The absence of myocardial calcium-independent phospholipase a2γ results in impaired prostaglandin e2 production and decreased survival in mice with acute trypanosoma cruzi infection

Janhavi Sharma
Saint Louis University

Christopher S. Eickhoff
Saint Louis University

Daniel F. Hoft
Saint Louis University

David A. Ford
Saint Louis University

Richard W. Gross
Washington University School of Medicine in St. Louis

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Please let us know how this document benefits you.

Recommended Citation
Sharma, Janhavi; Eickhoff, Christopher S.; Hoft, Daniel F.; Ford, David A.; Gross, Richard W.; and McHowat, Jane, "The absence of myocardial calcium-independent phospholipase a2γ results in impaired prostaglandin e2 production and decreased survival in mice with acute trypanosoma cruzi infection." Infection and Immunity. 81, 7. 2278-2287. (2013).
https://digitalcommons.wustl.edu/open_access_pubs/2552

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
The Absence of Myocardial Calcium-Independent Phospholipase A$_{2\gamma}$ Results in Impaired Prostaglandin E$_2$ Production and Decreased Survival in Mice with Acute Trypanosoma cruzi Infection

Janhavi Sharma, Christopher S. Eickhoff, Daniel F. Hoft, David A. Ford, Richard W. Gross and Jane McHowat

The Absence of Myocardial Calcium-Independent Phospholipase A$_2$γ Results in Impaired Prostaglandin E$_2$ Production and Decreased Survival in Mice with Acute Trypanosoma cruzi Infection

Janhavi Sharma,$^a$ Christopher S. Eickhoff,$^b$ Daniel F. Hoft,$^b$ David A. Ford,$^c$ Richard W. Gross,$^d$ Jane McHowat$^a$

Department of Pathology,$^a$ Division of Infectious Diseases, Department of Internal Medicine,$^a$ and Department of Biochemistry and Molecular Biology and Center for Cardiovascular Research,$^a$ Saint Louis University School of Medicine, St. Louis, Missouri, USA; Division of Bioorganic Chemistry and Molecular Pharmacology, Department of Medicine, and Department of Chemistry, Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA

Cardiomyopathy is a serious complication of Chagas’ disease, caused by the protozoan parasite Trypanosoma cruzi. The parasite often infects cardiac myocytes, causing the release of inflammatory mediators, including eicosanoids. A recent study from our laboratory demonstrated that calcium-independent phospholipase A$_2$γ (iPLA$_2$γ) accounts for the majority of PLA$_2$ activity in rabbit ventricular myocytes and is responsible for arachidonic acid (AA) and prostaglandin E$_2$ (PGE$_2$) release. Thus, we hypothesized that cardiac iPLA$_2$γ contributes to eicosanoid production in T. cruzi infection. Inhibition of the isoform iPLA$_2$γ or iPLA$_2$β, with the R or S enantiomer of bromoenol lactone (BEL), respectively, demonstrated that iPLA$_2$γ is the predominant isoform in immortalized mouse cardiac myocytes (HL-1 cells). Stimulation of HL-1 cells with thrombin, a serine protease associated with microthrombus formation in Chagas’ disease and a known activator of iPLA$_2$, increased AA and PGE$_2$ release, accompanied by platelet-activating factor (PAF) production. Similarly, T. cruzi infection resulted in increased AA and PGE$_2$ release over time that was inhibited by pretreatment with (R)-BEL. Further, T. cruzi-infected iPLA$_2$γ-knockout (KO) mice had lower survival rates and increased tissue parasitism compared to wild-type (WT) mice, suggesting that iPLA$_2$γ-KO mice were more susceptible to infection than WT mice. A significant increase in iPLA$_2$ activity was observed in WT mice following infection, whereas iPLA$_2$γ-KO mice showed no alteration in cardiac iPLA$_2$ activity and produced less PGE$_2$. In summary, these studies demonstrate that T. cruzi infection activates cardiac myocyte iPLA$_2$γ, resulting in increased AA and PGE$_2$ release, mediators that may be essential for host survival during acute infection. Thus, these studies suggest that iPLA$_2$γ plays a cardioprotective role during the acute stage of Chagas’ disease.

Worldwide, almost 10 million people are infected with Trypanosoma cruzi, the causative agent of Chagas’ disease (1). Initially confined mostly to South America, global migration, blood products, organ transplantation, and congenital transmission have contributed to the spread of the disease, particularly in areas of nonendemicity. Millions of people are now at risk for contracting the infection, and in the United States alone, it is estimated that more than 300,000 people are infected with T. cruzi (2).

The organ primarily affected by Chagas’ disease is the heart. The disease progresses from an acute stage, followed by the indeterminate stage, to the chronic stages. The acute stage of the disease may or may not be symptomatic, and if present, symptoms are usually nonspecific, such as fever, chills, and nausea. Cardiac involvement at the acute stage is rare but can occur in a small number of infected people, leading to myocarditis and sudden death. The indeterminate phase is devoid of any clinical presentation and may last for several decades. Among infected patients, approximately 30% develop chronic disease with cardiac involvement (3, 4). Chronic Chagas’ heart disease includes arrhythmias, thromboembolic events, and congestive heart disease, leading to sudden death (5). A key pathological feature of Chagas’ disease is inflammation, which is regulated by several mediators, including adhesion molecules, cytokines, eicosanoids, and nitric oxide (6–8). In the acute stage, these molecules are seen to be important for the development of host resistance, as well as regulating progression to the chronic stages of the disease (9). However, much remains unknown about the host-parasite interactions that contribute to Chagas’ disease via these mediators. A better understanding of the complex relationship between the parasite, its target organ, and the cascade of events following infection will give us valuable information regarding a major human disease that we still have very limited means to treat.

Following infection, the parasites move across the endothelium and vascular tissue to gain access to the myocardium. After penetrating cardiac myocytes, the parasites divide intracellularly before causing the cell to rupture, releasing more parasites that can then spread and infect other cells. This cycle of infection and multiplication of the parasites, resulting in host cell lysis, directly causes tissue injury in the myocardium. Myocytes also contribute indirectly to disease progression via cytokine, chemokine, and reactive oxygen species (ROS) production, accompanied by changes in myocyte morphology (8, 10, 11).

The release of arachidonic acid from membrane phospholipids, catalyzed by phospholipase A$_1$ (PLA$_1$), is the rate-limiting step in eicosanoid formation. Arachidonic acid is converted to prosta-
glands via the activity of cyclooxygenase enzymes. The accompanying lysophospholipid can be converted to platelet-activating factor (PAF) following a transacylation reaction. PAF is a potent molecule involved in the recruitment and activation of inflammatory cells under a variety of pathophysiological circumstances. Hence, using a single substrate, PLA2 enzymes can contribute to the generation of a variety of bioactive lipids with a well-established role in inflammation.

Several studies have described the presence of platelet aggregation and thrombus formation with the progress of Chagas’ disease (5, 12). Thrombin is an important serine protease involved in inflammation and thrombus formation with the progress of Chagas’ disease. Thrombin is published role in inflammation.

The generation of a variety of bioactive lipids with a well-established role in inflammation.

Parasites and infection of HL-1 cells. Tissue culture trypanosomes (TCT) from the Brazil strain of T. cruzi were propagated in 3T3 mouse embryonic fibroblasts grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 2% newborn calf serum. The 3T3 cells were infected with T. cruzi on reaching 60% confluence. Infected cells ruptured following parasite multiplication, releasing a large number of parasites. The supernatant containing the parasites was collected, and parasite numbers were determined using a Neubauer hemocytometer. HL-1 cells, grown to confluence in the appropriate culture dish, were counted, and a multiplicity of infection (MOI) of 0.2 was used to infect the cells. Parasites and HL-1 cells were fed Claycomb medium supplemented with 2% FBS, and the appropriate number of parasites was then added to each culture dish. To prepare Tulahuen strain blood form trypanosomes (BFT), BALB/c or C.B-17 SCID mice were infected subcutaneously with 5,000 to 20,000 BFT. After 12 to 16 days, the animals were sacrificed and bled by decapitation into 50-ml conical tubes containing 5 μl of heparin (1,000 U/ml)/animal. The concentration of BFT/ml was determined by hemocytometer counts with heparinized blood samples diluted 1:10 with phosphate-buffered saline (PBS).

**Mice and infections.** Animal protocols were in strict accordance with the National Institutes of Health guidelines for humane treatment of animals and were reviewed and approved by the Animal Care and Use Committee of Saint Louis University. C57Bl6 WT or iPLA2-/-KO mice were used for in vivo studies. iPLA2-/-KO mice were generated in the laboratory of Richard Gross and have been described previously (18). Mice were infected with the Tulahuen strain blood form trypanosomes (5,000 BFT; subcutaneous infection) and sacrificed 14 days postinfection. Hearts were collected and frozen in liquid nitrogen before processing for biochemical analysis.

**Phospholipase A2 activity.** HL-1 cells were grown to confluence and exposed to the different experimental conditions. At the end of the experimental intervention, the medium was rapidly removed; the cells were washed with ice-cold PBS; 1 ml of ice-cold PLA2 activity buffer containing (per liter) 250 mmol sucrose, 10 mmol KCl, 10 mmol imidazole, 5 mmol EDTA, and 2 mmol diethanolamine (DEA) with 10% glycerol, pH 7.8, was added to each well; and the cells were removed from the plate using a cell scraper. Similarly, mouse hearts were flash frozen and homogenized in PLA2 buffer following collection at day 14 postinfection.

The suspension was sonicated on ice six times for 10 s each time (using a microtip probe at 20% power output; 500 Sonic Dismembrator; Fisher Scientific), and the sonicate was centrifuged at 20,000 × g for 20 min to remove cellular debris and nuclei. The pellet was resuspended in PLA2 activity buffer, and activity was determined by incubating the enzyme (50 μg protein) with 100 μM (16:0, [3H]18:1) plasmalogen in a 50-μl reaction mixture containing: 10 mmol Tris, 4 mmol EGTA, 10% glycerol, pH 7.0, at 37°C for 5 min in a total volume of 200 μl. The radiolabeled phospholipid substrate was introduced into the incubation mixture by incubation in 5 μl ethanolic to initiate the assay. Reactions were terminated by the addition of 100 μl butanol, and the released radiola beled fatty acid was isolated by the application of 25 μl of the butanol phase to channeled Silica Gel G plates, development in petroleum ether-diethyl ether-acetic acid (70/30/1, vol/vol/vol), and subsequent quantification by liquid scintillation spectrometry. The protein content of each sample was determined by the Lowry method utilizing freez-dried bovine serum albumin as the protein standard.

**Measurement of total arachidonic acid release.** HL-1 cells grown in 35-mm culture dishes were incubated at 37°C with 3 μCi [3H]arachidonic acid for 18 h. This incubation resulted in >70% incorporation of radioactivity into the myocytes. After incubation, the myocytes were washed three times with Tyrode solution containing 0.36% bovine serum albumin to remove unincorporated [3H]arachidonic acid. The cells were incubated at 37°C for 15 min before being subjected to the experimental conditions. At the end of the stimulation period, the supernatant was removed and centrifuged at 2,000 × g to remove any detached myocytes. Myocytes attached to the tissue culture well were solubilized in 10% sodium dodecyl sulfate, and radioactivity in both supernatant and cells was quantified by liquid scintillation spectrometry.

**Measurement of PGF2α release from HL-1 cells.** Myocytes were grown to confluence in 16-mm tissue culture dishes. The cells were washed twice with Hanks balanced salt solution (HBSS) containing (per liter) 135 mmol NaCl, 0.8 mmol MgSO4, 10 mmol HEPES (pH 7.6), 1.2 mmol...
CaCl₂, 5.4 mmol KCl, 0.4 mmol KH₂PO₄, and 6.6 mmol glucose. After washing, 0.5 ml of HBSS with 0.36% bovine serum albumin (BSA) was added to each culture well. The myocytes were then subjected to the experimental conditions, and the surrounding buffer was removed from the cells after selected time intervals. Detached myocytes were removed by centrifugation at 2,000 × g, and PGE₂ release was measured immediately using an immunnoassay kit (R&D Systems, Minneapolis, MN).

**Measurement of PGE₂ content by MS.** PGE₂ was quantified by liquid chromatography-tandem mass spectrometry (LC–MS–MS) using a method adapted from Degoursey and coworkers (19). In brief, 25 mg of freeze-clamped and pulverized ventricular tissue was homogenized on ice in 125 μl of phosphate-buffered saline and 40 μl of methanol (containing deuterated PGE₂, internal standard). Eicosanoids were then extracted twice into a volume of 600 μl, and ethyl acetate was subsequently added to the tissue homogenate and thoroughly mixed for 30 s. The pooled ethyl acetate extracts were then dried under nitrogen and resuspended in 50 μl acetone:trifluoroacetic acid–water (20:8:1:2 vol/vol/vol) prior to LC–MS–MS analysis.

The supernatant was then dried under nitrogen, 0.5 ml of HBSS with 0.36% BSA was added to each culture well. The myocytes were then subjected to the experimental conditions, and the surrounding buffer was removed from the cells after selected time intervals. Detached myocytes were removed by centrifugation at 2,000 × g, and PGE₂ release was measured immediately using an immunnoassay kit (R&D Systems, Minneapolis, MN).

**Measurement of parasitemia.** Circulating parasites from samples of fresh blood collected from each mouse at day 14 postinfection were counted. Blood was obtained by cardiac puncture, 1.5 μl was placed under a 12-mm round coverslip, and 25 high-power (×40) fields were counted. This method of parasitemia estimation has a lower-sensitivity detection limit of 16,740 BFT/ml.

**Real-time PCR detection of T. cruzi in mouse tissue.** The hearts of WT and iPLA₂γ-KO mice were harvested 14 days postinfection with T. cruzi. DNA samples were purified using a DNeasy kit (Qiagen, Valencia, CA), and the total DNA concentration was adjusted to 40 ng/μl. Primers (5'-AACCCACGACGACCACA-3' and 5'-TGCAAGATATCGCA CAAAGTA-3') were used to specifically amplify a 65 bp fragment of cruzipain, as described previously (21). However, instead of SYBR green detection, as described in the previous method, the amplicon generation was detected using a 6-carboxyfluorescein−6-carboxytetramethylrhodamine (FAM-TAMRA) probe (5'-TGCACCAGAGCTTCCCCA-3'; Synthegen, Houston, TX) and TaqMan PCR master mix (Applied Biosystems, Carlsbad, CA) (a 900-nm concentration of each primer and 200 ng of sample DNA). A standard curve was generated using positive-control DNA harvested from a known concentration of T. cruzi epimastigotes grown in pure culture. These reactions were run in an Applied Biosytems 7500 Fast Instrument under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Analysis of T. cruzi molecular equivalents (mEq) was performed by using Applied Biosystems 7500 software v2.0.1.

**Statistical analysis.** Statistical comparison of values was performed by Student's t test or one-way analysis of variance, with post hoc analysis performed using Dunnet’s test. All results are expressed as mean ± standard error of the mean (SEM). Statistical significance was considered to be a P value of <0.05.

**RESULTS**

**Measurement of PLA₂ activity in cardiac myocytes.** HL-1 cells were incubated with increasing concentrations of either (R)- or (S)-BEL for 10 min, and iPLA₂ activity was measured. The R and S enantiomers of BEL are 10-fold more selective for iPLA₂ (22). PLA₂ activity was measured in the absence of Ca²⁺ (4 mM EGTA) and using [16:0, [3H] 18:1) plasme,-

**Arachidonic acid release.** HL-1 cells were pretreated with either (R)- or (S)-BEL (2 μM; 10 min) prior to the addition of T. cruzi (MOI, 0.2), after which arachidonic acid release was measured for up to 24 h. T. cruzi infection resulted in a significant increase in arachidonic acid release that was evident after 12 h (Fig. 2A). Increased arachidonic acid release in response to T. cruzi infection was not affected by (S)-BEL pretreatment, but a significant inhibition of T. cruzi-induced arachidonic acid release was observed with (R)-BEL pretreatment. In similar experiments, thrombin (0.1 IU/ml) was added to HL-1 cells and arachidonic acid release was measured for up to 30 min, with or without pretreatment with either (R)-or (S)-BEL. Arachidonic acid release from cardiac myocytes was increased in response to thrombin (Fig. 2B). Pretreatment with (R)-BEL inhibited thrombin-induced arachidonic acid release, whereas pretreatment with (S)-BEL did not affect arachidonic acid release significantly. Thus, iPLA₂γ is predominantly responsible for arachidonic acid release.
from mouse atrial myocytes following either *T. cruzi* infection or thrombin stimulation.

**PGE2 release.** Cardiac myocytes infected with *T. cruzi* show a significant increase in PGE2 release 24 to 48 h postinfection (Fig. 3A). Similar to what was seen for arachidonic acid release, (S)-BEL pretreatment did not affect PGE2 release; however, pretreatment with (R)-BEL resulted in significantly reduced PGE2 release. Likewise, thrombin (0.1 IU/ml)-treated cells had a significant increase in PGE2 release, observed over time (Fig. 3B). As with *T. cruzi* infection, (S)-BEL pretreatment did not affect thrombin-stimulated PGE2 release, however, (R)-BEL inhibits thrombin-stimulated PGE2 release (Fig. 3B). These data suggest that iPLA2/H9253 is critical in regulating release of PGE2 from cardiac myocytes following *T. cruzi* infection.

Arachidonic acid and PGE2 release were measured under similar experimental conditions using H9C2 cells, a rat cardiomyoblast cell line. H9C2 cells infected with *T. cruzi* also showed a significant increase in arachidonic acid and PGE2 release that was inhibited by pretreatment with (R)-BEL, but not by pretreatment with (S)-BEL (data not shown).

**PAF production.** In response to *T. cruzi* infection, there was a slight increase in PAF production that did not reach significance and was unaffected by pretreatment with either (R)- or (S)-BEL (Fig. 4A). However, incubating myocytes with thrombin (1 IU/ml) resulted in a significant increase in PAF production over time (Fig. 4B). In contrast to arachidonic acid and PGE2 release, (R)-BEL does not affect thrombin-mediated PAF production, whereas (S)-BEL significantly inhibits it. Thus, iPLA2γ does not contribute significantly to PAF production in cardiac myocytes, and the mediator is produced by iPLA2β activation in response to thrombin stimulation (Fig. 4B). Hence, in response to thrombin stimulation, PAF production is regulated by iPLA2β and not by iPLA2γ.

**RAW cell adherence.** To evaluate the functional impact of PAF production on recruitment of inflammatory cells, we measured the adherence of calcein-labeled mouse monocytes/macrophages, RAW 264.7 cells, to cardiac myocytes following *T. cruzi* infection or thrombin stimulation. Infection of cardiac myocytes with *T. cruzi* caused no significant changes in RAW 264.7 cell adherence (Fig. 5A). This is in agreement with the absence of PAF production...
in T. cruzi-infected myocytes (Fig. 4A). Pretreating RAW 264.7 cells with CV3988, a PAF receptor antagonist, completely inhibits macrophage/monocyte adherence to T. cruzi-infected HL-1 cells. Following thrombin stimulation, a significant increase in RAW 264.7 cell adherence was observed (Fig. 5B) with a time course similar to that observed for increased PAF production (Fig. 4B). RAW 264.7 cell adherence to thrombin-stimulated HL-1 cells was inhibited by (S)-BEL pretreatment, but not by pretreatment with (R)-BEL (Fig. 5B).

Studies in iPLA$_2$$\gamma$-KO mice. In vitro studies indicate that iPLA$_2$$\gamma$ is crucial for the production of arachidonic acid and PGE$_2$. To understand its physiological relevance in Chagas’ disease, WT and iPLA$_2$$\gamma$-KO mice were infected with T. cruzi and observed over a period of 42 days (6 weeks). By day 21, all the iPLA$_2$$\gamma$-KO mice died, but over 50% of the WT mice were still alive (Fig. 6). Thus, iPLA$_2$$\gamma$-KO mice are more susceptible to infection than WT mice. Given this time course, differences in pathology and biochemical changes may be pronounced just prior to the onset of mortality, i.e., day 14. Hence, in the subsequent experiments, mice...
were sacrificed 14 days postinfection; fresh blood samples were collected to measure parasitemia; and hearts were isolated to measure tissue parasitism, enzyme activity, and eicosanoid production.

**Parasitemia and tissue parasitism.** The number of circulating parasites was significantly higher in iPLA2/H9253-KO mice than in WT mice after 14 days of infection (Fig. 7A). Since microscopic examination of tissues has limited sensitivity, a PCR-based amplification technique was used to accurately identify the presence of parasites in tissues. Corresponding to the higher levels of circulating parasites, an increase in the number of parasites in the hearts of iPLA2γ-KO mice compared to WT mice was also observed (Fig. 7B).

**Biochemical changes in the heart.** A significant increase was observed in cardiac iPLA2 activity in infected WT mice compared to uninfected mice. Further, the iPLA2γ-KO mice showed no change in cardiac iPLA2 activity following infection (Fig. 8). Thus, T. cruzi infection leads to activation of iPLA2γ in the heart, the absence of which is detrimental to survival. Reduced iPLA2 activity in the KO hearts suggests that these mice could also have impaired eicosanoid production. On measuring the PGE2 content in the heart, the iPLA2γ-KO mice were found to have significantly less of the eicosanoid than WT mice (Fig. 9). These data are in agreement with our in vitro observations and demonstrate the importance of myocardial iPLA2γ and eicosanoid production following T. cruzi infection.

**DISCUSSION**

Despite knowing the etiology of Chagas’ disease for over a century, successful treatment strategies are still elusive. So far, only two drugs, benznidazole and nifurtimox, are widely used for the treatment of the disease, and both are known to cause severe side effects. Adverse effects include digestive problems, hematological changes, dermatological alterations and neurological alterations (23,24). Though both drugs are recommended, their efficacies are variable, and more information is needed to verify their ability to clear the parasites. The fact that only two drugs exist to treat a disease that has been prevalent for so long highlights the “neglected” status of Chagas’ disease. Several challenges have impaired the development of therapeutic strategies, and much remains unknown about vector-host interactions and the mechanisms behind the various pathological symptoms associated with the disease. Only recently has it been determined that the
chronic stages of the disease are associated with parasite persistence and that autoimmune mechanisms may not be responsible for organ damage, as hypothesized for several decades previously (25). Since the presence of parasites is critical for the disease, it is necessary to decipher the cascade of events that occur following infection, increasing our understanding and the potential to create drugs that can effectively treat the disease.

Several downstream metabolites of PLA2 have been implicated in Chagas’ disease, but little is known about the direct contribution of the enzyme to the pathogenesis of the disease. Bioactive lipids, such as eicosanoids and PAF, are potent inflammatory molecules produced by the action of PLA2 on membrane phospholipids. Previous studies have outlined the varying roles of eicosanoids in Chagas’ disease. Michelin et al. have suggested an immunosuppressive role for PGE2, upregulated via concanavalin A stimulation of COX-2 in T. cruzi-infected spleen cells (26). Abdalla et al. demonstrated that inhibition of COX-2-mediated PGE2 production resulted in reduced inflammation, parasite pseudocysts, and fibrosis in the hearts of infected mice, suggesting that PGE2 contributes to cardiac damage in the acute phase of infection (27). Cardoni et al. concluded from their studies that COX-2-derived mediators are protective during the acute stages of T. cruzi infection but could be responsible for tissue damage in the chronic stages. More recent studies by Ashton et al. have suggested that TXA2 is an important parasite-derived eicosanoid that contributes to parasite proliferation and hence the inflammatory response (28). Several studies also suggest that eicosanoids, in conjunction with cytokines, chemokines, and other inflammatory mediators, are essential for the progression and perpetuation of the chronic phase of the disease (9, 29). Since PLA2 is critical for arachidonic acid release and eicosanoid production, it could con-

![Figure 7](http://iai.asm.org/...)

![Figure 8](http://iai.asm.org/...)

![Figure 9](http://iai.asm.org/...)

**Sharma et al.**

2284 iai.asm.org Infection and Immunity

Downloaded from http://iai.asm.org on March 31, 2014 by Washington University in St. Louis
tribute to the pathogenesis of Chagas’ disease via the formation of these mediators.

The predominant phospholipase A2 in the heart, iPLA2, is known to have two major isoforms in mammalian cells, iPLA2β and iPLA2γ. While both isoforms are present in cardiac tissue, it has been determined in these studies and in previous work from our laboratory that most of the cardiac iPLA2 activity is attributable to the γ isoform of the enzyme (15). In order to differentiate between the functions of the two isoforms, pharmacological inhibitors, (R)- and (S)-BEL, are commonly used (22). BEL has been demonstrated to have 100-fold selectivity for iPLA2 versus cytosolic PLA2 (cPLA2) and secretory PLA2 (sPLA2) isoforms, and its enantiomers, (R)- and (S)-BEL, are 10-fold more selective for iPLA2γ and iPLA2β, respectively (30). Previously, we determined that iPLA2 has a preference for the arachidonoylplasmalogen phospholipid species, which are abundant in the sarcolemma of cardiac myocytes (14). Serine proteases, such as thrombin, were shown to activate iPLA2 via the protease-activated receptors (PARs) present on the myocyte surface, and based on pharmacological inhibition, iPLA2γ was established as the predominant isoform in cardiac myocytes (13, 16). Since arachidonic acid release, the rate-limiting step in eicosanoid production, is generated by the action of phospholipases, it is likely that iPLA2γ contributes to the production of this critical mediator. However, until the present study, the effect of T. cruzi on cardiac iPLA2 has not been investigated.

Other members of the PLA2 family also exist in the myocardium and are grouped based on their structures, substrate preferences, and requirements for calcium. cPLA2 enzymes are selective for arachidonoylcholine phospholipids but do not demonstrate any preference for sn-1 vinyl ether linkages (plasmalogens) and contribute to the formation of eicosanoids in response to extracellular signals (31). In the heart, cPLA2 is known to regulate cardiac hypertrophy and plays a protective role in ischemia reperfusion injury (32, 33). sPLA2s do not exhibit any preference for the sn-2 fatty acid but are proposed to play a role in eicosanoid formation in the heart (31). It has also been suggested that sPLA2 may be involved in regulating cardiac hypertrophy (34). iPLA2 enzymes are selective for plasmalogen phospholipids, which are selectively enriched in the sarcolemma of myocardial cells. Also, the majority of the myocardial arachidonic acid is esterified to plasmalogen species, making plasmalogens a rich source of the arachidonic acid required for eicosanoid production following hydrolysis by iPLA2. The contribution of any of the PLA2 isoforms to T. cruzi infection remains largely unknown. It is possible that both cPLA2 and sPLA2 contribute to the progression of Chagas’ heart disease; however, since the majority of the myocardial PLA2 activity is attributable to iPLA2, we have explored the role of this isoform in acute Chagas’ disease.

We used the mouse atrial cardiac myocyte cell line (HL-1 cells), as well as a knockout mouse model, to demonstrate the role of iPLA2 in acute T. cruzi infection. In mouse cardiac myocytes, the inhibition of iPLA2 activity is much more sensitive to (R)-BEL than to (S)-BEL, suggesting that iPLA2γ is the predominant isoform in these cells. An iPLA2γ-mediated increase in arachidonic acid and PGE2 release was also observed following treatment with thrombin or T. cruzi infection of cardiac myocytes. Hence, this isoform may be critical for controlling the rate-limiting step in eicosanoid production. The mouse model of infection revealed that iPLA2γ is important in acute infection, since the iPLA2γ-KO mice exhibited early mortality compared to WT mice. Prior to the onset of mortality in either group (day 14 postinfection), WT mice showed increased cardiac iPLA2 activity and less parasitemia and tissue parasitism, unlike that observed in the iPLA2γ-KO mice. Hence, the absence of iPLA2γ results in a higher parasite load and mortality, suggesting a protective role for the isoform in acute T. cruzi infection.

Previous studies by Mukherjee et al. suggest that COX-1 inhibition by aspirin leads to increased parasitemia and mortality in T. cruzi-infected mice. These findings were not mimicked entirely in COX-1 knockout mice, which show increased parasitemia, but not enhanced mortality. Based on these findings, it was hypothesized that COX-1-mediated inhibition of eicosanoids contributed to increased parasitemia but did not affect mortality and that aspirin-mediated increase in mortality is likely due to “off-target” effects of the drug. Our findings, in the absence of any pharmacological inhibitors, show that the reduced eicosanoids and increased parasite load associated with mortality are due to the absence of iPLA2γ.

Another important PLA2-derived mediator, PAF, is responsible for the activation and transmigration of inflammatory cells to relevant sites (35). The role of PAF in cardiac inflammation following T. cruzi infection is largely unknown. It has been suggested that PAF induces the production of nitric oxide in T. cruzi-infected macrophages and hence is protective (36). PAFR−/− mice infected with T. cruzi were shown to have an increased number of parasite pseudocysts and inflammation in the heart and hence are more susceptible to infection than WT mice (37). In this study, cardiac myocytes infected with T. cruzi did not show a significant increase in PAF production. Thrombin treatment however, did result in a considerable increase in PAF production, which is mediated by iPLA2β and not iPLA2γ.

The presence of microthrombi and platelet aggregation have been observed in Chagas’ heart, suggesting an increase in thrombin in the myocardium (12, 38). Pinazo et al. have also demonstrated that a prothrombotic state exists in chronic Chagas’ disease patients, as they have significantly increased endogenous thrombin potential and prothrombin fragment 1 + 2, which serves as a marker for thrombin generation (39). Thus, while the parasite itself may not directly affect PAF production in cardiac myocytes, other associated factors present in the vicinity of infection could enhance the process. These findings suggest a protective role for iPLA2β in acute infection via the production of PAF and its effects on macrophages, but this needs further investigation. The disparity in PAF production with T. cruzi infection versus thrombin pretreatment is not known but may be related to differences in intracellular events initiated by activation of the thrombin receptor and those initiated by infection with T. cruzi. In previous studies, we have demonstrated that there is an immediate increase in PAF production in response to thrombin stimulation of endothelial cells (35). Our data here show a similar increase in PAF production in cardiac myocytes stimulated with thrombin. We propose that this is due to tight coupling between the thrombin receptor and iPLA2β activation. Our data here suggest that thrombin activates HL-1 cell iPLA2γ and iPLA2β, whereas T. cruzi infection results in iPLA2γ activation only and may be related to more delayed intracellular processes. Regardless of changes in PAF production, RAW cell adherence remained unchanged, suggesting that increased PAF production does not play a direct role in inflammatory-cell adherence to cardiac myocytes.
In conclusion, these studies suggest that, following *T. cruzi* infection, the subsequent increase in arachidonic acid and eicosanoid levels is mediated by iPLA$_2$, the predominant PLA$_2$ isoform in these cells. Thrombin stimulation has a similar effect on iPLA$_2$ but, additionally, causes increased PAF production, mediated by iPLA$_2$B.

Several studies have suggested that eicosanoids produced during the acute stage of the disease are important immunomodulators, enabling the disease to progress into its chronic stages. Thus, iPLA$_2$ contributes to this process via eicosanoid production, which in turn affects parasite persistence and disease progression, forming a critical link between the parasite and the host. Our studies indicate that iPLA$_2$ activation is beneficial during acute infection, elucidated by the early mortality observed in iPLA$_2$-KO mice following infection. While many questions remain, clearly, iPLA$_2$ is critical in the setting of *T. cruzi* infection. Understanding its role during the acute stages of the disease may thus help us recognize important events during infection that may impact the chronic stages and present us with more points of intervention and treatment of Chagas’ disease.

**REFERENCES**


---

Infection and Immunity

2286  iai.asm.org

Downloaded from http://iai.asm.org on March 31, 2014 by Washington University in St Louis


