



## Current relevance of fungal and trypanosomatid glycolipids and sphingolipids: studies defining structures conspicuously absent in mammals

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*Manuscript received on July 31 2008; accepted for publication on February 20, 2009;  
presented by LUIZ R. TRAVASSOS*

### ABSTRACT

Recently, glycosphingolipids have been attracting attention due to their role on biological systems as second messengers or modulators of signal transduction, affecting several events, which range from apoptosis to regulation of the cell cycle. In pathogenic fungi, glycolipids are expressed in two classes: neutral monohexosylceramides (glucosyl- or galactosylceramide) and acidic glycosylinositol phosphorylceramides (the latter class carries longer glycan chains). It is worth to mention that monohexosylceramides exhibit significant structural differences in their lipid moieties compared to their mammalian counterparts, whereas the glycosylinositol phosphorylceramides exhibit remarkable structural differences in their carbohydrate moieties in comparison to mammal glycosphingolipids counterpart. We observed that glycosylinositol phosphorylceramides are capable of promoting immune response in infected humans. In addition, inhibiting fungal glycosphingolipid biosynthetic pathways leads to an inhibition of colony formation, spore germination, cell cycle, dimorphism and hyphal growth. Other pathogens, such as trypanosomatids, also present unique glycolipids, which may have an important role for the parasite development and/or disease establishment. Regarding host-pathogen interaction, cell membrane rafts, which are enriched in sphingolipids and sterols, participate in parasite/fungal infection. In this review, it is discussed the different biological roles of (glyco)(sphingo)lipids of pathogenic/opportunistic fungi and trypanosomatids.

**Key words:** glycosphingolipids, glycosylinositol phosphorylceramides, inositol phosphorylceramide, *Leishmania*, membrane rafts, pathogenic fungi.

### INTRODUCTION

This review is part of the celebration on the 75 years of Escola Paulista de Medicina/Universidade Federal de São Paulo. The development and establishment of research on different areas of the carbohydrate chemistry and biology in this University can be attributed to the great effort of Professor Carl P. Dietrich, who dedicated

himself to fostering the growth of young scientists and the glycobiology field in Brazil. His pioneering contributions in mid 1970's on the structure and biological role of glycosaminoglycans and proteoglycans provided then a new concept of carbohydrate-to-carbohydrate interaction in eukaryotic cells that may define various cellular aspects, including oncogenic transformation and development (Dietrich et al. 1977, 1983, Dietrich 1984). However, it took close to 20 years, through the independent studies from several laboratories, to substantiate the functional significance of carbohydrate-to-carbohydrate interaction in the cell recognition and growth (Kojima

In commemoration of the 75<sup>th</sup> anniversary of  
Escola Paulista de Medicina/ Universidade Federal de São Paulo.  
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and Hakomori 1991, Haseley et al. 2001, Matsuura and Kobayashi 2004). That concept, although greeted by considerable skepticism at the time, finds its place in the current notion on the importance of glycoconjugates in cellular function. A typical example is the key role of carbohydrate moieties of glycosphingolipid-enriched microdomains (GEMs) in cell-cell interaction and cell-matrix interaction (Hakomori 2000). In this context, this review is focused on structural studies of fungal and trypanosomatid glycosylinositol phosphorylceramides and neutral glycosphingolipids, as well as their functional roles in the fungus/parasite-host interaction.

#### GLYCOSPHINGOLIPIDS FROM PATHOGENIC FUNGI

In 1969, Steiner et al. described for the first time in *Saccharomyces cerevisiae* a glycosylated-inositol phosphorylceramide found in a fungus. Since then, especially in the last decades, finding new structures and defining the biosynthesis of these and other glycolipids from pathogenic or opportunistic fungi have become crucial due to the increasing mortality of immunosuppressed patients associated with fungal infections. In several cases, death is a direct consequence of current antifungal therapy ineffectiveness (Odds et al. 2003, Moore et al. 2000), leading therefore for a compelling search for development of antifungal therapeutic agents.

In the last 15 years, our laboratory has purified and characterized over 20 glycosphingolipids (GSL) from several pathogenic and opportunistic fungi, such as *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Sporothrix schenckii*, *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Cr. laurentii* and *Cr. albidus* (Tables I and II). Our group and other investigators found two major classes of GSLs present in fungi, namely monohexosylceramides (CMHs) and glycosylinositol phosphorylceramides (GIPCs). CMH class comprises glucosylceramide (GlcCer) and galactosylceramide (GalCer), having (4*E*,8*E*)-9-methyl-4,8-sphingadienine as long chain base (d19:2) in combination with either *N*-2'-hydroxyoctadecanoate (h18:0) or *N*-2'-hydroxy-(*E*)-3'-octadecenoate (h18:1) [(*E*) -  $\Delta^3$ -unsaturated fatty acid]. Table I summarizes the expression of GlcCer and GalCer in several fungi, and it is noteworthy that CMHs from mycelium forms of *P. brasiliensis* and *H. capsulatum* present a higher percentage

of unsaturated fatty acids (Toledo et al. 1999, 2001c). In addition, we previously proposed that activation/deactivation of a desaturase, responsible for the unsaturation modification of *P. brasiliensis* GlcCer, could represent a key step in a signaling cascade leading to transition from yeast to mycelium (or *vice versa*) that is initiated by change in temperature (Toledo et al. 2001c). Moreover, while GlcCer was detected in all analyzed fungi, with exception of *S. cerevisiae* and *Candida glabrata* (Saito et al. 2006), GalCer was only detected in *A. fumigatus* and yeast forms of *S. schenckii* (Table I). Interestingly, for these two fungi, we observed a higher percentage of GalCer bearing unsaturated fatty acid compared to GlcCer, suggesting that galactosyltransferase poorly uses saturated form of ceramide as acceptor. On the other hand, glucosyltransferases may use equally well saturated and unsaturated ceramides as acceptors for GlcCer biosynthesis (Toledo et al. 1999) (Table I). Regarding only *S. schenckii*, results suggest that activation of GalCer-synthase enzyme follows the transition of mycelium to yeast, or on the contrary, suppression of this activity follows the transition of yeast to mycelium (Toledo et al. 2000), inferring that some fungi have the ability to use a wide range of ceramide and glycosyl structures in order to survive and proliferate in a variety of substrata and growth conditions, as well as in infectivity processes.

The other class of GSLs found in fungi comprises about 20 distinct GIPC structures, with the discovery rate increasing over the last 10 years. GIPCs are acidic glycosphingolipids, widely distributed among fungal species (Dickson and Lester 2002). These molecules present a ceramide (*N*-acylsphingosine) moiety linked to a glycan chain of variable length and structure through an inositol-phosphate linker. Compared to CMHs, GIPCs present a less variable ceramide portion, containing t18:0 and t20:0 4-hydroxysphinganine (phytosphingosines), which are only found in fungi, and an h24:0 fatty acid (Scheme 1, Product '1'). These results suggest a dichotomy at the biosynthesis level for GIPCs and CMHs (Leipelt et al. 2001).

At the monosaccharide level, three distinct cores were determined in fungal GIPCs: i) GlcN $\alpha$ 1-2Ins, ii) Man $\alpha$ 1-6Ins, and iii) Man $\alpha$ 1-2Ins (Scheme 1, Products '3', '5' and '7', respectively). The differential expression

**TABLE I**  
Neutral glycosphingolipids found in fungi.

Dimorphic	GlcCer	GalCer	References
<i>P. brasiliensis</i> Y	+	–	Toledo et al. 1999
M	+(> $\Delta^3$ )	–	
<i>H. capsulatum</i> Y	+	–	Toledo et al. 2001c
M	+(> $\Delta^3$ )	–	
<i>S. schenckii</i> Y	+	+(> $\Delta^3$ )	Toledo et al. 2000
M	+	–	Cardoso et al. 1987
<b>Yeast</b>			
<i>Candida spp</i>	+	–	Leverly et al. 2000
<i>Cryptococcus spp</i>	+	–	Leverly et al. 2000
<b>Hyphae</b>			
<i>A. fumigatus</i>	+	+(> $\Delta^3$ )	Toledo et al. 1999 Villas-Boas et al. 1994
<i>A. nidulans</i>	+	–	Leverly et al. 2002

Y – Yeast; M – Mycelium; (+) – indicates presence; (–) – indicates absence; (>  $\Delta^3$ ) – indicates a higher levels of (*E*) –  $\Delta^3$  unsaturation of the fatty acid.

**TABLE II**  
Acidic glycosphingolipids found in fungi.

Fungus	GIPC	Structure	Reference
<i>P. brasiliensis</i>	Pb-2	Man $\alpha$ 3Man $\alpha$ 2InsPCer	Leverly et al. 1998
	Pb-3	Gal <i>f</i> $\beta$ 6(Man $\alpha$ 3)Man $\alpha$ 2InsPCer	
<i>A. fumigatus</i>	Af-4	Man $\alpha$ 2Man $\alpha$ 3(Gal <i>f</i> $\beta$ 6)Man $\alpha$ 2InsPCer	Toledo et al. 2007
<i>S. schenckii</i>	Ss-Y6	Man $\alpha$ 3Man $\alpha$ 6GlcNH $_2$ $\alpha$ 2InsPCer	Toledo et al. 2001a

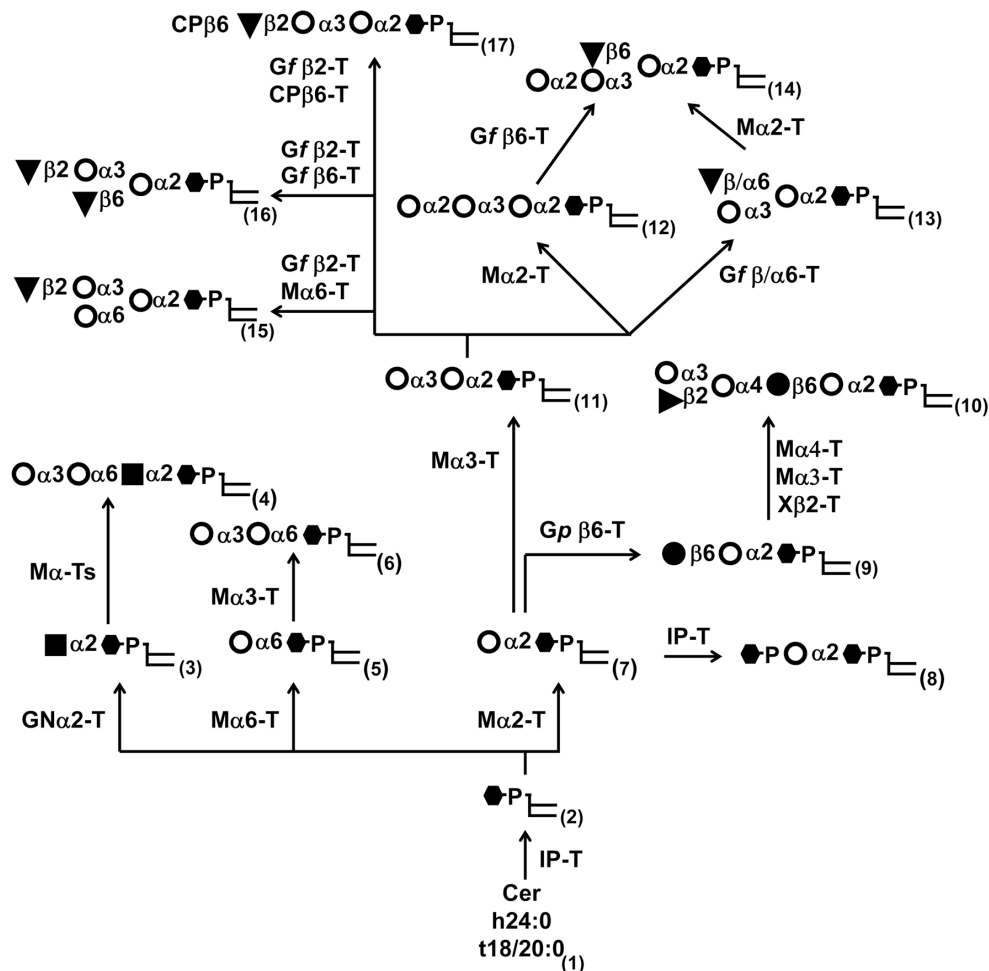
Ins = *myo*-inositol; P = phosphodiester; Cer = ceramide composed mainly by t18:0 4-hydroxysphinganine (phytosphingosine) and h24:0 fatty acid.

of these glycan cores seems to be dependent on fungal species and, apparently, strongly regulated during morphogenesis (Barr et al. 1984, Barr and Lester 1984, Leverly et al. 1998, Toledo et al. 2001a, b, 2007, Loureiro y Penha et al. 2001). While structure “iii” (Man $\alpha$ 2IPC) is the most common core, found from *S. cerevisiae* to a wide range of mycophatogens, to date, only *S. schenckii* seems to express all three cores in its GIPC structures (Toledo et al. 2001a, b), as seen on Table II. In fact, several GIPCs present long glycan moieties, which are built up, for example, on core “iii” (Scheme 1, Product 7), as it was described for *P. brasiliensis*, *H. capsulatum*, *S. schenckii*, *A. fumigatus*, *A. nidulans*, *C. albicans* and *Cr. neoformans*. Structure details are shown on Table II, and Scheme 1, which present a biosynthetic tree for the GIPCs known to date in fungi. Furthermore, recently,

more complex structures, built up on cores “i” (GlcN $\alpha$ 1-2Ins – product ‘4’, Scheme 1) and “iii” (Man $\alpha$ 1-2Ins – products ‘11’ to ‘17’, Scheme 1) have been found in *A. fumigatus* (Toledo et al. 2007, Simenel et al. 2008). As a result, together, these glycoconjugates constitute an extensive structural diversity that provides several evidences to propose different fungal glycolipids biosynthetic pathways, which can be inhibited by specific drugs without affecting mammal biosynthetic pathways.

#### INHIBITION OF FUNGAL GLYCOSPHINGOLIPID BIOSYNTHETIC PATHWAYS

Since fungal GSLs have unique structures not found in mammals and also are implicated in many fundamental cellular processes in fungi, including growth, differentiation and morphogenesis (Barr et al. 1984, Barr and



Scheme 1 – Glycosylinositol-phosphorylceramides from mycophagotogens. Scheme shows the possible biosynthetic pathways of different GIPCs already described in fungi. Product ‘1’ is the third set of ceramide expressed in fungi, yielding the synthesis of GIPCs. Product ‘1’ is the ceramide substrate to addition of inositol-phosphate, forming IPC (‘2’). Results gathered from the literature show three possible pathways using IPC as substrate to a series of specific glycosyl-transferases (T) shown at the scheme. The following GIPCs may be synthesized by: pathway **a** – leading to the synthesis of GlcNα2IPC (‘3’), which may be further glycosylated in GIPC ‘4’ (Toledo et al. 2001b, Aoki et al. 2004, Toledo et al. 2007); **b** – leading to the synthesis of Manα6IPC (‘5’), which may be further glycosylated in GIPC ‘6’ (Toledo et al. 2001b, Loureiro y Penha et al. 2001), and **c** – leading to the synthesis of a wide variety of GIPCs structures bearing the core Manα2IPC shown in ‘7’, which may be further glycosylated as follows: **i** – a second IP residue is added forming GIPC ‘8’ known as Man(IP)<sub>2</sub>Cer (Steiner et al. 1969); there is no description of longer chain derived from this structure; **ii** – may also be an addition of a galactopyranose residue leading to the synthesis of GIPC ‘9’, which may be further glycosylated by up to five specific mannose-T and a xylose-T, producing GIPCs described in *C. neoformans*; a common core is shown in ‘10’ (Heise et al. 2002); **iii** – a second residue of mannose may be added, leading to the synthesis of GIPC ‘11’; this product may also be further glycosylated by mannosea2-T (GIPC ‘12’) or galactofuranoseβ6-T (GIPC ‘13’); GIPC ‘14’ appears to be a product of glycosylation of the GIPC ‘12’ or ‘13’ (Toledo et al. 1995, 2001a, b, 2007, Barr et al. 1984, Barr and Lester 1984, Levery et al 1998, Bennion et al. 2003); GIPC ‘11’ is also glycosylated by a series of: **iv** – mannosea2-T and galactofuranoseβ2-T leading to the synthesis of GIPC ‘15’; **v** – galactofuranoseβ2/β6-T leading to the synthesis of GIPC ‘16’, and **vi** – galactofuranoseβ2-T and choline-phosphate-T leading to the synthesis of GIPC ‘17’, which was also described without the choline-phosphate unit. T = transferase; P = phosphate; C = Choline; Cer = Ceramide (=); ◆ = myo-inositol; ■ = Glucosamine (GN); o = Mannopyranose (M); ● = Galactopyranose (Gp); = Galactofuranose (Gf); ▼ = Xylose (X).

Lester 1984, Levery et al. 1998, 2002, Toledo et al. 2001a, b, 2007, Loureiro y Penha et al. 2001), blocking the biosynthesis of these GSLs has become a target for therapy development and understanding the fungal biology. Inhibitors for inositol phosphoceramide-synthase (IPC-synthase), for example, have been described in recent years. These enzymes catalyze the transfer of *myo*-inositol-1-phosphate from phosphatidylinositol to ceramide, resulting in synthesis of IPC (product '2', Scheme 1). Moreover, IPC-synthases are believed to be encoded by orthologous *AUR1/IPC1* genes, which were found in *S. cerevisiae*, *Schizosaccharomyces pombe*, *Candida* spp., *Aspergillus* spp. and *Cr. neoformans* (Nagiec et al. 1997, Hashida-Okado et al. 1996, 1998, Kuroda et al. 1999, Heidler and Radding 1995, 2000). Aureobasidin-A, khafrefungin and rustmicin are examples of IPC-synthase inhibitors, which present potent and selective antifungal activities, and also low toxicity in mammals, making them possible candidates as antifungal drugs (Takesako et al. 1993, Mandala et al. 1997, 1998, Georgopadakou 2000).

As shown by Levery et al. (2002), fungi expressing GlcCer are also highly susceptible to GlcCer-synthase inhibitors. *D-threo*-1-Phenyl -2-decanoylamino -3-morpholino -1-propanol (*D-threo* PDMP) (Radin et al. 1993) and its analogs, which have been widely applied for inhibition of mammalian GlcCer-synthase (GCS, UDP-Glc:ceramide  $\beta$ -glucosyltransferase) and are also capable to inhibit GlcCer-synthase in *A. fumigatus* and *A. nidulans*, affecting spore germination, cell cycle and hyphal growth. Furthermore, it is worth to mention that *A. fumigatus* expresses both GlcCer and GalCer and, therefore, theoretically, this fungus could prevent build up of free ceramide by utilizing it for synthesis of GalCer in the presence of *D-threo*-PDMP. However, it was observed that *A. fumigatus* susceptibility to this GlcCer-synthase inhibitor is similar to fungi expressing GlcCer only, suggesting that GlcCer performs other relevant functional roles in fungi which are not simply related to maintaining normal ceramide levels. Recently, we also observed that GlcCer-synthase inhibitors are able to strongly inhibit colony formation, spore germination, cell cycle and yeast to mycelium transformation in *P. brasiliensis*, *H. capsulatum*, *S. schenckii* and *C. neoformans*, clearly showing the importance of glucosyl-

ceramide on fungal biology. Therefore, it should be emphasized that these results indicate that the metabolic pathway of GlcCer synthesis represents a viable way to block fungal growth and differentiation, and since several structural aspects in fungal CMH lipid moiety differ of those from mammals (Levery et al. 2000), this glycolipid biosynthetic pathway should be explored with drugs that do not affect mammal GlcCer expression.

#### IMMUNE-RESPONSES TO FUNGAL GSLs

In addition to the importance of these glycolipids on the biology of the fungus, some of these glycoconjugates have shown to be highly antigenic. By HPTLC immunostaining, it was detected several GIPCs from *H. capsulatum*, *P. brasiliensis* and *A. fumigatus* reactive with sera of patients with histoplasmosis, paracoccidiodomycosis and aspergillosis (Barr and Lester 1984, Toledo et al. 1995, 2007, Bertini et al. 2007). By using immunochemical assays, it was observed that high antigenicity was due to presence of terminal galactofuranose residues in these glycolipids (Toledo et al. 1995, 2007, Bertini et al. 2007). Our group also demonstrated that GIPC Pb-3 (former Pb-1), which bears a terminal galactofuranose residue, is able to promote, in patients with paracoccidiodomycosis, a primary immune response with IgM production and subsequent switching to IgG1 (Bertini et al. 2007). We also observed that, after 5 months of antifungal treatment, there was a decline of symptoms and IgG1 titers, which makes Pb-3 a good marker for monitoring disease treatment. Since these antibodies may bind specifically to Fc $\gamma$  receptors of phagocytes and as Pb-3 is expressed on yeast surface (Suzuki et al. 1997), IgG1 antibodies could play an important role in fungal clearance by promoting phagocytes to remove *P. brasiliensis* from the organism.

Regarding the cellular immune response, we analyzed the effect of purified preparations of glycosphingolipids from *P. brasiliensis*, *A. fumigatus* and *S. schenckii* on murine T lymphocytes. Four pure GIPCs (Pb-3, Pb-2, Af-4 and Ss-Y6) and two pure CMHs (GlcCer and GalCer) from different fungi were tested by measuring concanavalin A (Con A)-induced [ $^3$ H]-thymidine uptake by normal BALB/c mouse lymphonode cells (Giorgio et al. 1992, 2003). Our analysis revealed that all four GIPCs inhibited T lymphocyte proliferation *in vitro* with

IC<sub>50</sub> values  $\leq 6\mu\text{M}$ . It was observed that the most efficient inhibitors were Pb-3, Pb-2, Af-4 and Ss-Y6, reaching 50% of inhibition with a concentration of  $\sim 5\mu\text{M}$ . On the other hand, neutral GSLs as CMH (GlcCer and GalCer) from fungi and from mammals reached 50% of inhibition in a concentration of  $\sim 20\mu\text{M}$ . These results clearly demonstrate that fungal glycolipids are able to inhibit T lymphocyte proliferation efficiently in a dose-dependent manner without inducing cell death, and also to elicit antibody production, suggesting that these GSLs may play an important role in modulating the host immune response.

#### FUNGI-HOST INTERACTION MECHANISMS: ROLE OF MEMBRANE RAFTS

For infection establishment in vertebrate host, some fungi also trigger host cell signaling pathways, which may lead to fungal invasion into host cells, host cell apoptosis or modulation of host immune response (see reviews Mendes-Giannini et al. 2005, Filler and Sheppard 2006, Netea et al. 2008). Belanger et al. (2002) and Monteiro da Silva et al. (2007), for example, showed that *C. albicans* and *P. brasiliensis* invasion was associated to activation of host tyrosine kinases activation. On the other hand, some events, such as apoptosis, may be activated or blocked depending on the species of fungi studied (Filler and Sheppard 2006, Mendes-Giannini et al. 2005). These final events may probably be due to the interaction between fungi and host molecules, such as extracellular matrix proteins, receptors, or even cell domains, such as membrane rafts (Maza et al. 2008, Netea et al. 2008, Filler and Sheppard 2006, Mendes-Giannini et al. 2005).

Recently, membrane rafts (also known as lipid rafts) were defined as small, cholesterol- and sphingolipid-enriched cell membrane domains (10–200 nm), which are highly dynamic, and where lipids and proteins move in and out, leading to cell signaling modulation events (Pike 2006). In addition, several membrane rafts present different lipid or protein compositions, leading consequently to distinct cell functions (Rajendran and Simons 2005, Zajchowski and Robbins 2002, Pike 2003).

Regarding pathogen-host relationship, in the last decade, several groups have demonstrated that bacte-

ria, virus and protozoa, such as *Listeria monocytogenes*, *Pseudomonas aeruginosa*, HIV and *Plasmodium falciparum*, interact with mammal host cell through membrane rafts. This leads to infection in the host organism by manipulating cell signaling events like rearrangement of the cytoskeleton or even fusion of phagosomes with lysosomes (see review by Mañes et al. 2003). Concerning to fungi, by using several approaches, such as membrane raft disruption with methyl- $\beta$ -cyclodextrin (m $\beta$ CD) and localization in cell membrane of the ganglioside GM1, a membrane raft marker, we demonstrated for the first time that epithelial cell membrane rafts are essential for *P. brasiliensis* adhesion to host cells, and as well as for activation of host cell protein kinases, such as Src-family kinases (Maza et al. 2008). The role of these and other kinases activation in *P. brasiliensis*-epithelial cell interaction is currently under investigation in our laboratory.

Membrane rafts are also present in fungi. We observed that membrane domain fractions from *H. capsulatum* and *P. brasiliensis* showed an enrichment of GIPCs, CMH, ergosterol and proteins, such as Pma1p, a known yeast membrane raft marker. In addition, it was verified that disruption of fungal membrane rafts promoted an inhibition by 40% of *H. capsulatum* infection in alveolar macrophages, suggesting that proteins and lipids organization integrity of *H. capsulatum* membrane rafts is essential to yeast-host cell interaction. These results indicate that the integrity of membrane microdomains of *H. capsulatum* would be essential in the process of macrophage infectivity by this fungus. Besides, the cell membrane organization and the identification of the components of these microdomains will contribute to clarify the mechanisms involved in the adhesion and late invasion of macrophages by *H. capsulatum* (L. Tagliari et al. unpublished data).

#### LEISHMANIA (GLYCO)(SPHINGO)LIPIDS: BIOLOGICAL ROLE AND ORGANIZATION

Our group has also been working extensively in characterizing and studying the organization and biological role of (glyco)(sphingo)lipids of *Leishmania* spp., the etiologic agent of leishmaniasis, which may present cutaneous lesions (localized or diffuse) or fatal visceral infection, depending on the *Leishmania* species. *Leishma-*

*nia* comprises two forms in its life cycle: promastigotes, found in the midgut of sandflies, and amastigotes, the obligatory intracellular form that proliferate inside of an acidified vacuole of mammal macrophages. Amastigotes of *L. (L.) amazonensis*, species that causes human cutaneous leishmaniasis, present on the parasite surface several GSLs, which are recognized by three monoclonal antibodies (mAbs), termed ST-3, ST-4 and ST-5. These mAbs were able to inhibit up to 80% of macrophage invasion by amastigotes, suggesting that GSLs are essential for *L. (L.) amazonensis* binding to macrophages (Straus et al. 1993, 1997). Moreover, Gal $\beta$ 1-3Galp disaccharide, which is part of one of the *L. (L.) amazonensis* amastigote GSLs, Gal $\beta$ 1-3Gal $\alpha$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-1Cer (referred herein as  $\beta$ -Gal-globotriaosylceramide), recognized by mAb ST-3, was also able to inhibit ~40% of macrophage invasion by these parasites, corroborating therefore the importance of these GSLs in *L. (L.) amazonensis* amastigote infection (Straus et al. 1993, 1997, Tanaka et al. 2007). Recently, a possible receptor for *L. (L.) amazonensis* amastigote GSLs was identified as being a 30 kDa protein from macrophage (Tanaka et al. 2007). Interestingly, this protein was not able to bind to *Leishmania (Leishmania) chagasi* amastigotes, which cause a visceral type of leishmaniasis and lack  $\beta$ -Gal-globotriaosylceramide, suggesting that GSLs of *L. (L.) amazonensis* amastigotes confer a particular specificity towards the host (Tanaka et al. 2007).

On the other hand, GSLs or glycoinositolphospholipid (GIPL) reactive with mAbs ST-3, ST-4 and ST-5 are absent in *L. (L.) amazonensis* promastigotes. Instead, these forms present other glycoconjugates, known as lipophosphoglycan (LPG) and proteophosphoglycan (PPG) which have epitopes recognized by mAbs ST-3, ST-4 and ST-5 (V.B. Valero et al., unpublished data). LPG and PPGs, along with glycosylphosphatidylinositol (GPI)-anchored proteins, and GIPLs are the major glycoconjugates found among several species of *Leishmania* (Descoteaux and Turco 1999, Guha-Niyogi et al. 2001, Handman 1999, Rittig and Bogdan 2000, Silveira et al. 2005, Suzuki et al. 2002, Spath et al. 2003). Recently, our group has demonstrated for *L. (L.) amazonensis* that GSLs and sterols of amastigotes, and GIPLs, sterols and IPC of promastigotes are preferentially dis-

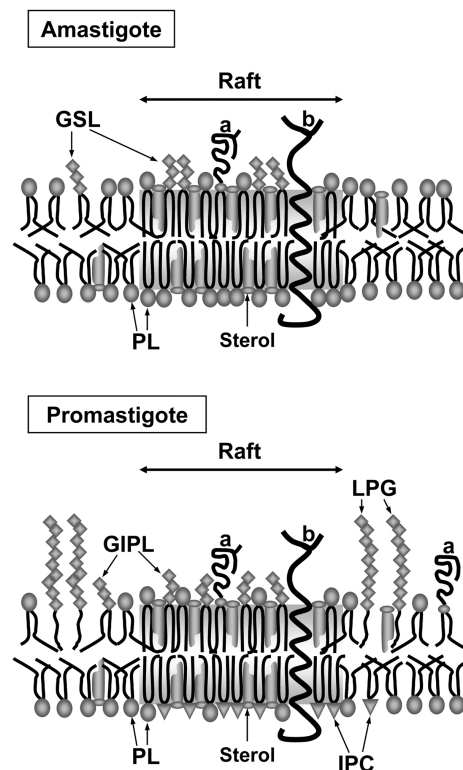


Fig. 1 – Distribution model of membrane raft components in *L. (L.) amazonensis*. This scheme shows membrane raft domains that are enriched in sphingolipids and sterols. Membrane rafts also may present transmembrane (b) or GPI-anchored (a) proteins, which could play important roles in cell signaling. In amastigotes, the major membrane raft components are GSLs and sterols. It is noteworthy that, after ultracentrifugation in sucrose density gradient, membrane raft-containing fractions present several proteins (52, 60, 140 and 160 kDa), which were detected by Western blot using sera from mice infected with *L. (L.) amazonensis*. Membrane rafts of promastigotes contain IPC and sterols as main constituents. As in amastigotes, in promastigote rafts, it was detected several proteins with different apparent molecular mass (46, 50, 62 and 92 kDa). (A.K. Tanaka et al., unpublished data). Abbreviations: GSL, glycosphingolipid; PL, phospholipid; GIPL, glycoinositolphospholipid; LPG, lipophosphoglycan; IPC, inositol phosphorylceramide.

tributed in membrane rafts (Fig. 1) (Suzuki et al. 2008). In a similar fashion, different species of *Leishmania*, *L. (V.) braziliensis* also presents membrane rafts enriched in sterol and IPC, in addition to phosphatidylinositol (PI) and phosphatidylethanolamine (PE) (Yoneyama et al. 2006). Treatment of *L. (V.) braziliensis* promastigotes with m $\beta$ CD, a membrane raft disruptor, inhibited

50% of macrophage infection when compared to non-treated parasites, suggesting that *Leishmania* membrane rafts participate in this interaction.

One major sphingolipid found in promastigotes of *Leishmania* is IPC (Kaneshiro et al. 1986, McConville and Bacic 1989). Recently, we demonstrated that Aureobasidin-A (AbA), an IPC synthase inhibitor, was able to decrease growth rate of promastigotes of *L. (L.) amazonensis*, *L. (L.) major* and *L. (V.) braziliensis*, and to decrease dramatically the number of amastigotes of *L. (L.) amazonensis* per infected macrophage (Tanaka et al. 2007, Suzuki et al. 2008). In addition, AbA-treated promastigotes were viable, suggesting that IPC is important for cell division events. On the other hand, as IPC were not detected in *L. (L.) amazonensis* amastigotes by conventional methods, and since these forms were highly susceptible to this drug, one could speculate that IPC, even in very low amounts, could be a key compound for the parasite survival in the host cell.

As for fungi, glycolipids containing terminal galactofuranose (Gal $f$ ) units are also present in *L. (L.) major*, and in other trypanosomatids, as *Trypanosoma cruzi*. In addition to being highly antigenic (patients with Chagas' disease present anti-Gal $f$  antibodies) (Golgher et al. 1993), these glycolipids play an important role in parasite-host interaction. For instance, Fab fragments of mAb MEST-1, which recognizes terminal Gal $f$  on GIPL-1 of *L. (L.) major* promastigotes, were able to inhibit the promastigotes and amastigotes invasion into murine macrophages (Suzuki et al. 2002). Moreover, for *T. cruzi*, Nogueira et al. (2007) demonstrated that Gal $f$ -containing GIPCs are important for epimastigote forms-midgut interaction with host cells, differentiation and proliferation, and Bertini et al. (2003) showed that trypomastigotes Gal $f$ -containing glycoproteins are important for Vero cells invasion by this form of *T. cruzi*.

Therefore, as discussed in this review, (glyco)-(sphingo)lipids, present in trypanosomatids and in pathogenic/opportunistic fungi, may be crucial for their interaction with host cells, differentiation, proliferation and host immune response. Understanding the mechanisms involved in these events, such as membrane raft formation, since its initial assembly at Golgi complex and endoplasmic reticulum, dynamic organization and glycolipid biosynthetic pathways may contribute to open new

vistas leading to the discovery of new and more efficient therapies.

#### CONCLUDING REMARKS

More than 20 glycosylinositol phosphorylceramides with new glycan sequences, as well as monohexosylceramides with unique modifications in the ceramide moiety, were described in pathogenic/opportunistic fungi in the last 10 years by our laboratory and other groups. The remarkable structural diversity of GIPCs observed in fungi is built up from three different cores: GlcN $\alpha$ 1-2Ins, Man $\alpha$ 1-2Ins and Man $\alpha$ 1-6Ins. Since carbohydrate determinants are synthesized in stepwise fashion in which each monosaccharide is transferred from a sugar nucleotide donor to the acceptor molecule (Tolmasky et al. 1982), it will be possible to assign glycosyltransferases not being present in mammals as potential targets for more efficient therapies. In a similar fashion, since several structural aspects of the lipid moiety of fungal CMH differ significantly of those from mammals (Leverly et al. 2000), this glycolipid biosynthetic pathway is an attractive target to be explored aiming the development of new drugs that will block fungal growth, but will not affect mammalian GlcCer expression. It is worth to mention that, similarly to mammal cells, fungal GIPCs and CMHs are not scattered in the plasma membrane but preferentially located in the glycosphingolipid enriched membrane microdomains (GEMs).

We also described that glycolipids play an important role in macrophage invasion by different species of *Leishmania*. These glycolipids are located in membrane rafts and disruption of such membrane microdomains with methyl- $\beta$ -cyclodextrin reduces significantly parasite infectivity. The importance of IPC-containing glycolipids on *L. (L.) amazonensis*, *L. (L.) major* and *L. (V.) braziliensis* assessed using AbA promoted a significant decrease in the parasite growth and a striking effect in the macrophage infection by *L. (L.) amazonensis*.

Based on the present results, it is possible to conclude that fungal and parasite membrane microdomains preferentially accumulate specific glyco(sphingo)lipids and other proteins/glycoproteins involved in adhesion, signal transduction, modulation of cellular and humoral responses of the host and other vital activities. Thus, a detailed knowledge on the structure of fungal and par-



asite glycolipids may provide ground to develop more specific and sensitive reagents/drugs for immunodiagnosis and treatment of systemic mycosis and leishmaniasis.

#### ACKNOWLEDGMENTS

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundo de Auxílio aos Docentes e Alunos da Universidade Federal de São Paulo (FADA-UNIFESP).

#### RESUMO

Recentemente, glicosíngolípídeos têm atraído atenção devido ao seu papel na biologia celular como segundo-mensageiro ou moduladores da transdução de sinal, afetando vários eventos, desde apoptose até a regulação do ciclo celular. Em fungos patogênicos, existem duas classes de glicolípídeos: monohexosil ceramidas neutras (glucosil- ou galactosilceramida) e glicosilinositol fosforilceramidas (os quais apresentam cadeias de carboidratos mais longas). É importante enfatizar que as monohexosil ceramidas exibem diferenças estruturais nas suas porções lipídicas quando comparadas às de mamíferos, enquanto que glicosilinositol fosforilceramidas exibem diferenças estruturais marcantes em suas porções carboidratos em comparação aos glicosíngolípídeos de mamíferos. Observamos também que glicosilinositol fosforilceramidas são capazes de promover resposta imune em indivíduos infectados. Além do mais, inibição das vias biossintéticas de glicosíngolípídeos de fungos acarreta a inibição da formação de colônias, germinação de esporos, ciclo celular, dimorfismo e crescimento de hifas. Outros patógenos, como os tripanosomatídeos, também apresentam glicolípídeos únicos, os quais apresentam um papel importante para o desenvolvimento do parasita e/ou para o estabelecimento da doença. Em relação à interação hospedeiro-patógeno, os “membrane rafts”, estruturas da membrana plasmática enriquecidas em esfingolípídeos e esteróis, têm participação fundamental na infecção do parasita/fungo. Nesta revisão, discutimos os diferentes papéis biológicos dos (glico)(esfingo)lipídeos de fungos patogênicos/opportunistas e de tripanosomatídeos.

**Palavras-chave:** glicosíngolípídeos, glicosilinositol-fosforilceramidas, inositol fosforilceramidas, *Leishmania*, “membrane rafts”, fungos patogênicos.

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