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MAJOR ARTICLE

Role of Prostaglandin $F_{2\alpha}$ Production in Lipid Bodies From *Leishmania infantum chagasi*: Insights on Virulence

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Lipid bodies (LB; lipid droplets) are cytoplasmic organelles involved in lipid metabolism. Mammalian LBs display an important role in host-pathogen interactions, but the role of parasite LBs in biosynthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has not been investigated. We report herein that LBs increased in abundance during development of *Leishmania infantum chagasi* to a virulent metacyclic stage, as did the expression of $PGF_{2\alpha}$ synthase (PGFS). The amount of parasite LBs and $PGF_{2\alpha}$ were modulated by exogenous arachidonic acid. During macrophage infection, LBs were restricted to parasites inside the parasitophorous vacuoles (PV). We detected $PGF_{2\alpha}$ receptor (FP) on the *Leishmania* PV surface. The blockage of FP with AL8810, a selective antagonist, hampered *Leishmania* infection, whereas the irreversible inhibition of cyclooxygenase with aspirin increased the parasite burden. These data demonstrate novel functions for parasite-derived LBs and $PGF_{2\alpha}$ in the cellular metabolism of *Leishmania* and its evasion of the host immune response.

Keywords. *Leishmania infantum chagasi*; lipid droplets; prostaglandin $F_{2\alpha}$; $PGF_{2\alpha}$ synthase; macrophage infection.

Lipid bodies (LBs; also called lipid droplets) are cytoplasmic organelles involved in the storage and processing of lipids and are present in most cell types and organisms [1, 2]. Accumulating evidence has placed mammalian lipid bodies as key organelles involved in regulation of eicosanoids in inflammatory and neoplastic cells [2]. LBs have been shown as stores of esterified arachidonate [3–5], sites of compartmentalization of eicosanoid-forming enzymes including cPLA2, cyclooxygenases and lipoxygenases [5–7], and major sites for eicosanoid generation during inflammatory and infectious conditions [8–10]. Several intracellular pathogens take advantage of the LB formation in the host cells. The increase in the number of host cell LBs and their recruitment to parasitophorous vacuoles have been demonstrated in infections with *Trypanossoma cruzi* [11], *Toxoplasma gondii* [12], *Plasmodium falciparum* [13], *Chlamydia trachomatis* [14], *Mycobacterium bovis BCG* [9], *Mycobacterium tuberculosis* [15], and *Mycobacterium leprae* [16]. The location of LBs close to phagolysosomes suggests that LBs could be used as a source of nutrients by pathogens. In addition, an increase in the LB number in the cytoplasm of macrophages is associated with release of PGE_2 and enhancement of *M. bovis* [9, 17], *M. leprae* [18, 19], and *T. cruzi* infection [10, 11]. All together, these findings argue that induction of LB formation by intracellular pathogens promotes their survival [20]. Notwithstanding the morphological similarity between

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the LBs in leukocytes and parasites, the function of parasite LBs and their role in eicosanoid production have not been demonstrated. Eicosanoids, such as prostaglandins (PG), are bioactive molecules produced from arachidonic acid (AA) metabolism by specific enzymes, such as cyclooxygenase (COX) and prostaglandin synthases. Prostaglandins have been implicated in the control of immune responses [21, 22]. Despite the absence of COX genes and homologous proteins in the Order *Trypasomatidae* protozoa, parasites such as *Leishmania* are capable of producing PGs [23, 24]. These parasites contain only prostaglandin $F_{2\alpha}$ synthase (PGFS) described as responsible for $PGF_{2\alpha}$ production [25]. $PGF_{2\alpha}$ acts directly on the $PGF_{2\alpha}$ receptor (FP) and triggers the activation of the COX pathway [26]. However, the question of whether PGF2α biosynthesis localizes in the LB parasite has not been investigated. Besides, it is unknown what is the role of $PGF_{2\alpha}$ and its FP in the *Leishmania*-host interplay.

In this study, we investigated the dynamics of LB formation and $PGF_{2\alpha}$ release in *Leishmania infantum chagasi* (*L. i. chagasi*). In addition, we investigated the role of the FP in macrophages during *L. i. chagasi* infection. Our findings demonstrated an increase in the expression of PGFS and LB formation during *L. i. chagasi* metacyclogenesis and showed that parasite-derived $PGF_{2\alpha}$ plays a critical role in macrophage infection.

MATERIALS AND METHODS

Animals

Inbred male BALB/c mice, age 3–5 weeks, were obtained from the animal facility of Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (CPqGM-FIOCRUZ, Brazil). The animals were kept at a temperature of 24°C, with free access to food and water and light and dark cycles of 12 hours each. All experiments using animals were in strict accordance with the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA). The Ethics Committee on the use of experimental animals (CEUA) of the Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (permit 27/2008) approved all protocols.

Wild-type Parasites

The *L. i. chagasi* promastigotes (MHOM/BR/00/1669) were serially passed through Syrian hamsters and isolated from spleens. Parasites were cultured in hemoflagellate-modified minimal essential medium (HOMEM) containing 10% HI-FCS for 7–9 days until the culture reached stationary phase. To obtain a pure population of logarithmic-phase promastigotes, the cultures were rediluted every 2 days for at least 3 consecutive cycles [27]. Metacyclic promastigotes were isolated from the stationary cultures using density gradient separation method described elsewhere [28]. Amastigotes were isolated from the spleens of the infected male Syrian hamsters and were incubated overnight in amastigote growth medium containing 20% FCS (GIBCO) at 37°C and 5% CO_2, pH 5.5 [29].

LcJ Parasites

The LcJ parasite line, derived from wild-type *L. i. chagasi*, converts between promastigote and amastigote forms in axenic culture. LcJ promastigotes were maintained in HOMEM, and amastigotes were maintained in a low pH medium with fetal calf serum, as reported elsewhere [30].

Western Blotting

Leishmania parasites (2×10^8/mL) at different stages were lysed using LyseM solution (Roche). Sample protein concentrations were measured using the BCA protein assay (Pierce). Total proteins (30 μg) were separated by 10% SDS-PAGE and were transferred to nitrocellulose membranes. The membranes were blocked in Tris-buffered saline (TBS) supplemented with 0.1% Tween 20 (TT) plus 5% dry milk for 1 hour before incubation overnight in murine anti-PGFS (1:1000) antiserum. After the removal of the primary antibody, the membranes were washed 5 times in TT and were incubated in the peroxidase-conjugated secondary antibody (1:5000) for 1 hour. The membranes were washed and developed using the ECL chemiluminscence kit (Amersham). The membranes were stripped in accordance with the manufacturer's instructions (Amersham) and reprobed with primary anti α-tubulin (1:1000) antibody as a loading control. The protein bands were detected using the ImageQuant LAS 4000 system (GE).

Culture and Infection of Bone Marrow Macrophages

Bone marrow cells were harvested from BALB/c mouse femurs and cultured at 37°C and 5% CO_2 in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% HI-FCS, 2 mM L-glutamine, 100 U/mL penicillin, and 50 mg/mL streptomycin (RP-10), and 20% L929 cell culture supernatant (American Tissue Type Collection, VA) as a source of macrophage colony-stimulating factor. After 7–9 days, differentiated adherent bone marrow derived macrophages (BMMs) were detached from the plate using 2.5 mg/mL trypsin plus 1 mM EDTA (Gibco) [29]. BMMs (3×10^5/well) were plated on coverslips in 24-well plates and cultured at 37°C, 5% CO_2 in RP-10 for 24 hours.

BMMs were either treated with 1, 10, and 50 μM of AL8810 isopropyl ester (Cayman Chemical) or with ethanol as the vehicle control. Alternatively, BMMs were treated with 10 μM aspirin (Cayman Chemical). Treated macrophages were infected with nonopsonized metacyclics promastigotes at a multiplicity of infection (MOI) of 10:1, LcJ promastigotes at a MOI of 20:1, or LcJ amastigotes at a MOI of 3:1. Macrophage binding was synchronized by centrifugation of BMMs and parasites for 3 minutes at 1200 rpm and 4°C, followed by placement at 37°C, 5% CO_2 at time = 0.

After 30 minutes extracellular parasites were removed by rinsing twice with HBSS without Ca^{++} and Mg^{++} ($HBSS^{-/-}$) followed by the addition of fresh RP-10. After specified times, some coverslips were fixed and stained with Diff Quik (Wright-Giemsa). Intracellular parasites were counted under light microscopy. Other coverslips were harvested after 1, 4, 8, 24, 48, or 72 hours, fixed in 2% paraformaldehyde and analyzed by confocal microscopy as described below.

Confocal Microscopy Analysis

Parasites were washed by centrifugation in $HBSS^{-/-}$ and subjected to cytospin onto glass slides, fixed in 2% paraformaldehyde, permeabilized in 0.1% Triton X-100 for 10 minutes, and rinsed with $HBSS^{-/-}$. The parasites were incubated overnight in anti-PGFS antiserum, and nonimmune mouse serum as the negative control.

Infected macrophages were fixed in 2% paraformaldehyde and permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 15 minutes and blocked with 5% dry milk for 1 hour. To stain parasitophorous vacuoles, BMMs were incubated with rat 1D4B anti-LAMP-1 (1:100) in 5% milk/PBS overnight at 4°C, washed, and incubated with secondary antibodies (1:200) Alexa Fluor 647 or 488-conjugated with goat anti- rat immunoglobulin G (IgG) for 1 hour at room temperature.

Both parasites and infected BMMs were stained for lipid bodies and nuclei. Cells were first incubated in BODIPY 493/503 (10 μM; molecular probes) at room temperature for 1 hour to stain the lipid bodies. Cells were washed and then stained with 5 ηg/mL ethidium bromide to stain the nuclei. Images were analyzed by confocal microscopy using a Zeiss 510 microscope equipped with ZEN2009 software (Carl Zeiss).

In addition, uninfected and infected macrophages were stained with anti-FP antibody (1:20) overnight at 4°C, washed, and incubated with Texas Red-conjugated with goat anti-rabbit IgG for 1 hour at room temperature. The FP staining was colocalized with anti-LAMPI and DAPI staining (Vector Laboratories). Samples were observed by AX-70 Olympus microscopy, and images were acquired using the software Image-Pro Plus (MediaCybernetics).

Measurement of $PGF_{2\alpha}$ Production

Supernatants from parasite cultures medium or infected macrophages were collected for measurement of $PGF_{2\alpha}$ by enzyme-linked immunoassay (EIA) according to the manufacturer's instructions (Cayman Chemical).

Transmission Electron Microscopy

Metacyclic *L. i. chagasi* or infected BMMs were centrifuged, and the pellets were resuspended and fixed with 1% paraformaldehyde plus 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) overnight at 4°C. A subset of metacyclic parasites were fixed using an imidazole-based technique to stain the neutral lipids [31] prior to fixation. All cells were washed using the 0.1 M phosphate buffer (pH 7.4) and embedded in molten 2% agar (Merck). Agar pellets containing the cells were post-fixed in a mixture of 1% phosphate-buffered osmium tetroxide and 1.5% potassium ferrocyanide for 1 hour and processed for resin embedding (PolyBed 812, Polysciences). The sections were mounted on uncoated 200-mesh copper grids and were viewed using a transmission electron microscope (JEOL JEM-1230).

Immunogold Electron Microscopy

The infected macrophages were processed for immunogold staining. Cells were fixed in 4% paraformaldehyde, 1% glutaraldehyde, and 0.02% picric acid in 0.1 M cacodilate buffer at 4°C. Free aldehyde groups were quenched in a 0.1-M glycine solution for 60 minutes. Cells were then dehydrated in a methanol series and embedded at progressively lowered temperatures in Lowicryl K4M. Thin sections containing the infected macrophages were stained with rabbit anti-FP antibody (1:20; Cayman Chemical) overnight at 4°C. After incubation the sections were washed with $HBSS^{-/-}$ and incubated with 10 ηm colloidal gold-Affini-Pure-conjugated anti-mouse or anti-rabbit IgG (H + L) for 1 hour at room temperature. The samples were examined using a transmission electron microscope (JEOL JEM-1230).

Statistical Analyses

Each experiment was repeated at least 3 times. The data are presented as the mean plus SEM (standard error of the mean) of representative experiments and were analyzed using the GraphPad Prism 5.0 software. The dose-response experiments were analyzed using 1-way analysis of variance (ANOVA) with post-test to linear trend, and comparisons between the 2 groups were analyzed using Student *t* test. The differences were considered statistically significant when $P \leq .05$.

RESULTS

Lipid Body Arrangement During *L. i. chagasi* Metacyclogenesis

Lipid bodies can be visualized using techniques to stain neutral lipids, such as osmium impregnation or BODIPY (a fluorescent probe) [32]. Both light and confocal microscopic analyses were used to visualize and enumerate the LBs content in the different developmental forms of *L. i. chagasi* (Figure 1*A*–*C*). We used the LcJ *L. i. chagasi* parasite cell line that converts between promastigote and amastigote forms in axenic culture [29]. Graphical representation of the numbers of lipid bodies per parasite showed that LcJ amastigotes contained more LBs per cell than LcJ promastigotes in logarithmic stage growth (Figure 1*C*). Similarly, wild-type (WT) *L. i. chagasi* amastigotes isolated from spleens of infected hamsters contained more LBs than promastigotes (Figure 1*C*). Remarkably, the LB content increased during metacyclogenesis, with the lowest numbers in logarithmic, higher in unpurified stationary, and highest content in isolated

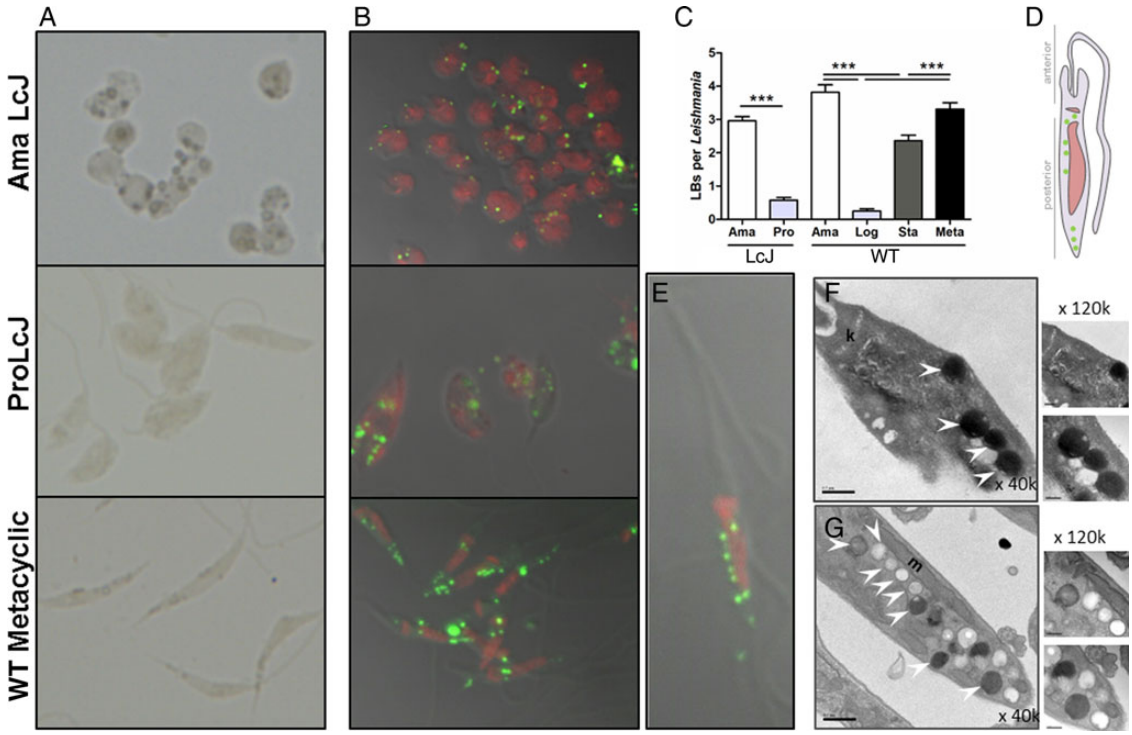

Figure 1. LB number during in vitro differentiation of *Leishmania infantum chagasi*. LcJ axenic parasite strain, which converts between amastigote and promastigote forms in vitro and WT metacyclic parasites were stained with (*A*) osmium tetroxide. *B*, Merged images of confocal microscopy of the parasites to LBs stained with BODIPY (*green*), DNA stained with ethidium bromide (*red*), and cell contours (DIC). *C*, Number of LBs in the different stages of *Leishmania* including the amastigote and promastigote LcJ axenic parasites strain and WT parasites. Bars represent the mean ± SEM from LB per parasite; n = 3; ***, $P < .001$ between groups (1-way ANOVA). *D*, Schematic representation of the arrangement of the LBs in most metacyclic forms, also shown microscopically in (*E*) by merge between LBs (*green*), DNA (*red*), and cell contours (DIC). Neutral lipids were detected using osmium imidazole-based (*F*) or conventional TEM (*G*). Lipid bodies are indicated with white arrowheads. Insets to the right of panels F and G show details of indicated LBs. Abbreviations: Ama, amastigote; ANOVA, analysis of variance; k, kinetoplast; LB, lipid body; Log, logarithmic; m, mitochondrion; Meta, metacyclic; Pro, procyclic; Sta, stationary; TEM, transmission electron microscopy; WT, wild-type.

metacyclic forms. The amount of LBs per metacyclic promastigote cell did not differ statistically from LB number per amastigote (Figure 1*C*). Next, we investigated the ultrastructural arrangement of LBs in the metacyclic forms of the *L. i. chagasi*, because this is the infective stage of the parasite. Confocal microscopy showed that the LBs were arranged in a linear sequence near to the cell nucleus (Figure 1*D* and 1*E*). Figure 1*D* shows a schematic of the arrangement of LBs in metacyclic promastigotes. In addition, we confirmed that the observed structures were LBs using osmium imidazole-based (Figure 1*F*) and conventional transmission electron microscopy (TEM; Figure 1*G*). The TEM analysis clearly showed the location of the LBs close to the mitochondrion and cell nucleus in the metacyclic forms (Figure 1*G*).

L. i. chagasi Lipid Bodies are Intracellular Sites for the Production of $PGF_{2\alpha}$

In *Trypasomatidae*, the only 2 enzymes in the eicosanoid synthesis pathway that have been described are phospholipase A_2 and PGFS [25]. To address whether PGFS is associated with LBs and whether this association correlates with virulence of parasite forms, we generated an anti-PGFS mouse antiserum against *L. chagasi* PGFS recombinant protein (see Supplementary Figure 1*A* and 1*B*). The LcJ promastigotes expressed higher levels of PGFS than amastigotes (Figure 2*A*). Strikingly, the PGFS expression in *L. i. chagasi* metcyclic forms was increased compared to WT amastigotes and procyclic forms (Figure 2*A*).

Lipid bodies are intracellular sites of eicosanoid synthesis in mammalian cells [33]. We tested if this is the case for *L. i. chagasi* by investigating the subcellular localization of PGFS in the metacyclic form of the parasite. Confocal microscopy showed that PGFS staining was strictly localized in the LBs (Figure 2*B*). To assay whether parasite LBs are the site of eicosanoid production, we incubated WT *L. i. chagasi* procyclic forms with different doses of arachdonic acid (AA; 3.75–30 μM), a major eicosanoid precursor. AA induced both LB formation and a dose-dependent release of $PGF_{2\alpha}$ by *Leishmania* (Figure 2*D* and 2*E*). However, there was no detectable effect on the cellular content of PGFS in the AA-stimulated *L. i. chagasi* procyclic forms (Figure 2*C*). These results suggest that: (i) *L. i. chagasi* LBs are the intracellular sites for $PGF_{2\alpha}$ production, (ii) the

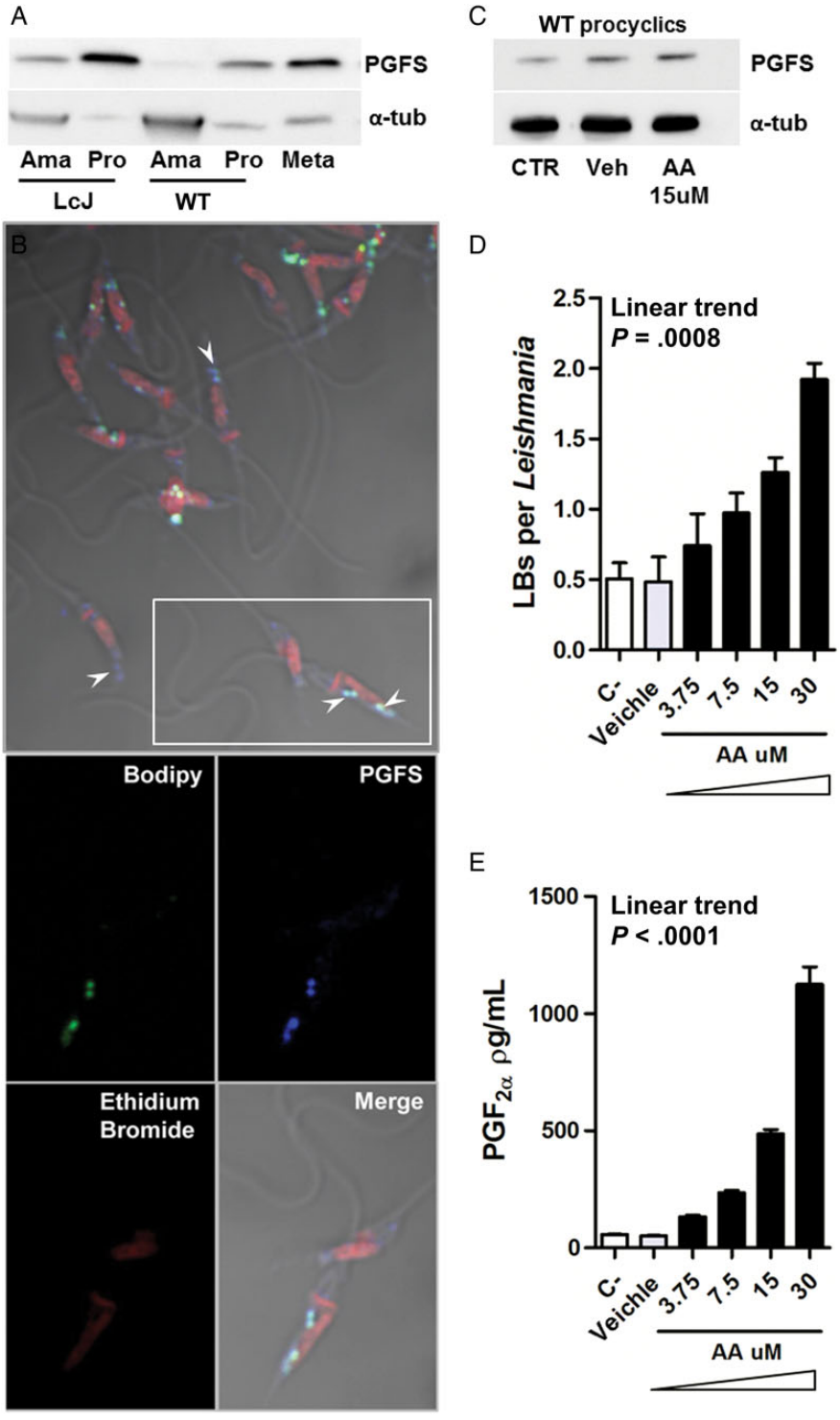

Figure 2. *Leishmania* LBs are intracellular sites for the production of $PGF_{2\alpha}$. *A*, Immunoblot comparing the abundance of PGFS at different stages in the WT or LcJ strain of *Leishmania infantum chagasi*, as described in the Methods section. Blots were incubated with polyclonal antiserum to recombinant PGFS (see Supplementary Figure 1*A* and 1*B*). *B*, upper panel shows merged image of metacyclic promastigotes visualized by confocal microscopy with anti-PGFS (*blue*), LBs stained with BODIPY (*green*), DNA stained with ethidium bromide (*red*), and cell contours (DIC). *B*, Lower panels show the left white box area as individual stains and a merged image to visualize PGFS colocalization with LBs. *C*, Immunoblot showing the abundance of PGFS in the procyclic forms of *L. i. chagasi* stimulated with AA, veh, or buffer (CTR) for 12 hours. *D*, Parasites were incubated with different doses of AA (3.75–30 μM) for 72 hours and then stained with osmium tetroxide to enumerate the LBs. *E*, Supernatants from promastigotes in panel *D* were harvested and $PGF_{2\alpha}$ levels were measured. Significance was tested by 1-way ANOVA with post-test linear trend. Bars represent the mean ± SEM, n = 3. Abbreviations: AA, arachidonic acid; ANOVA, analysis of variance; DIC, differential interference contrast; LB, lipid body; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGFS, prostaglandin $F_{2\alpha}$ synthase; SEM, standard error of the mean; veh, vehicle; WT, wild-type.

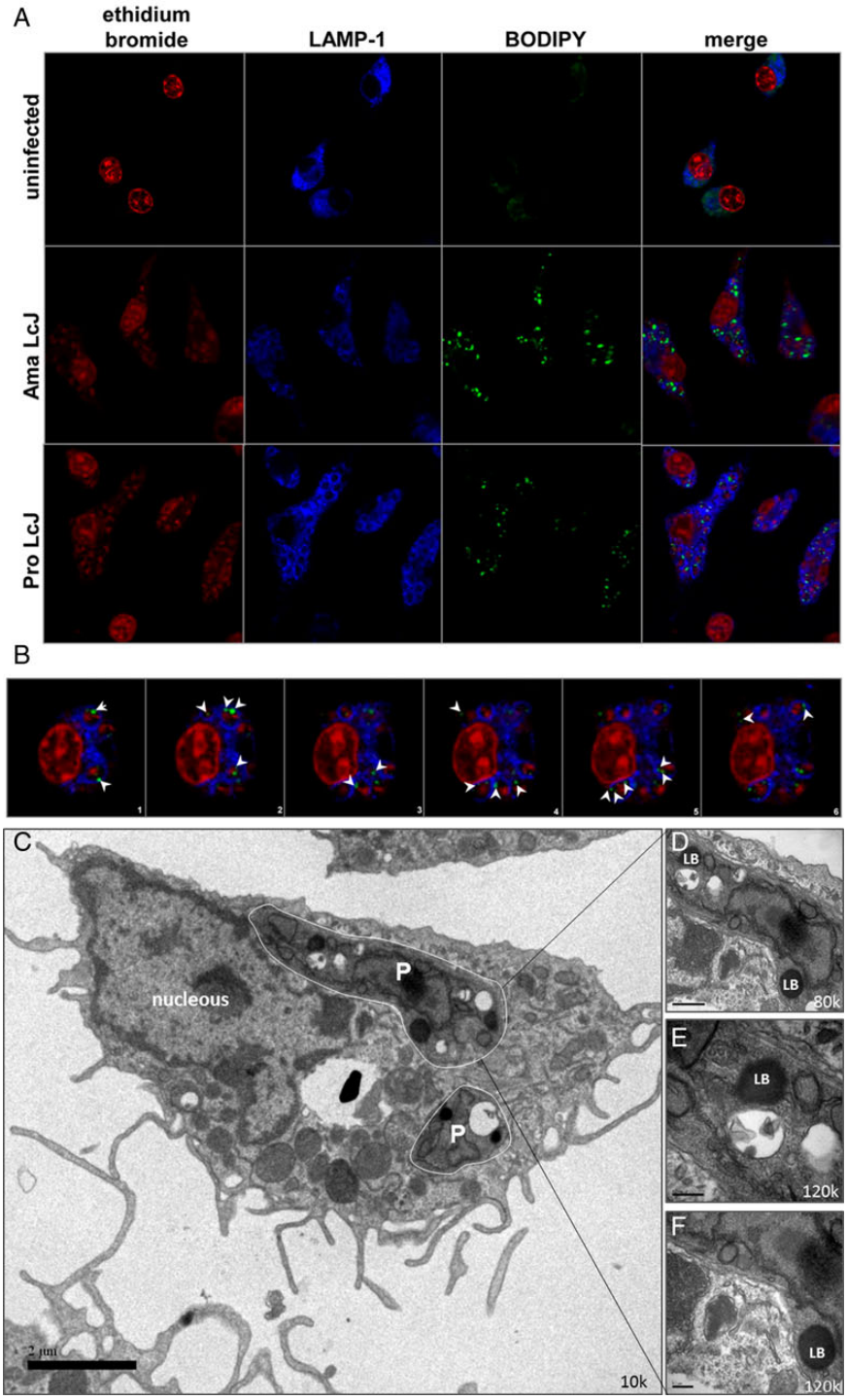

Figure 3. LBs are restricted to parasites during macrophage infection. *A*, images of BMMs infected with LcJ amastigotes and promastigotes for 24 hours. Nuclei were stained with ethidium bromide (*red*), PV membranes were stained with anti-Lamp1 (*blue*), and LBs were stained with BODIPY (*green*). *B*, A *z*-section sequence of images through an infected BMM. White arrowheads indicate the LBs inside PVs after 1 hour of LcJ amastigote infection. *C*, Transmission electron microscopic images of BMMs after 1 hour infection with metacyclic *Leishmania chagasi* shows an infected BMM with PV outlined in white (10k-fold increase). Panels *D* (80k-fold increase), *E*, and *F* (120k-fold increase) show details of LBs inside the parasites. Abbreviations: BMM, bone marrow derived macrophages; LB, lipid body; P, parasite; PV, parasitophorous vacuole.

promastigote production of $PGF_{2\alpha}$ increases in response to AA, and (iii) this prostaglandin is released from the parasite to the extracellular environment. Because compartmentalization is an important component of eicosanoid synthesis, a failure to

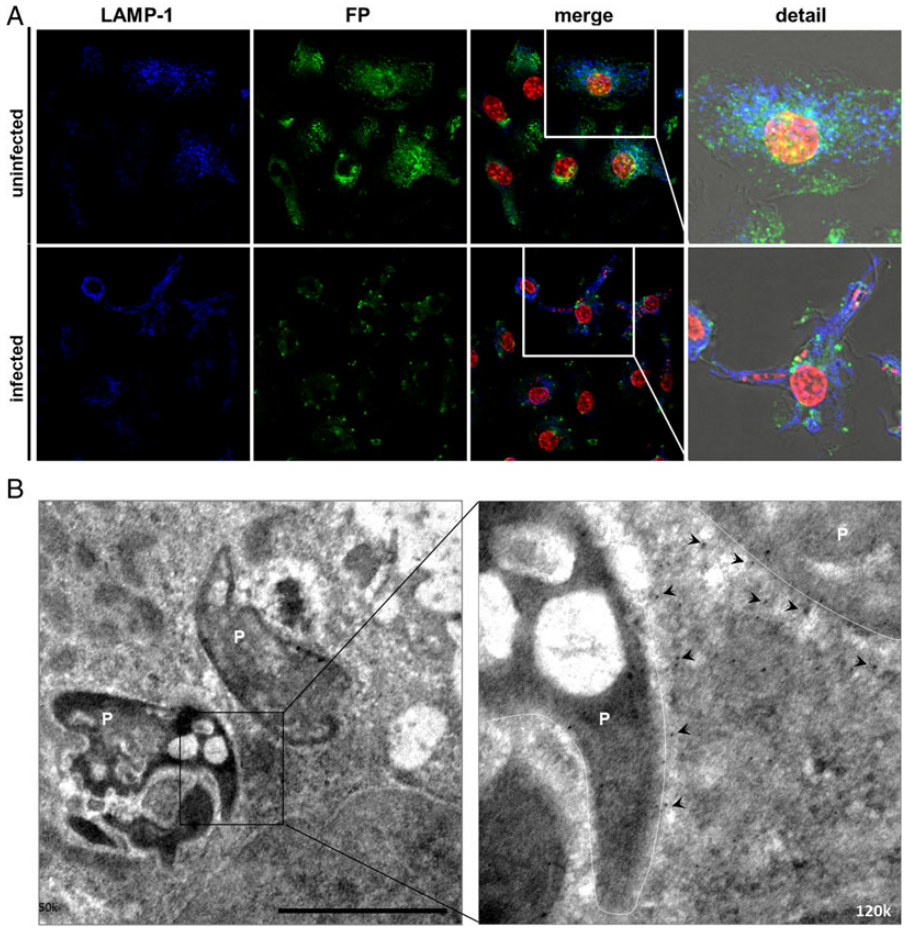

Figure 4. Localization of FP during early macrophage infection. *A*, BMMs were infected or not with metacyclic *Leishmania infantum chagasi* for 1 hour and FP localization was shown in the uninfected (*upper panels*) and infected cells (*lower panels*). Nuclei were stained with DAPI (*red*), PV membranes were stained with anti-Lamp1 (*blue*), and FP were stained using anti-FP or IgG control (*green*). Merge of fluorescence and DIC microscopy shows details of images from uninfected and infected. *B*, Right panel shows postembedding immunogold staining for FP (50k-fold increase), and left panel shows details of FP arrangement close to the PV outlined in white in the black box region from the right panel (120k-fold increase). FP staining is indicated by black arrowheads. Abbreviations: BMM, bone marrow derived macrophages; DIC, differential interference contrast; FP, prostaglandin $F_{2\alpha}$ receptor; IgG, immunoglobulin G; LB, lipid body; P, parasite; PV, parasitophorous vacuoles.

induce the total cellular abundance of the PGFS biosynthetic enzyme does not signify a failure to increase its activity.

Leishmania-driven $PGF_{2\alpha}$ Promotes *L. i. chagasi* Infection of Macrophages

Several intracellular pathogens induce LB formation and recruitment to parasitophorous vacuoles [33]. Intriguingly, our data suggest that the different developmental stages of *L. i. chagasi* forms did not induce host cell LB formation during infection of bone marrow-derived macrophages (BMMs). In contrast, we observed that the LB staining was restricted to the *L. i. chagasi* cell itself within the infected BMM (Figure 3*A*–*F* and see Supplementary Video 1). Because we have documented $PGF_{2\alpha}$ release from *L. i. chagasi*, we decided to assess the role of this eicosanoid in BMM infection. The distribution of FP was observed by confocal immunofluorescence in uninfected and *L. i. chagasi*-infected BMMs for 1 hour (Figure 4*A*). The FP staining in uninfected cell present diffuse in the cytoplasm, whereas in infected cell it was punctual and near to the early phagocytic vacuoles and to parasitophorous vacuoles containing parasites (Figure 4*A*). Using immunogold TEM to examine BMMs infected for 1 hour with *L.i. chagasi*, we observed that the FP, which recognizes $PGF_{2\alpha}$, was localized near to the parasitophorous vacuoles (Figure 4*B*).

In addition, the infected BMMs released $PGF_{2\alpha}$ after 48 hours postinfection (Figure 5*A*) but not PGE_2 (data not shown). Accordingly, pretreatment of BMMs with AL8810, a specific inhibitor of the FP, resulted in a dose-dependent decrease in *L. i. chagasi* infection (Figure 5*B*–*D*). Furthermore, inhibition of the FP decreased the infection index levels in BMMs

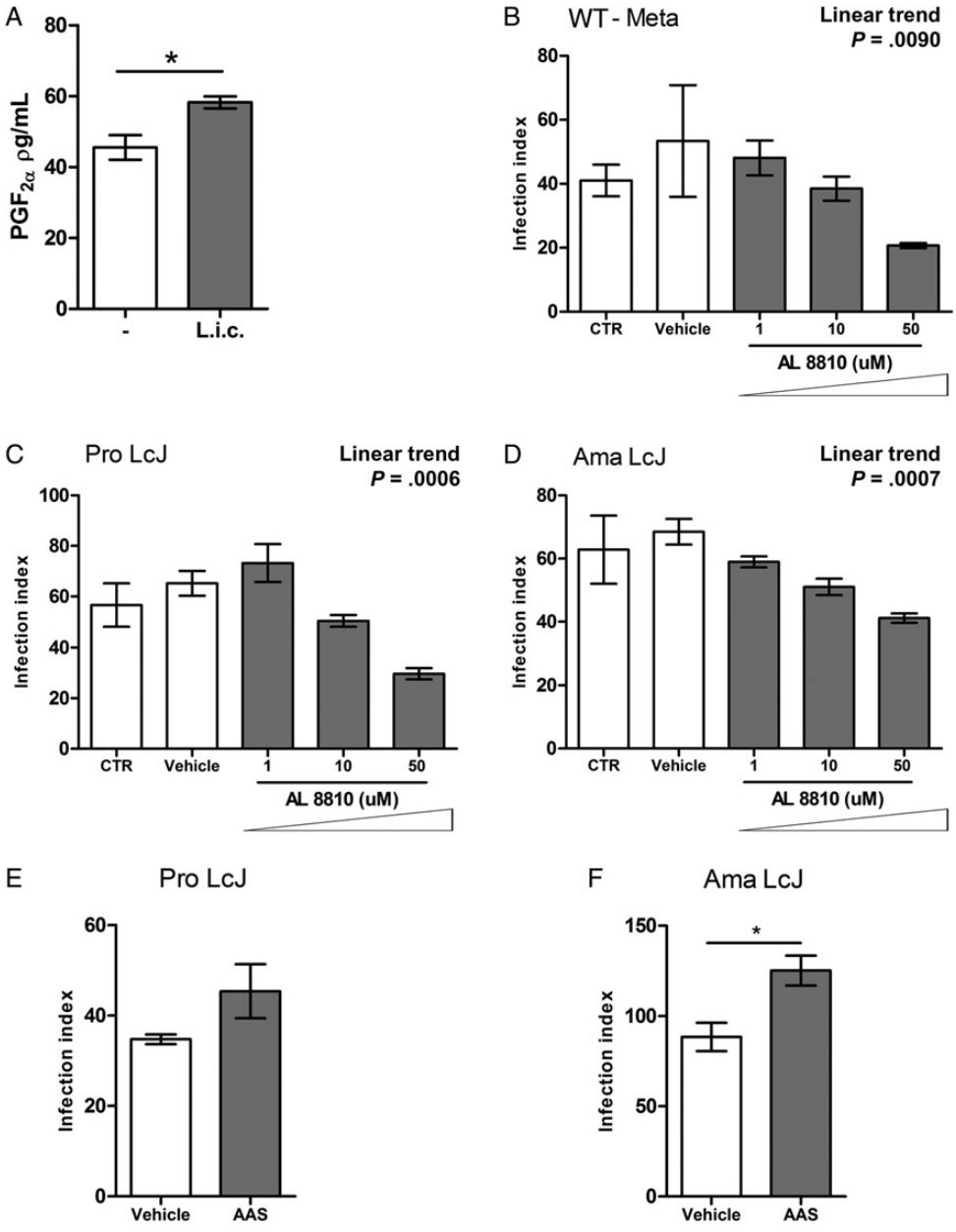

Figure 5. Role of the FP during *Leishmania infantum chagasi* infection. *A*, $PGF_{2\alpha}$ levels in supernatants harvested from BMMs infected with *L. i. chagasi* WT metacyclic for 48 hours. $^*P < .05$ (Student *t* test). BMMs were treated for 1 hour with 1, 10, and 50 μM AL8810, an FP antagonist, and infected with (*B*) WT metacyclic, (*C*) LcJ promastigotes, or (*D*) amastigotes forms of the parasite for 72 hours. Alternatively, BMMs were treated for 1 hour with 10 μM aspirin (ASS) and infected with (*E*) LcJ promastigotes or (*F*) amastigotes forms of the parasite for 72 hours. Infection indexes are illustrated (1-way ANOVA with post-test's linear trend). Bars represent the mean ± SEM, n = 3. Abbreviations: ANOVA, analysis of variance; BMM, bone marrow derived macrophages; FP, $PGF_{2\alpha}$ receptor; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SEM, standard error of the mean; WT, wild-type.

infected with all forms of parasites examined, that is, metacyclics, procyclics, and amastigotes (Figure 5*B–D*). To access the role of host-derived prostaglandins in the infection, we inhibited the host cell COX using aspirin, an irreversible COX inhibitor prior to infection. The cells were then washed and infected with *L.i. chagasi*. Of note, nonsteroidal antiinflammatory drugs (NSAIDs) including aspirin that inhibits COX activity in mammalian cells are not active to inhibit prostaglandin synthesis in parasites [34]. In this regard, the inhibition of COX enzyme of BMMs failed to change or even enhanced parasite burden during infection with of promastigotes and amastigotes parasites (Figure 5*E–F*). Taken together, these results indicate that *Leishmania* derived-$PGF_{2\alpha}$ plays an important role during infection.

DISCUSSION

Lipid bodies can play important roles as nutritional sources and in eicosanoid production during host-pathogens interactions [35, 36]. Eicosanoids released by macrophage LBs have the potential to modulate immune response [20, 33]. Despite of this, the role of eicosanoids produced by parasites and the cellular mechanism involved in their production have not been previously addressed. In the present study, we demonstrate that the LBs in *L. i. chagasi* are intracellular sites of prostaglandin production. Because LBs increase during both metacyclogenesis and in the intracellular amastigote form, we hypothesize that they could act as virulence factors. In addition, our data suggest that LBs in *L .i. chagasi* are responsible for the production of $PGF_{2\alpha}$ and they are important in the modulation of macrophage infection.

LBs have been implicated in the virulence of other infectious agents, such as *T. gondii* and *P. falciparum* [20]. The increase in the number of LBs in these parasites was demonstrated in vitro cultures and was associated with the acquisition of lipids, such as triacylglycerol (TAG), from the host cell during infection [37]. Herein, we demonstrated that *L. i. chagasi* increased the lipid storage in the LBs and amplified the expression of PGFS during metacyclogenesis, demonstrating that the parasites mobilize the eicosanoid machinery in the most infective stage of the parasite.

The biology of LBs in mammalian cells is relatively well understood. In leukocytes, LB formation is a coordinated process involving the activation of receptors and kinase proteins [2]. Similarly, recent studies in leukocytes have shown that *T. brucei* modulate the number of LBs via the activation of a specific parasite kinase named lipid droplet kinase LDK [38]. In the current study, we found that AA, a substrate of parasite PGFS, increases both the number of LBs and the release of $PGF_{2\alpha}$ by *L. i. chagasi*. Previous studies have shown that AA induces parasites to release prostaglandins, such as PGE_2, PGD_2 and $PGF_{2\alpha}$ [23, 25, 34]. Our data suggest that *L. i. chagasi*-derived $PGF_{2\alpha}$ may be important for parasite virulence because the expression of PGFS in the parasite increase during metacyclogenesis. In addition, PGFS is expressed predominantly in LBs, indicating that LBs are the major intracellular site for the production of prostaglandins in *L. i. chagasi*.

It has been reported that the host cell LBs are an important source of TAG and cholesterol for pathogens [35]. Indeed, pathogens can recruit host cell LBs to their parasitophorous vacuoles during infection [10, 14]. A recent study suggested that *Leishmania* may use a similar mechanism to acquire lipids and to induce foam cell formation [39]. However, our data demonstrated that the LBs formed during the *L. i. chagasi* infection are mainly from the parasites because the LBs are located inside the parasites within the parasitophorous vacuoles in the infected macrophages. Further studies will be necessary to elucidate how *Leishmania* acquires lipids from the host cells for its metabolism.

The role of $PGF_{2\alpha}$ in the immune response is not well understood. Macrophages can produce $PGF_{2\alpha}$ during inflammation [40] or during *L. donovani* infection [41]. $PGF_{2\alpha}$ binds and activates the FP to enhance COX-2 expression in the 3T3-L1 cell line, and the autocrine signaling of this mediator increases PGE_2 and $PGF_{2\alpha}$ levels [26]. Herein, we demonstrate that the FP is localized in the early phagocytic vacuoles and surface parasitophorous vacuoles during macrophages infection with metacyclic forms of *L. i. chagasi*. In addition, the *L. i. chagasi*-infected macrophages release $PGF_{2\alpha}$ and the inhibition of the FP in the macrophages diminishes the *L. i. chagasi* parasite load 72 hours after infection.

The role of eicosanoids derived from host cells has been demonstrated during *Leishmania* infection [42–44]. LTB_4 production during infection contributes to parasite killing [42, 43, 45]. On the other hand, PGE_2 is related with survival of the parasites in the intracellular environment [44, 46]. However, the contribution of eicosanoids derived from parasites during the infection have been poorly addressed. In this work, macrophages treated with AAS, an irreversible inhibitor of COX enzyme, followed by infection with nontreated *L. i. chagasi*, failed to revert the increased parasite load, suggesting that prostaglandins mostly derived from intracellular *L. i. chagasi* are related with parasite persistence.

Our findings demonstrate that LBs and PGFS from *L. i. chagasi* are upregulated in the metacyclic forms of the parasites as well as $PGF_{2\alpha}$ and its FP display an important role in the *Leishmania*-host interplay. They also suggest that parasite derived eicosanoids may enhance the survival of the parasite inside macrophages. Further studies will be necessary to elucidate how intracellular *Leishmania* could acquire lipids from the host cells and if and how they in turn release eicosanoid precursors into the infected macrophage cytoplasm. Ultimately, this could reveal a major mechanism through which the parasite controls the inflammatory microbicidal state of the infected host cell.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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