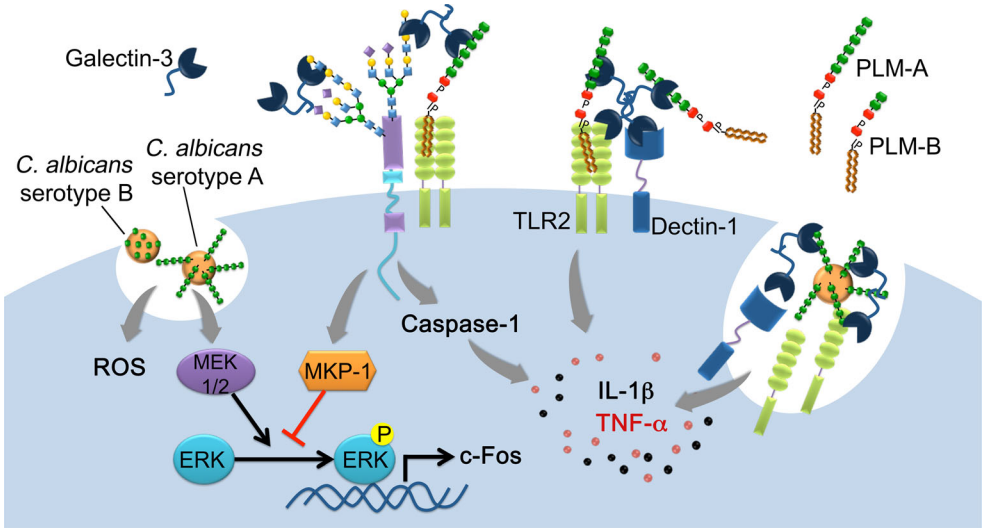

Fig. 3 The illustration shows the proposed mechanism for PLM-induced dysregulation of signaling pathways involved in phagocytosis of *C. albicans* serotype A (presenting long chain of β-1,2-mannosides) and serotype B (bearing short chains of oligomannosides). In addition, the proposed interaction of PLM/galectin-3 complexes with known cell surface receptors (TLR2 and dectin-1) and unknown glycoproteins is also illustrated. We believe that PLM/galectin-3 oligomeric complexes may favor the binding of PLM to TLR2 and/or to other glycosylated receptors, thus enhancing PLM-induced cell activation

elevated galectin-3 serum levels [49], it is tempting to suppose that the interaction between these two soluble molecules may contribute to exacerbate the inflammatory response during *Candida* infection. It has also been shown that *Candida parapsilosis* and *C. albicans* induce galectin-3 secretion from neutrophils [50] and human gingival epithelial cells [51] which functions as a proinflammatory autocrine/paracrine signal for these cells. Interestingly, recent data by Collette et al. [52] describe a small, aqueous, heat-stable compound of 3,000 Da secreted by the yeasts in contact with macrophages, which alters nitric oxide (NO) generation induced by LPS and IFN-γ. Whether PLM could participate in this mechanism leading to altered induction of iNOS (inducible nitric oxide synthase) remains to be examined.

Open issues

Although the presence of sphingolipids and sphingolipid-metabolizing enzymatic activities has been demonstrated in *C. albicans*, their role in the physiology, biology, and pathobiology of this human pathogen has yet to be elucidated. On the other hand, the description of PLM as a modulator of the immune cell function highlights the importance of these molecules in the pathogenesis of fungal infections and raises some unanswered questions. For example, soluble PLM is able to induce the production of pro-inflammatory cytokines by macrophages, but we still do not know how to explain the control mechanism(s) of PLM secretion. In addition, it is not known whether the interaction between soluble PLM and cell surface galectin-3 may somehow interfere with the M1 or M2 activation programs in macrophages. Galectin-3 has already been implicated in the regulation of M2 activation [53] whereas the specific role PLM plays in the macrophage M1/M2 polarization is still under investigation. Finally, since a link between *C. albicans* and Crohn's disease has already been established [54, 55], a possible role of PLM in triggering and/or exacerbating chronic inflammatory disorders is also under investigation.

Conclusions

The (glyco)sphingolipids are a family of (glyco)lipids that play essential roles both in the development of fungi and fungal-induced pathogenesis. The synthesis of (glyco)sphingolipids is very well characterized in yeast and represents a unique opportunity for the understanding of (glyco)lipid synthesis and for the search of potential inhibitors of various enzymatic steps in the biosynthetic pathway. We have demonstrated that PLM, a glycosphingolipid from *C. albicans*, is an important component of the inflammatory response against this pathogen. More important, *C. albicans* is able to evade the immune system and cause chronic persistent symptoms and we believe that PLM accounts at least in part for regulating the host immune response to favor survival of the fungus. Therefore, the characterization of the PLM biosynthesis pathway [4] and the further identification of selective inhibitors may open novel therapeutic perspectives to prevent invasive candidiasis in high-risk patients and/or pathogen-induced chronic inflammatory disorders [55].

Acknowledgments This work was supported by Inserm and by the European community's Seventh Framework Program (FP7–2007–2013) under grant agreement no. HEALTH-F2–2010–260338 'ALLFUN'. We would like to thank Sofia Nascimento dos Santos for her assistance on the preparation of the illustrations.

All the experiments described in the paper comply with the current laws of the country in which they were performed.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Heung LJ, Luberto C, Del Poeta M (2006) Role of sphingolipids in microbial pathogenesis. Infect Immun 74:28–39
2. Trinel PA et al (1993) Isolation and preliminary characterization of the 14- to 18-kilodalton *Candida albicans* antigen as a phospholipomannan containing beta-1,2-linked oligomannosides. Infect Immun 61:4398–43405
3. Trinel PA et al (2002) *Candida albicans* phospholipomannan, a new member of the fungal mannose inositol phosphoceramide family. J Biol Chem 277:37260–37271
4. Mille C et al (2012) Members 5 and 6 of the *Candida albicans* BMT family encode enzymes acting specifically on beta-mannosylation of the phospholipomannan cell-wall glycosphingolipid. Glycobiology 22:1332–1342
5. Mille C et al (2004) Inactivation of CaMIT1 inhibits *Candida albicans* phospholipomannan beta-mannosylation, reduces virulence, and alters cell wall protein beta-mannosylation. J Biol Chem 279:47952–47960
6. Bozza S, Clavaud C, Giovannini G (2009) Immune sensing of *Aspergillus fumigatus* proteins, glycolipids, and polysaccharides and the impact on Th immunity and vaccination. J Immunol 183: 2407-2414
7. Dickson RC et al (1990) Isolation of mutant *Saccharomyces cerevisiae* strains that survive without sphingolipids. Mol Cell Biol 10:2176–2181
8. Dickson RC et al (1997) Synthesis of mannose-(inositol-P)2-ceramide, the major sphingolipid in *Saccharomyces cerevisiae*, requires the IPT1 (YDR072c) gene. J Biol Chem 272:29620–29625
9. Jenkins GM et al (1997) Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae*. J Biol Chem 272: 32566–32572
10. Dickson RC, Sumanasekera C, Lester RL (2006) Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*. Prog Lipid Res 45:447–465
11. Cowart LA, Obeid LM (2007) Yeast sphingolipids: recent developments in understanding biosynthesis, regulation, and function. Biochim Biophys Acta 1771:421–431
12. Ko J, Cheah S, Fischl AS (1994) Regulation of phosphatidylinositol: ceramide phosphoinositol transferase in *Saccharomyces cerevisiae*. J Bacteriol 176:5181–5183
13. Funato K, Riezman H (2001) Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. J Cell Biol 155: 949–959
14. Perry RJ, Ridgway ND (2005) Molecular mechanisms and regulation of ceramide transport. Biochim Biophys Acta 1734:220–234
15. Levine TP, Wiggins CA, Munro S (2000) Inositol phosphorylceramide synthase is located in the Golgi apparatus of *Saccharomyces cerevisiae*. Mol Biol Cell 11:2267–2281
16. Nagiec MM et al (1997) Sphingolipid synthesis as a target for antifungal drugs. Complementation of the inositol phosphorylceramide synthase defect in a mutant strain of *Saccharomyces cerevisiae* by the AUR1 gene. J Biol Chem 272:9809–9817
17. Lisman Q et al (2004) Protein sorting in the late Golgi of *Saccharomyces cerevisiae* does not require mannosylated sphingolipids. J Biol Chem 279:1020–1029
18. Uemura S et al (2007) Regulation of the transport and protein levels of the inositol phosphorylceramide mannosyltransferases Csg1 and Csh1 by the $Ca2^{+}$-binding protein Csg2. J Biol Chem 282:8613–8621
19. Malathi K et al (2004) Mutagenesis of the putative sterol-sensing domain of yeast Niemann Pick C-related protein reveals a primordial role in subcellular sphingolipid distribution. J Cell Biol 164:547–556
20. Heidler SA, Radding JA (2000) Inositol phosphoryl transferases from human pathogenic fungi. Biochim Biophys Acta 1500:147–152
21. Prasad T et al (2005) Functional analysis of CaIPT1, a sphingolipid biosynthetic gene involved in multidrug resistance and morphogenesis of *Candida albicans*. Antimicrob Agents Chemother 49:3442–3452
22. Trinel PA et al (1999) The *Candida albicans* phospholipomannan is a family of glycolipids presenting phosphoinositolmannosides with long linear chains of beta-1,2-linked mannose residues. J Biol Chem 274:30520–30526
23. Trinel PA et al (2002) Beta-1,2-mannosylation of *Candida albicans* mannoproteins and glycolipids differs with growth temperature and serotype. Infect Immun 70:5274–5278
24. Jouault T et al (1998) Early signal transduction induced by *Candida albicans* in macrophages through shedding of a glycolipid. J Infect Dis 178:792–802
25. Fradin C et al (2008) Beta-1,2 oligomannose adhesin epitopes are widely distributed over the different families of *Candida albicans* cell wall mannoproteins and are associated through both N- and O-glycosylation processes. Infect Immun 76:4509–4517
26. Shibata N, Kobayashi H, Suzuki S (2012) Immunochemistry of pathogenic yeast, *Candida* species, focusing on mannan. Proc Jpn Acad Ser B Phys Biol Sci 88:250–265
27. Mille C et al (2008) Identification of a new family of genes involved in beta-1,2-mannosylation of glycans in *Pichia pastoris* and *Candida albicans*. J Biol Chem 283:9724–9736
28. Munro CA et al (2005) Mnt1p and Mnt2p of *Candida albicans* are partially redundant alpha-1,2-mannosyltransferases that participate in O-linked mannosylation and are required for adhesion and virulence. J Biol Chem 280:1051–1060
29. Fabre E et al (2014) Characterization of the recombinant *Candida albicans* beta-1,2-mannosyltransferase that initiates the beta-mannosylation of cell wall phosphopeptidomannan. Biochem J 457:347–360
30. Trinel PA et al (2005) *Candida albicans* serotype B strains synthesize a serotype-specific phospholipomannan overexpressing a beta-1,2-linked mannotriose. Mol Microbiol 58:984–998
31. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the Drosophila toll protein signals activation of adaptive immunity. Nature 388:394-397
32. Ariizumi K et al. (2000) Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. J Biol Chem 275:20157-20167
33. Fradin C, Poulain D, Jouault T (2000) Beta-1,2-linked oligomannosides from *Candida albicans* bind to a 32-kDa macrophage membrane protein homologous to the mammalian lectin galectin-3. Infect Immun 68:4391–4398
34. Tada H et al. (2002) *Saccharomyces cerevisiae*- and *Candida albicans*-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. Microbiol Immunol 46:503-512
35. Porcaro I et al. (2003) Mannose receptor contribution to *Candida albicans* phagocytosis by murine E-clone J774 macrophages. J Leukoc Biol 74:206-215

36. Rouabhia M et al (2005) Virulence of the fungal pathogen *Candida albicans* requires the five isoforms of protein mannosyltransferases. Infect Immun 73:4571–4580
37. McGreal EP et al (2006) The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. Glycobiology 16:422–430
38. Thornton BP et al. (1996) Analysis of the sugar specificity and molecular location of the beta-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18) J Immunol 156:1235-1246
39. Esteban A et al (2011) Fungal recognition is mediated by the association of dectin-1 and galectin-3 in macrophages. Proc Natl Acad Sci U S A 108:14270–14275
40. Brown GD, Gordon S (2001) Immune recognition. A new receptor for beta-glucans. Nature 413:36–37
41. Jouault T et al (2006) Specific recognition of *Candida albicans* by macrophages requires galectin-3 to discriminate *Saccharomyces cerevisiae* and needs association with TLR2 for signaling. J Immunol 177:4679–4687
42. Jouault T et al (2003) *Candida albicans* phospholipomannan is sensed through toll-like receptors. J Infect Dis 188:165–172
43. Li M, Chen Q, Shen Y, Liu W (2008) *Candida albicans* phospholipomannan triggers inflammatory responses of human keratinocytes through toll-like receptor 2. Exp Dermatol 18:603–810
44. Devillers A et al (2013) Deficient beta-mannosylation of *Candida albicans* phospholipomannan affects the proinflammatory response in macrophages. PLoS One 8:e84771
45. Jouault T, Lepage G, Bernigaud A et al (1995) Beta-1,2-linked oligomannosides from *Candida albicans* act as signals for tumor necrosis factor alpha production. Infect Immun 63:2378–2381
46. Fermino ML et al (2011) LPS-induced galectin-3 oligomerization results in enhancement of neutrophil activation. PLoS One 6:e26004
47. Ibata-Ombetta S et al (2001) Role of extracellular signal-regulated protein kinase cascade in macrophage killing of *Candida albicans*. J Leukoc Biol 70:149–154
48. Ibata-Ombetta S et al (2003) *Candida albicans* phospholipomannan promotes survival of phagocytosed yeasts through modulation of bad phosphorylation and macrophage apoptosis. J Biol Chem 278: 13086–13093
49. ten Oever J et al. (2013) Circulating galectin-3 in infections and non-infectious inflammatory diseases. Eur J Clin Microbiol Infect Dis 32: 1605-1610
50. Linden JR et al (2012) The role of galectin-3 in phagocytosis of *Candida albicans* and *Candida parapsilosis* by human neutrophils. Cell Microbiol 15:1127–1142
51. Tamai R, Kiyoura Y (2014) *Candida albicans* and *Candida parapsilosis* rapidly up-regulate galectin-3 secretion by human gingival epithelial cells. Mycopathologia 177:75–79
52. Collette JR, Zhou H, Lorenz MC (2014) *Candida albicans* suppresses nitric oxide generation from macrophages via a secreted molecule. PLoS One 9:e96203
53. MacKinnon AC et al (2008) Regulation of alternative macrophage activation by galectin-3. J Immunol 180:2650–2658
54. Standaert-Vitse A et al (2006) *Candida albicans* is an immunogen for anti-*Saccharomyces cerevisiae* antibody markers of Crohn's disease. Gastroenterology 130:1764–1775
55. Rehaume LM, Jouault T, Chamaillard M (2010) Lessons from the inflammasome: a molecular sentry linking *Candida* and Crohn's disease. Trends Immunol 31:171–175