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Leishmania (Mundinia) species differ in glycoconjugate composition and induce variable levels of NO *via* TLR2 and TLR4

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ABSTRACT

Leishmania (Mundinia) species have attracted increasing attention due to their ancestral phylogenetic position, broad distribution, and ability to exploit non-sand fly vectors. However, the structure and immunomodulatory role of their surface glycoconjugates remain poorly understood. We comparatively characterized the biochemical and functional properties of glycoconjugates from representative *Mundinia* species during interaction with murine macrophages. Lipophosphoglycans (LPGs) and glycoinositolphospholipids (GIPLs) were extracted, purified, and preliminary characterized from *Leishmania enriettii, L. orientalis, L. martiniquensis,* and *L. macropodum*. Immunoblotting analysis of LPG using MG1 antibody detected different levels of glucose side chains branching-off the repeat units of *Mundinia* species, except *L. enriettii*. In contrast, GIPLs were conserved and galactosylated (suggestive of type II) by fluorophore-assisted carbohydrate electrophoresis (FACE). Structural polymorphisms were assessed in relation to nitric oxide (NO) production by murine macrophages, including TLR2- and TLR4-dependent responses. Both glycoconjugates induced variable levels of NO production *via* TLR4, with secondary involvement of TLR2. Although we found polymorphisms in the sugar moieties, a clear correlation between structure and activity could not be found.

1. Introduction

Mundinia is the most recently established subgenus in *Leishmania*, formally proposed in 2016 and currently regarded as the most basal lineage of the genus [1]. Phylogenetic and genomic studies support the division of *Leishmania* into four subgenera – *Leishmania*, *Viannia*, *Sauroleishmania*, and *Mundinia* – highlighting the evolutionary distinctiveness of the latter [2]. Although its taxonomy has undergone recent revisions, whole genome sequencing (WGS) analyses have consistently confirmed the phylogenetic placement of several species within this subgenus [3]. Since its initial description, additional species have been formally recognized, and *Mundinia* currently comprises at least six representatives with a broad geographic distribution: *Leishmania* (*M.*) *enriettii* [4], *L.* (*M.*) *macropodum* [5], *L.* (*M.*) *martiniquensis* [6], *L.* (*M.*) *orientalis* [7], *L.* (*M.*) *chancei* and *L.* (*M.*) *procaviensis* [8,9]. This taxonomic expansion, together with increasing reports of *Mundinia*-associated infections, has positioned this subgenus as an emerging group of human/veterinary pathogens and a valuable model for investigating evolutionary trajectories within Trypanosomatidae [3,5,10,11]. Notably, the ecological and biological diversity observed across these species – including variation in host range and transmission modes – suggest that key molecular traits may exhibit lineage-specific adaptations.

Representative species of the *Mundinia* subgenus are recognized as etiological agents of both visceral (VL) and cutaneous leishmaniasis (CL) in immunocompetent and immunocompromised individuals [7,12–15]. However, for most species, the transmission cycle remains poorly understood, particularly regarding vector competence and mammalian reservoirs [10,11,16]. In Brazil, guinea pigs (*Cavia porcellus*), a domesticated species of Andean origin, are the only confirmed hosts of *L. enriettii*, while the role of native Caviidae, such as *Galea spixii* or *C. aperea*, remains hypothetical [17,18]. Infected *C. porcellus* develop cutaneous lesions with abundant parasitized macrophages [4], and the infection may transiently visceralize in spleen, lungs, and trachea [19].

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Outside South America, *L. macropodum* was described in red kangaroos (*Osphranter rufus*) in Australia, and its transmission is believed to involve biting midges of the genus *Forcipomyia* [20]. Experimental studies further demonstrated that *Culicoides sonorensis* can support the development and transmission of some *Mundinia* species [21,22]. Although it was unable to transmit *L. enriettii*, other sand flies such as *Pintomyia monticola* may act as a suspected vector in Brazil [23]. Despite these advances, important gaps remain in the epidemiology of *L. enriettii*, particularly in natural transmission cycles. A recent study failed to detect this parasite in wild hosts in Paraná, reinforcing the hypothesis that *C. porcellus* may be its primary reservoir [24], which is consistent with its apparent refractoriness to other *Mundinia* species [25].

Despite the plethora of molecular and epidemiological data, relatively little is known about the biochemical composition of *Mundinia* surface molecules and their role in host–parasite interactions. Glycoconjugates (LPG and GIPL) from *L. enriettii* have been characterized and were shown to be pro-inflammatory *via* TLR4/TLR2 in murine macrophages [26]. Here, as part of a wider study on *Mundinia* biology, we proposed a preliminary and functional analysis of their glycoconjugates during interaction with murine macrophages.

2. Methods

2.1. Parasite culture and molecular typing

Four World Health Organization reference strains of *L. enriettii* (MCAV/BR/1945/L88), *L. orientalis* (MHOM/TH/2014/LSCM4), *L. martiniquensis* (MHOM/MQ/92/MAR1), and *L. macropodum* (MMAC/AU/2004/AM-2004) were used. Promastigotes were cultured at 25 °C in M199 medium supplemented with 10% foetal bovine serum (FBS) (Invitrogen/Thermo Fisher Scientific, Carlsbad, USA), penicillin (200 U/mL), and streptomycin (200 µg/mL) (both from Merck KGaA, Darmstadt, Germany), as previously described [26]. Parasite species was confirmed by molecular typing targeting the ITS1 intergenic region [27]. The resulting sequences were deposited in the GenBank database (PV816353–PV816356).

2.2. Immunoblotting analyses of LPGs

LPGs and GIPLs were extracted from late log-phase promastigotes and purified by phenyl-Sepharose chromatography using ESOAK (H_2O/ethanol/diethyl ether/pyridine/NH4OH; 15:15:5:1:0.017) [26]. Purified LPGs were resuspended in endotoxin-free water and analyzed by immunoblotting. For western-blot analysis, 5 µg of LPG was applied to 0.45 µm nitrocellulose membranes (Amersham Protran, Marlborough, USA), blocked for 1 h with 5% skimmed milk, and incubated overnight with mAb CA7AE (1:1000), which recognizes the Gal(β1,4)Man(α1)-PO_4 epitope [28], and mAb MG1 (1:1000), specific for β-glucose side chains [29]. Membranes were washed three times in PBS (3 × 5 min) and incubated for 1 h with peroxidase-conjugated anti-mouse IgG (1:10,000) (Bio-Rad, Hercules, CA, USA).

2.3. Preliminary biochemical analyses of glycoinositolphospholipids

To determine monosaccharide composition, depolymerized and desalted samples were fluorescently labelled with 0.1 M AMAC (2-aminoacridone) in 5% acetic acid and 1 M cyanoborohydride (Sigma-Aldrich/Merck, St. Louis, USA). Labelled sugars were analysed by fluorophore-assisted carbohydrate electrophoresis (FACE) and visualized under UV light. Monosaccharides (D-galactose, D-glucose, and D-mannose) were used as standards [26,30].

2.4. Ethics and functional assays

Experimental animals were maintained at the Animal Facility of the Instituto René Rachou (Fiocruz Minas). All procedures were conducted in accordance with Brazilian regulations, including the guidelines of the Brazilian College for Animal Experimentation (COBEA; Law 11.794/2008), and were approved by the Institutional Animal Ethics Committee (CEUA; protocol LW-15/22). Thioglycolate-elicited peritoneal macrophages from C57BL/6 mice (WT) and respective knockouts for TRL2 (-/-) and TLR4 (-/-) were used. After injection of 2 mL of sterile 3% thioglycolate solution, they were recovered 72 h later by sequential peritoneal washes using cold RPMI medium. Cell viability was assessed by trypan blue exclusion. A total of 3×10^5 cells per well were seeded in 96-well plates and incubated for 1 h at 37 °C in a 5% CO_2 atmosphere for adhesion. Adherent cells were primed with interferon-gamma (IFN-γ, 3 U/mL) for 18 h and subsequently stimulated with LPGs (10 µg/mL) from each *Leishmania* (*Mundinia*) strain for 48 h. Lipopolysaccharide (LPS, 100 ng/mL) and *Staphylococcus aureus* extract were used as positive controls for TLR4 (-/-) and TLR2 (-/-), respectively [26]. Nitrite (NO) levels were determined by the Griess reaction. All incubations were performed at 37 °C in a humidified 5% CO_2 atmosphere. Culture media were supplemented with 2 mM glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin, and 10% foetal bovine serum [26].

2.5. Statistical analysis

Data normality was assessed using the Shapiro–Wilk test. Normally distributed data were analysed using one-way analysis of variance (ANOVA) followed by Student's *t*-test, as appropriate. Statistical analyses were performed using GraphPad Prism v. 8.0 (GraphPad Software, San Diego, USA). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. LPGs from Mundinia species displays variations in glucosylation levels based on antibody reactivity

Following extraction and purification, LPG repeat units were analysed by immunoblotting using mAbs CA7AE and MG1. Most species were detected by CA7AE, except *L. orientalis* (Fig. 1A). As expected, LPG from *L. infantum* (type I, positive control) was also recognized (Fig. 1A). With MG1, an interesting cline from *L. macropodum* (fainted reaction) to *L. orientialis* (strong reaction) was observed suggesting different levels of glucosylation (Fig. 1B). As expected, *L. enriettii* LPG, who is devoid of side chains [26] was not recognized by MG1. Consistent with those observations, glucosylation decreased reactivity with CA7AE suggesting that side-chains branching-off the repeat unit backbone hindered access to this antibody (Fig. 1A). Altogether, these results indicate the presence of glucose side chains in LPGs from all *Mundinia* species analysed, except *L. enriettii*.

3.2. GIPLs from Mundinia spp displayed no variations in monosaccharide composition

To investigate monosaccharide composition, GIPLs were analysed by FACE. revealing a predominance of galactose residues, with no major qualitative differences among species (Fig. 1C). The four *Mundinia* GIPLs are predominantly composed of galactose (galactosylated), resembling type II-like features. Notably, a reduced level of galactosylation was observed in GIPLs from *L. macropodum* compared to the other *Mundinia* species, even when a threefold higher amount of material was loaded (Fig. 1C, arrows).

3.3. Glycoconjugates elicited different levels of nitric oxide (NO) production

To evaluate whether LPGs/GIPLs affect macrophage function, purified glycoconjugates were added to IFN-γ-primed thioglycolate-elicited peritoneal macrophages. All LPGs and GIPLs induced NO production,

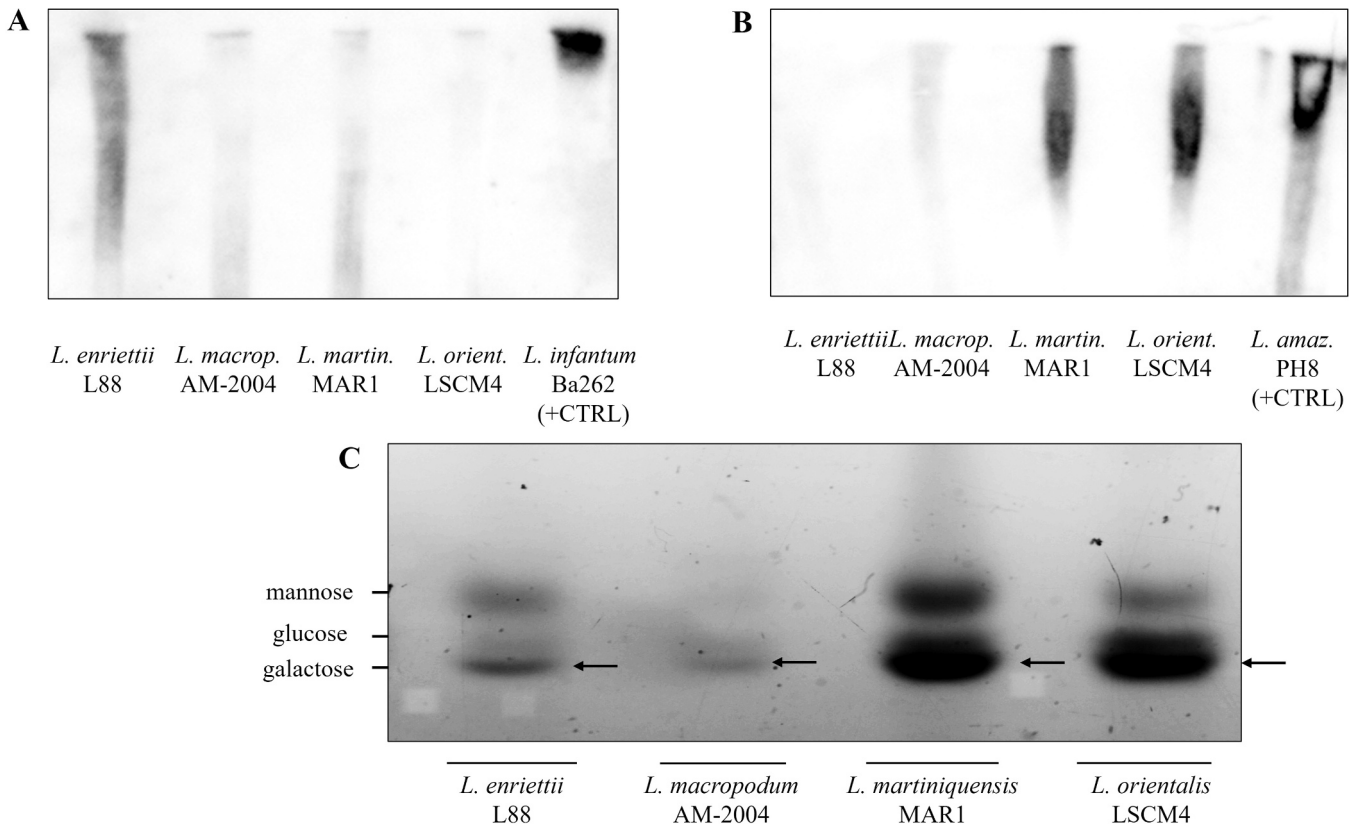


Fig. 1. Immunoblotting of purified liposphosphoglycans (LPGs). Purified LPGs (5 ug/lane) from *Mundinia* spp were probed with mAbs CA7AE e MG1 (1:1000) for western-blots (panels A and B). LPGs purified from *L. amazonensis* (PH8) and *L. infantum* (Ba262) were used as positive controls. Fluorophore-assisted carbohydrate electrophoresis (FACE) of monossacharides derived from glycoinositolphospholipids (GIPLs) (panel C) from *Mundinia* spp. Arrows indicate galactosylation.

primarily *via* TLR4 and secondarily *via* TLR2. *L. enriettii* LPG induced the highest NO levels across all mouse lineages (WT, TLR2$^{-/-}$, TLR4$^{-/-}$), whereas *L. macropodum* LPG/GIPLs induced the lowest NO levels in knockout mice. In WT macrophages, LPGs and GIPLs triggered variable NO levels. As expected, *L. infantum* LPG/GIPLs and LPS (positive control for TLR4) induced NO via TLR4, while *S. aureus* extract (positive control for TLR2) stimulated NO via TLR2 (Figs. 2A and 2B).

4. Discussion

Leishmania glycoconjugates (LPG/GIPLs) play a pivotal role during the interaction not only with vertebrate but also invertebrate hosts. LPG is widely recognized as a multifunctional virulence factor with immunomodulatory properties, whereas GIPLs have been associated with inhibitory or regulatory effects on host responses [31]. Over the past decades, considerable effort has been devoted to understanding how structural variations in these molecules influence host–parasite interactions. In this context, *Mundinia* species provide a valuable underexplored system. Here, we focused on preliminary biochemical and functional analysis of *L. enriettii, L. macropodum*, *L. orientalis* and *L. martiniquensis* glycoconjugates.

The first biochemical characterization of glycoconjugates in a *Mundinia* species showed that *L. enriettii* LPG lacks side chains (type I), while its GIPLs are highly galactosylated (suggestive of type II) [26]. It is important to mention that these data are preliminary, and FACE analysis are not sufficient to confirm linkage types. This should await further confirmation by other techniques including methylation analysis and NMR. Type I LPGs have also been described in several other *Leishmania* species, including *L infantum* [32], *L. donovani* [33], *L. braziliensis* [34–36] and *L. amazonensis.*[37].

Here, we found interspecies polymorphisms in the LPGs of *Mundinia* species indicating an increase in glucosylation from *L. macropodum* to *L. orientalis* based on MG1 reactivity. Glucosylated LPGs are commonly found in other *Leishmania* species, including *L. infantum* [32,38] and *L. amazonensis* [39]. The finding of variable glucose branching off the repeat units of some *Mundinia* LPGs represents an interesting feature of this group. On the other hand, no qualitative differences in GIPLs were detected among species, but the galactose content in *L. macropodum* appeared to be lower. Together, these data indicate that *Mundinia* glycoconjugates combine conserved features with lineage-specific structural adaptations.

We next investigated whether these structural differences translate into functional variation during interaction with host immune cells. Pioneering studies indicated that TLR2 is the receptor for *L. major* LPG [40]. Subsequentially, particularly for the New World *Leishmania*, we demonstrated that both TLR4 and TLR2 are important for macrophage activation, especially in *L. infantum* [41], *L. braziliensis* [35] and *L. amazonensis* [37]. Regarding *L. enriettii*, TLR2/TLR4 were shown to be important for NO and cytokine production involving MAPK signalling and NF-kB translocation [26].

A distinguished feature of *L. enriettii* LPGs/GIPLs was that they were the most proinflammatory glycoconjugates inducing the production of TH1-associated mediators including NO, TNF-α, IL-6 and IL-12. The cutaneous lesions of *L. enriettii* start to heal after 6 weeks post-infection and this may be linked to a higher stimulation of the immune system [19,26,42]. Here, this pattern was shown among *Mundinia* species, where not only LPGs but also GIPLs from *L. enriettii* were the most proinflammatory. Interestingly, *L. macropodum* LPG/GIPLs had the lowest ability to induce NO production in murine macrophages. A clear correlation between structural polymorphisms in the sugars and functional activity could not be ascertained for all species. For example, *L. enriettii* LPG (devoid of glucose sidechains) induced the highest NO

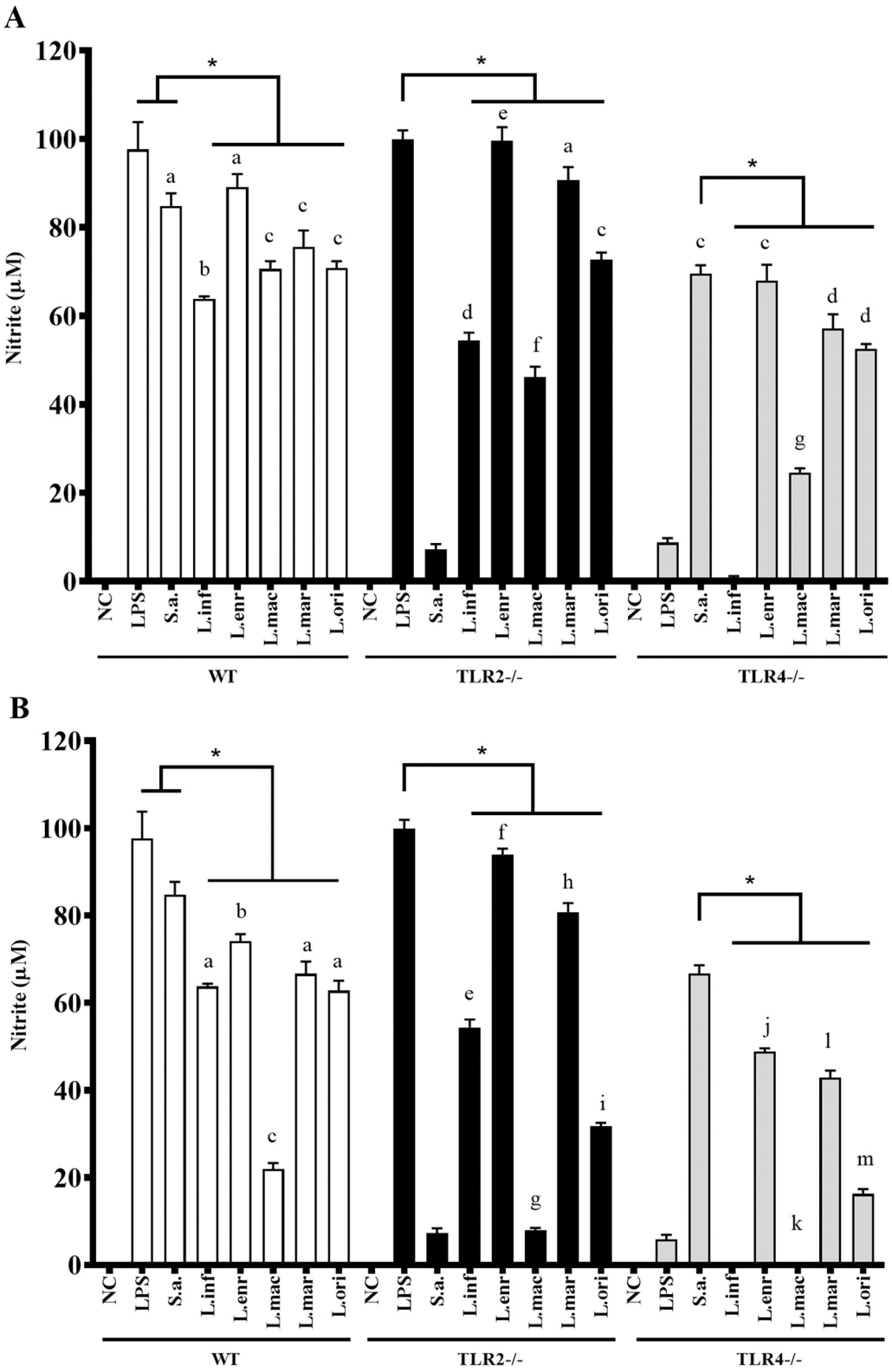


Fig. 2. Nitrite production (uM) by murine macrophages stimulated by lipophosphoglycans (A) and glycoinositolphospholipids (GIPLs) (10 µg/mL) from *Mundinia* spp. Wildtype (WT, white bars), TLR2 (-/-) (dark bars) and TLR4 (-/-) (grey bars) knockouts. Legend: NC, negative control; LPS, lipopolysaccharide (TLR4 agonist, 100 ng/mL); S.a., *Staphylococcus aureus* (TLR2 agonist, 100 ng/mL); L. inf., *L. infantum* LPG; L. enr., *L. enriettii*; L. mac., *L. macropodum*; L. mar., *L. martiniquensis*; L, ori, *L. orientalis*. Asterisks and different letters indicate statistical differences ($P < 0.05$). The same letters above bars do not indicate statistical differences ($P > 0.05$).

levels compared to glucosylated LPGs. Similarly, GIPLs also had variations in NO induction although no clear polymorphisms were detected in the sugar moieties. This led us to hypothesize that those variations cannot be explained based only on sugar moieties, but also to differences in the lipid cores or a combination of both.

Despite belonging to the same subgenus *Mundinia*, *L. enriettii*, *L. orientalis*, *L. martiniquensis*, and *L. macropodum* display biochemical and functional polymorphisms in their surface glycoconjugates. These reinforce that structural diversity in *Mundinia* glycoconjugates contributes directly to species-specific immunomodulatory profiles, likely influencing host–pathogen interactions, disease outcomes, and perhaps vector competence.

CRediT authorship contribution statement

Rodrigo Pedro Soares: Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Vyacheslav Yurchenko:** Writing – original draft, Validation, Investigation, Conceptualization. **Petr Volf:** Writing – original draft, Validation, Investigation, Conceptualization. **Paula Monalisa Nogueira:** Writing – original draft, Methodology, Investigation, Conceptualization. **Felipe Dutra-Rêgo:** Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supporting information

All relevant data supporting the findings of this study are included within the manuscript. DNA sequences were deposited in the GenBank database (PV816353-PV816356). The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

[1] O.A. Espinosa, M.G. Serrano, E.P. Camargo, et al., An appraisal of the taxonomy and nomenclature of trypanosomatids presently classified as Leishmania and Endotrypanum, Parasitology 145 (2018) 430–442.
[2] A.Y. Kostygov, A. Karnkowska, J. Votýpka, et al., Euglenozoa: taxonomy, diversity and ecology, symbioses and viruses, Open. Biol. 11 (2021) 200407.
[3] A.Y. Kostygov, A.T.S. Albanaz, A. Butenko, et al., Phylogenetic framework to explore trait evolution in Trypanosomatidae, Trends Parasitol. 40 (2024) 96–99.
[4] J. Muniz, H. Medina, Cutaneous Leishmaniasis in the Guineapig, Hospital 33 (1948) 7–25.
[5] A.M. Dougall, B. Alexander, D.C. Holt, et al., Evidence incriminating midges (Diptera: Ceratopogonidae) as potential vectors of Leishmania in Australia, Int. J. Parasitol. 41 (2011) 571–579.
[6] N. Desbois, F. Pratlong, D. Quist, et al., Leishmania (Leishmania) martiniquensis n. sp.(Kinetoplastida: Trypanosomatidae), description of the parasite responsible for cutaneous leishmaniasis in Martinique Island (French West Indies), Parasite 21 (2014) 12.
[7] N. Jariyapan, T. Daroontum, K. Jaiwong, et al., Leishmania (Mundinia) orientalis n. sp.(Trypanosomatidae), a parasite from Thailand responsible for localised cutaneous leishmaniasis, Parasit. Vectors 11 (2018) 351.
[8] G. Kwakye-Nuako, M.-T. Mosore, D. Boakye, et al., Description, biology, and medical significance of Leishmania (Mundinia) chancei n. sp.(Kinetoplastea: Trypanosomatidae) from Ghana and Leishmania (Mundinia) procaviensis n. sp. (Kinetoplastea: Trypanosomatidae) from Namibia, J. Parasitol. 109 (2023) 43–50.
[9] H. Almutairi, M.D. Urbaniak, M.D. Bates, et al., Chromosome-Scale Assembly of the Complete Genome Sequence of Leishmania (Mundinia) procaviensis Isolate 253, Strain LV425, Microbiol. Resour. Announc. 12 (2023) e01306-22.
[10] L.F. Paranaiba, L.J. Pinheiro, A.C. Torrecilhas, et al., Leishmania enriettii (Muniz & Medina, 1948): A highly diverse parasite is here to stay, PLoS. Pathog. 13 (2017) e1006303.
[11] P. Volf, J. Sadlova, Mysterious Leishmania martiniquensis parasites and their relatives of the subgenus Mundinia: Emerging pathogens reshaping our understanding of leishmaniases, PLoS. Pathog. 22 (2026) e1014057.
[12] L. Bualert, W. Charungkiattikul, P. Thongsuksai, et al., Autochthonous disseminated dermal and visceral leishmaniasis in an AIDS patient, southern Thailand, caused by Leishmania siamensis, Am. J. Trop. Med. Hyg. 86 (2012) 821.
[13] C. Supsrisunjai, T. Kootiratrakarn, P. Puangpet, et al., Disseminated autochthonous dermal leishmaniasis caused by Leishmania siamensis (PCM2 Trang) in a patient from central Thailand infected with human immunodeficiency virus, Am. J. Trop. Med. Hyg. 96 (2017) 1160.
[14] S. Anugulruengkitt, W.N. Songtaweesin, N. Thepnarong, et al., Case report: simple nodular cutaneous leishmaniasis caused by autochthonous Leishmania (Mundinia) orientalis in an 18-month-old girl: the first pediatric case in Thailand and literature review, Am. J. Trop. Med. Hyg. 108 (2022) 44.
[15] G. Kwakye-Nuako, M.-T. Mosore, C. Duplessis, et al., First isolation of a new species of Leishmania responsible for human cutaneous leishmaniasis in Ghana and classification in the Leishmania enriettii complex, Int. J. Parasitol. 45 (2015) 679–684.
[16] L.F. Paranaiba, L.J. Pinheiro, D.H. Macedo, et al., An overview on Leishmania (Mundinia) enriettii: biology, immunopathology, LRV and extracellular vesicles during the host–parasite interaction, Parasitology 145 (2018) 1265–1273.
[17] F. Dutra-Rêgo, M.S. Otto, A.C.V.M.R. Lima, et al., Expanding the known geographic range of Leishmania (Mundinia) enriettii: clinical and molecular characterization in naturally infected guinea pigs from southern Brazil, Pesqui. Veter-.-. ária Bras. 45 (2025) e07652.
[18] V.B. Richini-Pereira, P.M. Marson, E.Y. Hayasaka, et al., Molecular detection of Leishmania spp. in road-killed wild mammals in the Central Western area of the State of São Paulo, Brazil, J. Venom. Anim. Toxins Incl. Trop. Dis. 20 (2014) 27.
[19] E.V. Alves-Sobrinho, L. de J. Pinheiro, L.F. Paranaíba, et al., Leishmania enriettii visceralises in the trachea, lungs, and spleen of Cavia porcellus, Mem. Inst. Oswaldo Cruz 117 (2022) e220065.
[20] E. Panahi, M. Shivas, S. Hall-Mendelin, et al., Utilising a novel surveillance system to investigate species of Forcipomyia (Lasiohelea)(Diptera: Ceratopogonidae) as the suspected vectors of Leishmania macropodum (Kinetoplastida: Trypanosomatidae) in the Darwin region of Australia, Int. J. Parasitol. Parasites Wildl. 12 (2020) 192–198.
[21] V. Seblova, J. Sadlova, B. Vojtkova, et al., The biting midge Culicoides sonorensis (Diptera: Ceratopogonidae) is capable of developing late stage infections of Leishmania enriettii, PLoS. Negl. Trop. Dis. 9 (2015) e0004060.
[22] T. Becvar, B. Vojtkova, P. Siriyasatien, et al., Experimental transmission of Leishmania (Mundinia) parasites by biting midges (Diptera: Ceratopogonidae), PLoS. Pathog. 17 (2021) e1009654.
[23] E. Luz, M. Giovannoni, A.M. Borba, Infecção de Lutzomyia monticola por Leishmania enriettii, Fac. Med. Univ. Fed. Paraná 9 (1967) 121–128.
[24] V.T. Soccol, E.A. de Castro, L. Reifur, et al., Guinea pigs naturally infected by Leishmania enriettii: clinical analyses, parasite isolation and identification, Braz. Arch. Biol. Technol. 64 (2021) e21210095.
[25] T. Becvar, P. Siriyasatien, P. Bates, et al., Development of Leishmania (Mundinia) in guinea pigs, Parasit. Vectors 13 (2020) 181.
[26] L.F. Paranaíba, R.R. de Assis, P.M. Nogueira, et al., Leishmania enriettii: biochemical characterisation of lipophosphoglycans (LPGs) and glycoinositolphospholipids (GIPLs) and infectivity to Cavia porcellus, Parasit. Vectors 8 (2015) 31.
[27] G. Schönian, A. Nasereddin, N. Dinse, et al., PCR diagnosis and characterization of Leishmania in local and imported clinical samples, Diagn. Microbiol. Infect. Dis. 47 (2003) 349–358.
[28] D.L. Tolson, L.F. Schnur, A. Jardim, et al., Distribution of lipophosphoglycan-associated epitopes in different Leishmania species and in African trypanosomes, Parasitol. Res. 80 (1994) 537–542.
[29] R. Lira, S. Sundar, A. Makharia, et al., Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of Leishmania donovani, J. Infect. Dis. 180 (1999) 564–567.
[30] R.P.P. Soares, T. Barron, K. McCoy-Simandle, et al., Leishmania tropica: intraspecific polymorphisms in lipophosphoglycan correlate with transmission by different Phlebotomus species, Exp. Parasitol. 107 (2004) 105–114.
[31] R.R. de Assis, I.C. Ibraim, P.M. Nogueira, et al., Glycoconjugates in New World species of Leishmania: polymorphisms in lipophosphoglycan and glycoinositolphospholipids and interaction with hosts, Biochim. Et. Biophys. Acta (BBA)-General. Subj. 1820 (2012) 1354–1365.
[32] J.M. Coelho-Finamore, V.C. Freitas, R.R. Assis, et al., Leishmania infantum: lipophosphoglycan intraspecific variation and interaction with vertebrate and invertebrate hosts, Int. J. Parasitol. 41 (2011) 333–342.
[33] D.L. Sacks, P.F. Pimenta, M.J. McConville, et al., Stage-specific binding of Leishmania donovani to the sand fly vector midgut is regulated by conformational changes in the abundant surface lipophosphoglycan, J. Exp. Med. 181 (1995) 685–697.

[34] R.P.P. Soares, T.L. Cardoso, T. Barron, et al., Leishmania braziliensis: a novel mechanism in the lipophosphoglycan regulation during metacyclogenesis, Int. J. Parasitol. 35 (2005) 245–253.
[35] T. da S. Vieira, J.N. Rugani, P.M. Nogueira, et al., Intraspecies polymorphisms in the lipophosphoglycan of L. braziliensis differentially modulate macrophage activation via TLR4, Front. Cell. Infect. Microbiol. 9 (2019) 240.
[36] C.A. Cardoso, G.V. Araujo, C.M. Sandoval, et al., Lipophosphoglycans from dermotropic Leishmania infantum are more pro-inflammatory than those from viscerotropic strains, Mem. Inst. Oswaldo Cruz 115 (2020) e200140.
[37] F.D. Rêgo, C. d A. Cardoso, P.O.L. Moreira, et al., Leishmania amazonensis from distinct clinical forms/hosts has polymorphisms in Lipophosphoglycans, displays variations in immunomodulatory properties and, susceptibility to antileishmanial drugs, Cell. Biol. Int. 46 (2022) 1947–1958.
[38] R.P.P. Soares, M.E. Macedo, C. Ropert, et al., Leishmania chagasi: lipophosphoglycan characterization and binding to the midgut of the sand fly vector Lutzomyia longipalpis, Mol. Biochem. Parasitol. 121 (2002) 213–224.
[39] P.M. Nogueira, A.C. Guimarães, R.R. Assis, et al., Lipophosphoglycan polymorphisms do not affect Leishmania amazonensis development in the permissive vectors Lutzomyia migonei and Lutzomyia longipalpis, Parasit. Vectors 10 (2017) 608.
[40] M.J. de Veer, J.M. Curtis, T.M. Baldwin, et al., MyD88 is essential for clearance of Leishmania major: possible role for lipophosphoglycan and Toll-like receptor 2 signaling, Eur. J. Immunol. 33 (2003) 2822–2831.
[41] I.C. Ibraim, R.R. de Assis, N.L. Pessoa, et al., Two biochemically distinct lipophosphoglycans from Leishmania braziliensis and Leishmania infantum trigger different innate immune responses in murine macrophages, Parasit. Vectors 6 (2013) 54.
[42] L.J. Pinheiro, L.F. Paranaíba, A.F. Alves, et al., Salivary gland extract modulates the infection of two Leishmania enriettii strains by interfering with macrophage differentiation in the model of Cavia porcellus, Front. Microbiol. 9 (2018) 969.