Leishmania braziliensis prostaglandin F$_{2\alpha}$ synthase impacts host infection

Eliza Vanessa Carneiro Alves-Ferreira$^1$, Tiago Rodrigues Ferreira$^1$, Pegine Walrad$^2$, Paul M. Kaye$^2$ and Angela Kaysel Cruz$^1$*

Abstract

Background: Prostaglandins (PG) are lipid mediators derived from arachidonic acid metabolism. They are involved in cellular processes such as inflammation and tissue homeostasis. PG production is not restricted to multicellular organisms. Trypanosomatids also synthesize several metabolites of arachidonic acid. Nevertheless, their biological role in these early-branching parasites and their role in host-parasite interaction are not well elucidated. Prostaglandin F$_{2\alpha}$ synthase (PGF2S) has been observed in the *Leishmania braziliensis* secreted proteome and in *L. donovani* extracellular vesicles. Furthermore, we previously reported a positive correlation between *L. braziliensis* PGF2S (*Lbr*PGF2S) expression and pathogenicity in mice.

Methods: *Lbr*PGF2S gene expression and PGF2α synthesis in promastigotes were detected and quantified by western blotting and EIA assay kit, respectively. To investigate *Lbr*PGF2S localization in amastigotes during bone marrow-derived macrophage infection, parasites expressing mCherry-*Lbr*PGF2S were generated and followed by time-lapse imaging for 48 h post-infection. PGF2S homolog sequences from *Leishmania* and humans were analyzed in silico using ClustalW on Geneious v6 and EMBOSS Needle.

Results: *Leishmania braziliensis* promastigotes synthesize prostaglandin F$_{2\alpha}$ in the presence of arachidonic acid, with peak production in the stationary growth phase under heat stress. *Lbr*PGF2S is a cytoplasmic protein enriched in the secretory site of the parasite cell body, the flagellar pocket. It is an enzyme constitutively expressed throughout promastigote development, but overexpression of *Lbr*PGF2S leads to an increase of infectivity in vitro. The data suggest that *Lbr*PGF2S may be released from intracellular amastigotes into the cytoplasm of bone marrow-derived macrophages over a 48-hour infection period, using time-lapse microscopy and mCherry-PGF2S (mChPGF2S)-expressing parasites.

Conclusions: *Lbr*PGF2S, a parasite-derived protein, is targeted to the host cell cytoplasm. The putative transfer of this enzyme, involved in pro-inflammatory lipid mediator synthesis, to the host cell suggests a potential role in host-parasite interaction and may partially explain the increased pathogenicity associated with overexpression of *Lbr*PGF2S in *L. braziliensis*. Our data provide valuable insights to help understand the importance of parasite-derived lipid mediators in pathogenesis.

Keywords: *Leishmania braziliensis*, *Lbr*PGF2S, Macrophage, Host-parasite, PGF2α

*Correspondence: akcruz@fmrp.usp.br
$^1$ Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, Brazil
Full list of author information is available at the end of the article
**Background**

*Leishmania (Viannia) braziliensis* is the most virulent agent of localized cutaneous (LCL) and mucocutaneous (MCL) leishmaniasis in Brazil [1]. Mucosal commitment occurs in approximately 5–10% of patients infected with *L. braziliensis* [2] and this clinical form is most often diagnosed months or years after the primary clinical manifestations. Otorhinolaryngological examination of patients from an endemic area in Brazil detected the parasite in the nasal mucosa during early infection, in the absence of mucosal lesions [3]. The mechanisms that facilitate mucosal involvement during *L. braziliensis* infection are poorly understood.

In a previous study, we performed gene expression analyses on two pairs of mucosal and cutaneous *L. braziliensis* isolates, in which proteomic profile differences between isolates were detected [4]. Comparative proteomic analysis revealed a consistently differential pattern of prostaglandin F2α synthase expression (*LbrM.31.2410; LbrPGF2S*), with higher protein abundance in cutaneous isolates compared to mucosal isolates [4]. Data deposited on TriTrypDB (tritrypdb.org) indicate that *LbrPGF2S* has been identified in the secretome of *L. braziliensis* and exosomes derived from *Leishmania donovani*. In addition, the Tropical Disease Research Targets Database (tdr-targets.org) indicates that the *Leishmania major* PGF2S (*LmPGF2S*) homolog has 13 putative antigenic epitopes with 77.8% antigenicity. The structure of *LmPGF2S* protein has been resolved by crystallization [5] facilitating future study for drug design using this protein.

The synthesis and functions of prostaglandins (PGs) are well characterized mainly in terms of mammalian physiology. In mammals, prostaglandin synthases catalyze the production of prostaglandins using arachidonic acid (AA) metabolites as substrates. AA is removed from membranes by the action of phospholipase A2 and converted into prostaglandin H2 (PGH2) by cyclooxygenases (COX-1 or COX-2). PGH2 is then converted into several metabolites, such as PGD2, PGE2 and PGE2α, by prostaglandin synthases (e.g. PGF2S) [6]. In mammals, PGF2α is mostly related to ovulation, luteolysis, uterine contraction and the onset of labor [7]. However, it has been recently reported that PG production is not restricted to mammals, occurring in trypanosomatids [6] and other protozoan parasites, such as *Plasmodium falciparum* [8] and *Entamoeba histolytica* [9] [10]. High levels of PGF2α have been detected in *Trypanosoma brucei*, catalyzed by TbPGF2S from PGH2 [11]. The prostaglandin F2α synthase activity has been demonstrated for three trypanosomatids orthologous genes. Roberts and colleagues [12] have shown that, in spite of the lack of credible cyclooxygenase genes, these parasites use arachidonic acid as a substrate to produce PGF2α. Additionally, these and other authors demonstrated that some of these kinetoplastid aldo-keto reductases metabolize toxic ketoaldehydes, playing a role as detoxification agents and possibly acting in cellular defense [12, 13]. However, the importance of these pathways for parasite biology and host interaction remains under-explored.

We have shown a positive correlation between *Lbr*-PGF2S ectopic overexpression in *L. braziliensis* and the rate of *in vitro* infection [4], suggesting that *LbrPGF2S* has a role in parasite virulence. In addition, a study on *Leishmania infantum chagasi* showed that PGF2S is highly expressed in metacyclic promastigotes [14]. These authors also observed an increase in lipid bodies (sites for prostaglandin synthesis in mammalian cells) in macrophages infected with *L. infantum*. In our study, we examined PGF2α production in *L. braziliensis*, the expression profile of *LbrPGF2S* during promastigote development and its localization. Our results are indicative that *LbrPGF2S* might be transferred from intracellular parasites into the cytoplasm of mouse macrophages. This work, in conjunction with others [4, 14], lend weight to the hypothesis that PGs are parasite virulence factors.

**Methods**

**Culture of parasites and infections**

Promastigotes of all the *Leishmania braziliensis* wild type strains: BA778 (MHOM/BR/00/BA778), *Lb*2903 (MHOM/BR/75/M2903), H3227 (MHOM/BR/94/H3227), and transfectant strains Lb2903[mChPGF2S::SSU] and Lb2903[mCherry::SSU] were cultured at 26°C in 1× M199 medium supplemented with 0.04 M HEPES, 0.1 mM adenine, 50 µg/ml biotin, 0.25% hemin, 25 mg/ml penicillin, 2.5 mg/ml streptomycin and 5 µg/ml biotin. Transfectants were kept in liquid medium containing G418 (4 µg/ml, 4× LD50).

**Sequence alignment analysis**

The protein sequences of *LbrPGF2S* homologs were obtained from TriTrypDB (http://tritrypdb.org/tritrypdb/). Multiple alignments were performed using ClustalW and Geneious v6 [15] (Biomatters Ltd, Auckland, New Zealand). Global alignment and quantification of identity/similarity were performed using the online version of Needle (ebi.ac.uk/Tools/psa/emboss_needle). To compare the protein 3D structure available in the PDB (http://www.rcsb.org/pdb/) for PGF2S from *L. major* (pdb 4g5d) and AKR1C3 (pdb 4yvv), RCSB’s online Comparison Tool and jFatCat_rigid alignment algorithm were used. Alignments were visualized in Geneious v6.

**SDS-PAGE and immunoblotting analysis**

Promastigotes (1 × 10⁷) were harvested from the culture on days 2, 3, 4, 5, 6, 7 and 8, and resuspended in...
Laemmli sample buffer (500 mM Tris-HCl pH 6.8; 20% glycerol; 0.001% bromophenol blue; 2% SDS; 0.28 M β-mercaptoethanol). To evaluate promastigote secretion, 50 ml of a 7-day-old culture supernatant was collected, filtered through 0.22 μm syringe filters and the proteins precipitated with 10% TCA. Protein extracts were homogenized, denatured at 95 °C for 5 min and loaded on a 12.5% acrylamide gel. Western blotting assays were then performed according to Alves-Ferreira et al. [4].

Overexpression target construction and transfection

The LbrPGF2S CDS (coding DNA sequence) was amplified from the genomic DNA (gDNA) of L. (V.) braziliensis strain MHOM/BR/75/M2904 using primers LbrPGF2S-nostart-BglII-For (5′-TCA AGA TCT GCT GGG GCC ATC AAC GTT GGT AAG ACC G -3′) and LbrPGF2S-BamHI-Rev (5′-TCA GGA TCC TCA GAA CTG CGC CTC ATC A -3′). The PCR products were digested with BglII and BamHI enzymes and cloned into the pmCherry-C1 (Addgene, Cambridge, MA, USA) plasmid digested with same enzymes. The mCherry-PGF2S plasmid was then digested with Pmel and Ndel enzymes. Promastigotes were transfected with mCherry-PGF2S or mCherry linear fragments by electroporation [16]. Transfected colonies were extracted from M199-agar medium and the mCherry-PGF2S-BamHI::SSU(BglII) strain MHOM/BR/75/M2903 using primers LbrPGF2S-BamHI-Rev (5′-TCA GGA TCC TCA GAA CTG CGC CTC ATC A -3′). The PCR products were digested with BglII and BamHI enzymes and cloned into the pmCherry-C1 (Addgene, Cambridge, MA, USA) plasmid digested with same enzymes. The mCherry-PGF2S plasmid was then digested with Pmel and Ndel enzymes. Promastigotes were transfected with mCherry-PGF2S or mCherry linear fragments by electroporation [16]. Transfected colonies were extracted from M199-agar medium in the presence of the G418 antibiotic (Sigma-Aldrich, St. Louis, MO, USA). The G418 LD50 was determined for the Lb2903 strain and at four-fold LD50 drug concentration (4 μg/ml). Parasites overexpressing LbrPGF2S ectopically were produced and kept as described in [4].

Bone marrow-derived macrophage (BMDM) production and in vitro infection

BMDMs were produced following the protocol described elsewhere [17]. The macrophages were infected with late-stationary-phase Leishmania promastigotes (MOI 10:1). For prostaglandin receptor (FP) inhibition, infected macrophages were treated with prostaglandin F2α dimethyl amide (Cayman Chemical, Michigan, USA), an FP receptor antagonist, for up to 24 h or 48 h.

Immunofluorescence

Promastigotes (in early logarithmic phase) were harvested by centrifugation at 2500 × rpm for 10 min, washed in PBS and fixed in 2% paraformaldehyde for 10 min. The fixed cells were centrifuged, suspended in 1 M glycine solution and attached to coverslips. Permeabilization was performed using 0.3% Triton X-100 for 10 min and blocking with 2% BSA in PBS for 1 h. We have previously generated an anti-LbrPGF2S chicken IgY antibody by immunizing chickens with His-LbrPGF2S heterologously expressed in Escherichia coli [4]. The parasites were then incubated in anti-Lbr-PGF2S and anti-tubulin (Millipore, Massachusetts, USA) antibodies at 1:10,000 in 1% BSA-PBS for 1 h and washed three times in PBS-T. Secondary anti-chicken conjugated CF488A (Sigma-Aldrich) or anti-mouse conjugated Alexa 555 was added at 1:1000 dilution, supplemented with 60 μM DAPI. Coverslips were washed in PBS-T and MilliQ water and then mounted with ProLong Gold Antifade Reagent (Invitrogen, California, USA). Images were acquired on a Zeiss LSM 510 confocal microscope.

To acquire time lapse images of in vitro infection, bone marrow derived macrophages were infected with Lb2903[mChPGF2S::SSU] and Lb2903[mCherry::SSU] parasites for 2 h. The cultures were then washed three times with PBS and complete RPMI medium (RPMI-1640, 20% FCS) was added. The culture was kept in the BioStation IMQ (Nikon, Tokio, Japan) incubation chamber at 33 °C and 5% CO2 for up to 48 h. The images were captured using a fluorescent field (587 nm excitation and 610 nm emission) and bright field to produce 18 min videos. The images were converted to TIFF format and both images and video processed using ImageJ (Fiji, LOCI, Madison, EUA) and GIMP (GNU Image Manipulation Program).

Dosage of prostaglandin F2α

Prostaglandin F2α was measured in the supernatant (filtered through 0.22 μm syringe filters) of the L. braziliensis culture on growth days 3 and 7 using the immunoenzymatic EIA assay PGF2α KIT (Cayman Chemical, Michigan, USA), according to manufacturer’s instructions.

Results

PGF2S ortholog sequences are highly conserved among Leishmania spp.

Firstly, we confirmed high sequence homology between prostaglandin F2α synthase genes in Leishmania spp. by performing a multiple sequence alignment for Lbr-PGF2S (LbrM.31.2410) orthologs across different Leishmania genomes available in TriTrypDB (tritrypdb.org) (Fig. 1a). We then compared the protein sequence (Fig. 1b) and the 3D structure (Fig. 1c) of LbrPGF2S and the best
### a

|     | LbrM.31.2410 | LmF.31.2150 | LmM.30.2150 | Lj.31.2210 | LtaP31.2590 |
|-----|--------------|-------------|-------------|------------|-------------|
|     | MIWVKTATNENP | MWVKTATNENP | MWVKTATNENP | MWVKTATNENP | MWVKTATNENP |
|     | NPKFELOG6DSP | NPKFELOG6DSP | NPKFELOG6DSP | NPKFELOG6DSP | NPKFELOG6DSP |
|     | RCERFNVAGNNV | RCERFNVAGNNV | RCERFNVAGNNV | RCERFNVAGNNV | RCERFNVAGNNV |
|     | YAKYChSVEVG | YAKYChSVEVG | YAKYChSVEVG | YAKYChSVEVG | YAKYChSVEVG |
|     | ACYVRVP | ACYVRVP | ACYVRVP | ACYVRVP | ACYVRVP |

### b

|     | LbrM.31.2410 | LmF.31.2150 | LmM.30.2150 | Lj.31.2210 | LtaP31.2590 |
|-----|--------------|-------------|-------------|------------|-------------|
|     | MINVGTAMNAD | MINVGTAMNAD | MINVGTAMNAD | MINVGTAMNAD | MINVGTAMNAD |
|     | NSVNVIRAGS | NSVNVIRAGS | NSVNVIRAGS | NSVNVIRAGS | NSVNVIRAGS |
|     | G - EATENAV | G - EATENAV | G - EATENAV | G - EATENAV | G - EATENAV |
|     | NKLHGVYIKVR | NKLHGVYIKVR | NKLHGVYIKVR | NKLHGVYIKVR | NKLHGVYIKVR |
|     | NV5MAG | NV5MAG | NV5MAG | NV5MAG | NV5MAG |

### c

![3D protein structure](image-url)
characterized human PGF2α, AKR1C3 (NP_003730.4), and found 51.4% similarity and 34.3% identity. Additionally, *in silico* analysis of protein domains revealed the presence of two aldo/keto reductase domains (IPR18170) and a putative secretion cleavage signal near the C-terminus in the *Leishmania* PGF2S, suggesting that the protein might undergo proteolysis for subsequent export (Fig. 1b). A third aldo/keto reductase domain was detected in the C-terminal region of AKR1C3, but not in *Lbr*PGF2S, probably due to I247V mutation in this enzyme. PGF2S orthologs in *L. major* (LmjF31.2150), *L. mexicana* (LmxM30.2150), *L. infantum* (LinJ31.2210) and *L. tarentolae* (LtaP31.2590) carry the predicted third Aldo-Keto domain present in the protein sequences from other organisms. In contrast, PGF2S from *Leishmania braziliensis* (LbrM31.2410) and all the other *Leishmania* strains from the subgenus Viamia (deposited on TriTrypDB) lost the third domain, all of them carry a valine in the isoleucine position, which differs from the strains of the subgenus *Leishmania*.

**Evaluation of *Lbr*PGF2S expression and PGF2α production in promastigotes.**

To evaluate whether *Lbr*PGF2S protein levels are modulated during axenic promastigote differentiation, whole cell extracts were fractionated by SDS-PAGE, and *Lbr*-PGF2S analyzed by immunoblotting. No significant changes in PGF2S abundance were observed from day 1 to day 8 (from log to stationary phase, enriched for procyclic and metacyclic forms, respectively), indicating that *Lbr*-PGF2S is constitutively expressed in promastigotes during *in vitro* growth (Fig. 2a). We then analyzed the presence of *Lbr*PGF2S in the supernatant of stationary promastigote culture (day 7 post-inoculum). Promastigotes were removed by centrifugation and supernatants filtered through a 0.22 μm syringe filter, excluding parasites but not vesicles, such as exosomes. *Lbr*PGF2S was detected by immunoblotting in both parasite lysates and culture supernatants (Fig. 2b). Indirect immunofluorescence using anti-*Lbr*PGF2S antibody revealed cytoplasmic distribution of *Lbr*PGF2S with a noticeably stronger signal near the flagellar pocket (Fig. 2c), a分泌orygine in trypanosomatids. To confirm that axenic *L. braziliensis* promastigotes produce PGF2α, the levels of PGF2α in the culture supernatant were quantified at growth days 3 and 7 using an immunoenzymatic assay. Prior to evaluation, the cells were kept at either 26 °C or 37 °C for 4 h in the presence or absence of arachidonic acid (AA). Under all the conditions tested, we observed an increment in PGF2α levels in the supernatant when AA was added. The effect was particularly evident in samples kept at 37 °C (Fig. 2d).

Next, to investigate *Lbr*PGF2S expression in amastigotes, mouse bone marrow-derived macrophages (BMDM) were used for *in vitro* infection with wild type (WT) *L. braziliensis* late-stationary phase promastigotes and transfectants overexpressing *Lbr*PGF2S ectopically (*Lb[pXLbrPGF2S]*). After 48 h of infection, *Lbr*PGF2S was detected in WT intracellular amastigotes and at higher levels in macrophages infected with the overexpressing parasites (Fig. 3).

**Detection of *Lbr*PGF2S in host cell cytoplasm.**

To robustly demonstrate the localization of *Lbr*PGF2S in amastigotes and infected host macrophages in real time during *in vitro* infection, we generated *L. braziliensis* lines expressing *Lbr*PGF2S fused with fluorescent protein mCherry (mChPGF2S). These constructs overcome the problem associated with cross-reaction of the *Lbr*PGF2S antibody with macrophage molecules. Plasmids pSSU-Neo-mCherry (control) and pSSU-Neo-mChPGF2S were used to generate lines *Lb*2903[mCherry::SSU] and *Lb*2903[mChPGF2S::SSU] (hereinafter referred as *Lb*[mCherry] and *Lb*[mChPGF2S], respectively) (Additional file 1: Figure S1a). Correct integration of mCherry constructs in the ribosomal locus was confirmed by PCR (Additional file 1: Figure S1b). We then confirmed the presence of both the SSU-integrated mChPGF2S (~61kDa) and the endogenous *Lbr*PGF2S (~31kDa) using anti-*Lbr*PGF2S antibody (Additional file 1: Figure S1c).

Similarly to parasites overexpressing wild type *Lbr*-PGF2S ectopically [4], *Lb*[mChPGF2S] stationary phase promastigotes infected BMDMs more effectively than *Lb*[mCherry], the control parasites (Additional file 2:...
Figure S2). To investigate the impact of the PGF2α pathway on the *L. braziliensis in vitro* infection we performed an inhibition assay by treating infected BMDM with an FP receptor antagonist for 24 h (prostaglandin F2α dimethyl amide). FP inhibition decreases the percentage of infected macrophages with *L. braziliensis* wild type (Strain H3227) (a 10-fold decrease at 10 µg/ml) and the number of amastigotes per cell (a 18.5-fold decrease at 10 µg/ml) (Additional file 3: Figure S3).
To investigate \textit{Lbr}PGF2S distribution, macrophages were infected with late stationary phase promastigotes from \textit{Lb}[mCherry] and \textit{Lb}[mChPGF2S] parasites, followed by time-lapse imaging for 48 h. Fluorescence of mChPGF2S was detected mainly inside intracellular promastigotes in the early stages of infection. Surprisingly, at \(~18\) h post-infection (pi), mChPGF2S was observed in the macrophage cytoplasm and subsequently found dispersed. Note that at this time the parasites had not fully differentiated into amastigotes, although most gene expression changes take place in this initial phase of differentiation [18]. This staining pattern was not observed in control \textit{Lb}[mCherry] intracellular parasites (Fig. 4, Additional file 4: Video S1, Additional file 5: Video S2, Additional file 6: Video S3, Additional file 7: Video S4). The distribution of mChPGF2S fluorescence throughout the host cell cytoplasm was more clearly observed in macrophages with a higher number of intracellular parasites (Fig. 5a, Additional file 8: Video S5, Additional file 9: Video S6). Nevertheless, even macrophages infected with only two mChPGF2S parasites exhibited dispersion of the fluorescence around the entire cell body, while fluorescence in macrophages infected with mCherry parasites was restricted to the vacuole region (promastigotes and amastigotes) (Fig. 5b).

**Discussion**

This study shows that \textit{Lbr}PGF2S, which might contribute to \textit{Leishmania} virulence profiles in the mammalian host [4], is found in host macrophage cytoplasm infected...
with *L. braziliensis*, suggesting direct interaction with the host cell. In addition, *LbrPGF2S* expression was also detected in *L. braziliensis* promastigotes in axenic culture throughout promastigote growth and in the supernatant. The protein might be secreted through the flagellar pocket, as indicated by the strong signal detected under immunofluorescence. These results corroborate and extend previous studies that have identified PGF2S in the secreted proteome of *L. braziliensis* [19].

Although PGF2α synthesis by recombinant *LbrPGFS* was not measured herein, other researchers have shown that PGF2S homologs in *L. major*, *L. tropica*, *L. donovani*, *L. infantum*, *T. cruzi* and *T. brucei* catalyze PGF2α synthesis *in vitro* [6, 12]. Since we detected the production of PGF2α by promastigotes in axenic culture, we suggest that *LbrPGF2S* has the same catalytic function. To the best of our knowledge, we are the first, however, to observe that *LbrPGF2S* is expressed in amastigotes and localized in the host cell cytoplasm infected with *Leishmania*. Other authors have shown that vesicles released by *Leishmania* during *in vitro* or *in vivo* infection interfere with host cell response [20]. Recently, it has been shown that promastigotes secret exosomes into the midgut lumen of the sand fly vector and these extracellular vesicles are regurgitated with parasites into the skin during blood meal, modulating the immune response to *Leishmania* infection. Interestingly, the PGF2S protein was found among the 124 proteins identified by proteomic analysis in vesicles from *L. infantum*-infected midguts [21]. Although other experiments are needed to confirm *LbrPGF2S* secretion, our results are indicative of the transfer of the enzyme into the cytoplasm of infected macrophages. Other proteins secreted to the host cell cytoplasm, such as gp63, were shown to modulate phagocytic cell response [22, 23]. The process by which PGF2S reaches the cytoplasm of the host cell remains unknown, but we have shown that inhibition of the prostaglandin receptor affected negatively the infection profile of BMDM *in vitro*.

Although most studies on PGF2α are related to mammalian female reproduction [7, 24], their role in leukocyte migration has been shown, with high potential to act as a neutrophil chemoattractant *in vitro* [25] and *in vivo* [26]. In addition, AKR1C3 is abundant in keratinocytes in the suprabasal layer of the epidermis and regulates the synthesis of PGF2α in the presence of calcium, contributing to the pro-inflammatory response *in vitro* [27]. Although we have shown that *LbrPGF2S* and AKR1C3 protein sequences share 51.4% similarity and 34.3% identity, modelling using the Research Collaboratory for Structural Bioinformatics (RCSB) PDB suggests

![Figure 4](image-url). Parasite-derived mChPGF2S accesses the host cell cytoplasm. Time-lapse images were captured from mouse bone marrow-derived macrophages infected with *Lb[mCherry]* and *Lb[mChPGF2S]* promastigotes, as indicated in the figure. Cyan was used as a pseudocolor; numbers at the bottom right of each panel indicate hour:minutes after infection. See videos in Additional files 4, 5, 6, 7, 8, 9. Scale-bar: 5 µm.
that the protein structures of LbrPGF2S and AKR1C3 are quite similar, sharing at least two aldo/keto reductase domains and indicating that both proteins may retain the core function of PGF$_{2\alpha}$ production. Thus, the work of other researchers using different Leishmania species [6, 14] and our own data suggest that the PGF$_{2\alpha}$ signaling pathway could be involved in Leishmania pathogenicity.

Fig. 5 Detection of mChPGF2S in highly-infected macrophages. a Detection of mCherry fluorescence in the host cell cytoplasm. Time-lapse images of macrophages infected with more than three mChPGF2S parasites. Cyan was used as pseudocolor; numbers at the bottom right of each panel indicate hour:minutes after infection. b Close image analysis of mCherry and mChPGF2S localized fluorescence intensity in infected macrophages. Numbers at the top of each image indicate hour:minutes after infection. c Overall intracellular fluorescence in infected macrophages was quantified using FIJI (ImageJ) software up to 30 h pi. Scale-bars a, 10 µm.
Conclusions
To the best of our knowledge, this is the first study to report that *L. braziliensis* promastigotes and amastigotes can express *Lbr*PGF2S and that it is possibly found in the infected host cell cytoplasm. Based on our results and those in the literature, we propose that the production of PGF2S improves the fitness of the parasite and might play a role in the mammalian host-parasite interaction. *Lbr*PGF2S could be a new *Leishmania* virulence factor and is, therefore, a potentially attractive target for drug discovery or vaccine development.

Supplementary information

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Authors’ contributions
EVCAF, TRF and AKC performed the experimental design. EVCAF and TRF performed and analyzed the experiments. EVCAF and AKC wrote the manuscript. PW and PMK contributed reagents, materials and space. EVCAF, TRF, PW, PMK and AKC reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data supporting the conclusions of this article are available in the article and its additional files.

Ethics approval and consent to participate
The cells samples use in this study, and the use of mice to recover cells derived from bone marrow, was approved by the Animal Research Ethics Committee (CETEA) at the Ribeirão Preto Medical School, University of São Paulo. Protocol no. 159/2011 was certified consistent with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) in 8/27/2012.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil. 2Centre for Immunology and Infection, Department of Biology and Hull York Medical School, University of York, York, UK.

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