Trypanosoma cruzi produces the specialized proresolving mediators Resolvin D1, Resolvin D5 and Resolvin E2

Running Title: *T. cruzi* and Resolvins

Romain A. Colas[1*‡], Anthony W. Ashton[2‡], Shankar Mukherjee[3], Jesmond Dalli[1*], Oscar B. Akide-Ndunge[3], Huan Huang[3], Mahalia S. Desruisseaux [3,4], Fangxia Guan[3.7], Linda A. Jelicks[5], Fabiane Matos dos Santos[3], Jyothi Nagajyothi[6], Michael Zingman[3], Jinet Reyes[3], Louis M. Weiss[3,4], Charles N. Serhan[1], Herbert B. Tanowitz [3,4,#]

[1]Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[2]Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, N.S.W. Australia

Departments of [3]Pathology, [4]Medicine, [5]Physiology & Biophysics, Albert Einstein College of Medicine, Bronx, NY

[6]Public Health Research Institute Center at the International Center for Public Health, New Jersey Medical School - Rutgers, The State University of New Jersey

[7]School of Life Sciences, Zhengzhou University, Zhengzhou,China

*Present Address: Lipid Mediator Unit, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, London, United Kingdom

**Present Address: Columbia University College of Physicians & Surgeons, NY, NY

‡RAC and AWA contributed equally

Corresponding Author

Herbert B. Tanowitz, MD

Department of Pathology

Albert Einstein College of Medicine

1300 Morris Park Avenue

Bronx, NY 10461

Phone: +1 (718) 430-2142

Email: herbert.tanowitz@einstein.yu.edu

ABSTRACT

Trypanosoma cruzi is a protozoan parasite that causes Chagas disease (CD). CD is a persistent, life-long infection affecting many organs, most notably the heart where it may result in acute myocarditis and chronic cardiomyopathy. The pathological features include myocardial inflammation and fibrosis. In the Brazil-strain infected CD-1 mouse that recapitulates many of the features of human infection, we found increased plasma levels of resolvin D1 (RvD1), a specialized pro-resolving mediator of inflammation, both during the acute and chronic phases of the infection (>100 days post infection) as determined by ELISA. Additionally, ELISA on lysates of trypomastigotes of both the Tuliahan and Brazil strains revealed elevated levels of RvD1 when compared with lysates of cultured epimastigotes of *T. cruzi*, tachyzoites of *Toxoplasma gondii*, and trypomastigotes of *T. brucei*, cultured L_6E_9 myoblasts and culture media containing no cells. Lysates of *T. cruzi* –infected myoblasts also displayed increased levels of RvD1. Lipid mediator metabolomics confirmed that the trypomastigotes of *T. cruzi* produced RvD1, RvD5 and RvE2, which have been demonstrated to modulate the host response to bacterial infections. Plasma levels of RvD1 maybe both host and parasite derived. Since *T. cruzi* synthesizes specialized pro-resolving mediators of inflammation as well as pro-inflammatory eicosanoids, such as thromboxane A_2, one may speculate that by using these lipid mediators to modulate its microenvironment, the parasite is able to survive.

Key words: *Trypanosoma cruzi*, Chagas disease, Resolvins, Resolvin D1, Resolvin D5, Resolvin E2, inflammation, immune modulation

List of Abbreviations:

ELISA, Enzyme Linked ImmunoSobrant Assay; FFA, free fatty acid; AA, arachidonic acid; DHA, docosahexenoic acid; EPA, eicosapentaenoic acid; HDHA, hydroxyl-docosahexenoic acid; HETE, hydroxy-eicosatetraenoic acid; HEPE, hydroxy-eicosapentaenoic acid; PG, prostaglandin; TXA_2, thromboxane A_2; TP, Receptor for TXA_2; FP, receptor for $PGF_{2\alpha}$; LT, leukotriene; LX, lipoxin; SPM, specialized pro-resolving mediator; RvD, resolvins derived from DHA; RvE, resolvins derived from EPA; PD, protectin; MaR, maresin; EFA, essential fatty acid; LO, lipoxygenase; IFN-γ, interferon -γ; PBMC, peripheral blood mononuclear cell; H&E, Hematoxylin and Eosin; MRI, magnetic resonance imaging; ECG, electrocardiogram; LM, lipid metabolite; LC, Liquid chromatography; MS, mass spectrometry; dpi, days post-inoculation; NDGA, nordihydroguaiaretic acid; TcOYE, *T. cruzi* old yellow enzyme; TbPGFS, *T. brucei* $PGF_{2\alpha}$ synthase.

INTRODUCTION

The parasite *Trypanosoma cruz*i is the etiologic agent of Chagas disease. It is endemic in Mexico, Central and South America where millions of persons are infected or at risk (1). In recent years, due to migration of individuals from endemic areas, there has been increased recognition of Chagas disease in the United States, Europe and other non-endemic areas (2-4). Additionally, vector transmission of this parasite has been documented in the United States (5). Although any nucleated cell in the body can be infected, the cardiovascular system is among the organs targeted by this parasite causing acute myocarditis and chronic cardiomyopathy.

Acute infection with *T. cruzi* results in an intense inflammatory response associated with an increased expression of pro-inflammatory mediators including cytokines, chemokines and endothelin-1(6). We have also previously described the release of the bioactive lipid mediators prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2) from *T. cruzi*, although TXA_2 is preferentially synthesized (7). TXA_2 is a pro-inflammatory, vasoconstrictor with pro-thrombotic activity through enhanced platelet activation-aggregation. $PGF_{2\alpha}$ is a pro-inflammatory vasoconstrictor substance like TXA_2 (8); however, it opposes platelet activation by TXA_2 and platelet activating factor (9). Both receptors (TP and FP) couple to $G_{\alpha q}$ and $G_{\alpha 13}$, activate the small GTPase Rho, and promote cellular activation; however, FP expression on leukocytes is not well documented suggesting the effects of FP on inflammation are likely indirect while the effects of TP are direct (8). A previous report indicates that TP can be activated by $PGF_{2\alpha}$ (10), suggesting a single focal point for the action of pro-inflammatory prostaglandins in Chagas disease and re-enforcing the prominence of TP to the pathogenesis of disease.

Acute inflammation is a natural host protective mechanism mounted by the body in response to

injury or invading pathogens. When self-limited, it restores homeostasis (11); however, if left uncontrolled, excessive inflammation can lead to chronic tissue damage (12). Resolution of inflammation is an active process orchestrated by a genus of potent molecules known as specialized pro-resolving mediators (SPM)(13) . SPM, that include the resolvins, protectins and maresins, are bioactive autacoids with both anti-inflammatory and pro-resolving properties. They are enzymatically produced from essential fatty acids (EFA), with distinct stereochemistries. RvD1 biosynthesis involves sequential oxygenations of DHA by 15-lipoxygenase (LO) and 5-LO (14). Using a total organic synthetic approach the complete stereochemistry of RvD1 has been established as 7S,8R,17S-trihydroxy- 4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (15). RvD1 displays potent leukocyte-directed actions in pico-nanogram concentration range *in vivo* (14). In *E. coli*-induced peritonitis, RvD1 reduces bacterial titers and hypothermia, increases survival and enhances microbial containment and killing by phagocytes (16). The aspirin triggered epimer of RvD1, has been found to regulate the host immune response in Chagas disease patients by decreasing IFN-γ and the percentage of necrotic cells in the peripheral blood mononuclear cell (PBMC) pool, and reducing the rate of *T. cruzi* antigen-stimulated PBMC proliferation in cultured cells (17). The source and relevance of resolvins to *T cruzi* infection is currently uninvestigated.

The full complement of the *T. cruzi* lipidome is yet to be determined. We asked whether *T. cruzi* infection resulted in up regulation of SPM expression and whether there were contributions to the pro-resolution pathway from both host and parasite. We hypothesized that infection with *T. cruzi* results in an increased expression of resolvins both *in vitro* and *in vivo*. Indeed, herein we demonstrate that mice infected with *T. cruz*i display increased levels of resolvin D1 (RvD1) and that the trypomastigote form of the parasite contains RvD1, RvD5 and RvE2. Thus, it is of

interest that *T. cruzi* synthetized both pro-inflammatory (TXA_2) and SPM (resolvins) that are essential regulators of host response that promote transition to chronic infection. This observation may have new implications for the pathogenesis of Chagas disease.

Materials and Methods

Animal Ethics Statement: All animal experiment were performed with approval of the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

Parasitology and Pathology: The Brazil and Tulahuen strains of *T. cruzi* were maintained by serial passage in C3H mice (Jackson Laboratories, Bar Harbor, ME). For studies with the Brazil strain 8-week old male CD-1 mice were infected with $5x10^4$ parasites. Parasitemia was determined by counting in an hemocytometer chamber as previously described (18). Trypomastigotes of both the Brazil and Tulahuen strains of *T. cruzi* were propagated in a myoblast cell line (L_6E_9) as previously described(19). The RH strain of *Toxoplasma gondii* was maintained in cultured human foreskin fibroblasts. The *T. brucei brucei* Lister 427 strain was provided to us by Dr. George Cross (The Rockefeller University, New York, NY). Hearts were fixed in 10% (v/v) neutral buffered formalin and sections (5 μm) were stained with Hematoxylin and Eosin (H&E).

Cardiac Magnetic Resonance Imaging (MRI): For magnetic resonance imaging (MRI), mice were anesthetized with isoflurane inhalation (2% (v/v) in medical air administered via a nose cone). MRI compatible electrocardiogram (ECG) electrodes were inserted in the left front and right rear paws and the ECG signal was used as a trigger signal with a Small Animal Instruments physiological monitoring system (Stony Brook, NY). Mice were positioned in a 35-mm ID 1H volume coil [Molecules2Man Imaging Co., Cleveland, OH(20)]. Body temperature was maintained at 34~35°C using warm air with feedback from a body surface thermocouple and a small respiratory sensor balloon, taped onto the abdomen, provided for respiratory gating. Images were acquired using a 9.4 T Varian Direct Drive animal magnetic resonance imaging and spectroscopic system (Agilent Technologies, Inc. Santa Clara, CA). One mm thick slices were

acquired in short-axis orientation using an ECG-triggered and respiratory gated multi-frame tagged cine sequence. The imaging parameters used were field of view (FOV) of 40×40 mm^2, matrix size of 256×256, TE of 2.6 ms, TR of 5.5 ms, flip angle of 25°, number of averages of 2.

General considerations regarding ELISA and LC-MS/MS assays: *T. cruzi* epimastigotes were cultured in LIT medium which we have assayed and contains no resolvins (by both ELISA and LC-MS-MS). They were washed 3 times in PBS (containing no resolvins) and lysates were made from these epimastigotes. Trypomastigotes of *T. cruzi* were obtained from infected myoblast cultures which we confirmed contained no resolvins (by both ELISA and LC-MS-MS). Lysates of *T. brucei* trypomastigotes and tachyzoites of the RH strain of *Toxoplasma gondii* prepared in similar fashion contained no resolvins indicating the resolvin content of *T. cruzi* was not an artifact of culture conditions. Positive controls for the LC-MS/MS were the known authentic standards for the resolvins. Negative controls were the L_6E_9 myoblasts which we found to have no detectable amounts of resolvins. The limits of detection of this LC-MS-MS system ~ 10-15 picomoles. Resolvin (Rv)D1 was determined in lysates of $1x10^6$ trypomastigote of both the Tulahuen and Brazil strains of *T. cruzi* and trypomastigotes of *T.b. brucei* according to manufacturer's specifications (Cayman Chemical Company, Ann Arbor, MI, USA). Blood was collected from infected CD-1 mice by retro-orbital bleed, centrifuged and frozen at −80°C until use. RvD1 levels for all experiments were determined according to absorbance measured between 405 - 420 nm via a microplate reader.

Lipid mediator (LM) metabololipidomics: LM metabololipidomics was performed as described (Colas et al, 2014). After hypotonic lysis, parasites were further sonicated and snap frozen (stored at -80°C until analyzed). Briefly, parasite lysates were placed in ice-cold methanol (containing deuterium labeled internal standard (d_5-RVD2, d_4-PGE_2, d_5-LXA_4 and d_4-LTB_4,

500pg each) to facilitate quantification and recovery. Samples were held at -20°C for 45 minutes to allow protein precipitation and centrifuged. Supernatants were collected and placed onto an automated extraction system (Biotage) using a C18 column. Bioactive LMs were collected and injected into an LC system (Shimadzu) coupled to a Qtrap 5500 (ABSciex). Identification was conducted using published criteria including matching retention time to synthetic standards and a minimum of 6 diagnostic ions in the MS-MS mode as reported (21).

Statistical analysis: All other data are expressed as the mean (±SEM) and were analyzed using GraphPad Prism statistics software (GraphPad Software Inc., San Diego, CA). For analysis of differences between groups the Student's *t* test was performed. A level of significance of 5% was chosen to denote differences between means.

Results

Animal studies

Parasitology, Pathology and Cardiac Imaging

Parasitemia was first detected at 15 days post infection (dpi), peaked at 4-5x10^5 trypomastigotes/ml at 35 to 40 dpi and waned by 60 dpi when parasitemia was undetectable (Figure 1A). Mortality was 50% during acute infection (Figure 1B). At 100 dpi cardiac imaging demonstrated an enlarged heart and a significant increase in the right ventricular internal diameter consistent with previous reports (20) (Figure 1C and D). Histopathological examination of the heart during the acute phase revealed myonecrosis and parasite pseudocysts (Figure 1E). During the chronic phase there was cardiac myocyte hypertrophy, chronic inflammation and fibrosis, consistent with previous reports (6).

Plasma Resolvin D1 increases during acute *T. cruzi* infection

There was a rapid rise in RvD1 (μg/ml of plasma) in infected mice that was evident at 20 dpi and remained elevated out to 140 dpi during the chronic phase of infection (Figure 2A). This profile suggests that RvD1 is derived both from parasite and host. To assess this hypothesis we examined RvD1 levels in parasite lysates.

Resolvin D1 levels in parasite extracts as determined by ELISA

Trypomastigotes of both the Brazil and Tulahuen strains of *T. cruzi* had significantly greater amounts of RvD1 compared to epimastigotes of the Tulahuen (Figure 2B) and Brazil (data not shown) strains. RvD1 was not detected at significant levels in trypomastigotes of *T. brucei*, tachyzoites of *T. gondii* or from uninfected L_6E_9 myoblasts. In contrast, myoblasts infected with

the Tulahuen strain of *T. cruzi* (which contained intracellular amastigotes) produced significant amounts of RvD1 compared to uninfected myoblasts. Although production was lower than in free trypomastigotes these data suggest that intracellular amastigotes synthesize RvD1 or that infection stimulates production by myoblasts. To unequivocally determine that RvD1 was being produced we performed LC-MS-MS analysis, the gold standard for identification of bioactive lipid mediators.

LM metabololipidomics

The above results clearly indicated that RvD1 is present in trypomastigotes of *T. cruzi*. Figure 3 demonstrates the characteristics LC-MS-MS spectra used for the identification of RvD1, RvD5 and RvE2 in parasite extracts. The resulting lipidomic profiles are representative of 3 separate parasite lysates from both Tulahuen and Brazil strains. Extracts of uninfected L_6E_9 myoblasts and LIT media were negative for lipid species suggesting all identified species were parasite derived (data not shown). Both strains had the precursor lipids docosahexenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (Table 1) indicating that biosynthesis of all pro-inflammatory and pro-resolving mediators were possible. DHA and its bioactive metabolome were present in significantly higher quantities indicating a potential preference for biosynthesis of mediators derived from this fatty acid. Consistent with earlier reports from our laboratory and others(8) we identified the arachidonic acid metabolites PGE_2, PGD_2 and $PGF_{2\alpha}$ in *T. cruzi* lysates (Table 2). The presence of 5/12/15-HETE and 5/12/15/18-HEPE in extracts (Table 3) suggests active lipoxygenase pathways are present in the parasite with arachidonic acid and EPA as the respective precursor FFAs(22). Potential downstream pro-inflammatory end products might also include leukotrienes; however, no leukotriene B_4 production was identified

(Table 3). Thus, the primary class of pro-inflammatory lipid species produced by *T. cruzi* is prostaglandins.

An active lipoxygenase pathway would also initiate and promote the biosynthesis of pro-resolving lipid mediators such as lipoxins, resolvins, maresins and protectins. When we examined lysates by LC-MS-MS no evidence of lipoxins, protectins or maresins were observed (Table 4). However, their pathway markers were identified indicating that trypomastigotes of *T. cruzi* have the enzymatic capability to produce these mediators. Of interest, RvD1 and RvD5 were biosynthesized by both the Tulahuen and Brazil strains of *T. cruzi* (Table 4). Moreover, the Brazil strain produced significant levels of RvE2 (Table 4) indicating that only the Brazil strain utilized EPA to produce pro-resolving mediators. The precursor of RvE2 (18-HEPE) is lower in the Brazil strain perhaps reflecting increased use of this precursor for RvE2 synthesis. These studies clearly indicate that RvD1 is present in trypomastigotes of *T. cruzi* and that D and E series resolvins are the likely primary lipid mediators through which *T. cruzi* dampen the host response to infection.

238 **Discussion**

239 The heart is an important focus of infection by *T. cruzi*, although other tissues and organs are
240 infected as well. Acute *T. cruzi* infection is characterized by an upregulation of pro-inflammatory
241 cytokines and chemokines and an influx of inflammatory cells. In the setting of Chagas disease,
242 as in other disease states, the acute inflammatory response is a “double-edged" sword, in that, it
243 is needed to control the infection, but also leads to tissue injury. The chronic phase of Chagas
244 disease is accompanied by the appearance of chronic inflammatory cells and cardiac remodeling
245 (i.e., fibrosis). The present study clearly demonstrates, for the first time, that *T. cruzi*
246 trypomastigotes and amastigotes produce RvD1 which likely contributes to the resolution of
247 inflammation. This was confirmed by both ELISA and LM metabolomic profiling. In addition,
248 *T. cruzi*-infected mice display elevated circulating RvD1 levels during the acute phase of
249 infection and well into the chronic phase when peripheral parasitemia has waned and, in the
250 mouse model, cardiomyopathy is present. Lysates of trypomastigotes of the Brazil and Tulahuen
251 strains contained RvD5 while trypomastigotes of the Brazil strain contained RvE2. We did not
252 identify RvD1 in a variety of other protozoan parasites (*T. gondii* and *T. brucei*) and non-infected
253 mammalian cells.

254 Historically, the resolution of inflammation was considered a passive process resulting from the
255 loss or dilution of pro-inflammatory mediators from the extracellular milieu. It is now
256 understood, however, that the resolution of inflammation is instead, an active and programmed
257 event (13). The mediators of the resolution of inflammation, SPM (include the resolvins,
258 protectins and maresins), are produced enzymatically from essential fatty acids (EFA). RvD1 is
259 one of these bioactive mediators and as noted in *E. coli*-induced peritonitis, RvD1 reduces
260 bacterial titers and hypothermia, increases survival and enhances microbial containment and

killing by phagocytic cells (16). RvD5 has also been shown to be involved in the setting of experimental *E. coli* by enhancing phagocytosis. Combination of RvD5 with the antibiotic ciprofloxacin was able to accelerate the antibiotic effect and the same was observed in the setting of experimental Staphylococcal infection (16). RvE2, identified by LM metabololipidomics, has potent leukocyte-directed actions that regulates chemotaxis of human neutrophils, enhancing phagocytosis and anti-inflammatory cytokine production. RvE2 rapidly down-regulates surface expression of human leukocyte integrins in whole blood and dampened responses to platelet-activating factor. These actions appear to be mediated by leukocyte G-protein-coupled receptors. Collectively, these observations indicate that RvE2 can stimulate host-protective actions throughout initiation and resolution in the innate inflammatory responses (23).

The ability of the parasite and host to liberate pro-resolving mediators is likely to alter the course of disease. The identification of 15- and 5(S),15 (S)-HETE in *T cruzi* extracts suggests 15-LO activity (which has been previously reported in parasites such as *T. gondii*)(24), however, this has not been previously documented and no data is available comparing disease progression in $15LO^{-/-}$ mice or with pharmacological antagonists of 15LO activity. Conversely, a comparison of 5LO activity has been performed (25). 5LO activity is essential for synthesis of many pro-resolving mediators (such as E-series resolvins and lipoxins). Direct comparison of 5-LO null mice (intact parasite RvD1 synthesis) and infected mice treated with NDGA (1.25 mg/mouse/day), a 5LO inhibitor (inhibiting both mouse and parasite RvD1) indicated that NDGA treatment promoted earlier development of parasitemia and greater lethality than the 5LO null mice (25) although number of cardiac amastigote nests and anti-oxidant protection were similar. These differences suggest that NDGA may have inhibited parasite- and host-derived 5LO activity and that parasite 5LO derivatives may play a significant role in suppressing early

parasite growth during acute infection and promote survival of the host through the acute infection. Our data suggest that of the available mediators only RvE2 production would be affected by such a difference (as no lipoxin nor leukotriene production was observed in the *T. cruzi* strains tested). These data indicate the liberation of SPMs from the parasite may be important in mediating the transition from acute to chronic disease and promoting host survival during experimental *T. cruzi* infection.

Earlier, our groups reported that *T. cruzi* synthesizes the lipid mediators TXA_2 and $PGF_{2\alpha}$(7), TXA_2 is both a potent vasoconstrictor and a pro-inflammatory mediator synthesized by both trypomastigotes and amastigotes (7, 22). The majority of TXA_2 in the serum of infected mice is derived from the parasite (7). In the present report, we have demonstrated that trypomastigotes and amastigotes of both the Brazil and Tulahuen strains of *T. cruzi* strains contain RvD1, RvD5 and RvE2, important pro-resolving mediators. Plasma RvD1 levels are elevated in *T. cruzi* (Brazil strain) infected mice and it is possible that the source of the RvD1 is, in part, from the parasite itself. The synthesis of pro-inflammatory and pro-resolving lipid mediators suggests that the parasite is able to modulate its microenvironment through these metabolites. If the net result is a damping down of the inflammatory response, this may be important in the perpetuation of the infection into the chronic phase. There are other anti-inflammatory factors present during *T. cruzi* such as interleukin-10. In that regard, it was recently demonstrated that there is an interaction between RvD1 and interleukin-10 in the resolution of inflammation in adipose tissue in the experimental obese state (26). These observations suggest that the addition of such an anti-parasitic regimen may ameliorate some of the consequences of this infection. Resolvins dampen the inflammatory response and promote resolution of infections, which prevents fibrosis, and enables parasite persistence and the perpetuation of the parasite life cycle

(27).

The biosynthesis of lipid mediators by *T. cruzi* is increasingly complex but may provide advantages for longevity of infection. The parasite genome encodes terminal synthases for some eicosanoid species and the ability of *T. cruzi* to elevate the host plasma levels of lipid mediators in mice knocked out for the respective terminal synthase (such as the TXA_2 synthase null mice) suggest these pathways are active during disease (7). While *T. cruzi* proteins such as $PGF_{2\alpha}$ synthase are highly homologous to other prokaryotic enzymes (such as yeast old yellow enzyme (TcOYE) and *T. brucei* synthase (TbPGFS))(28-30) their primary sequences bare significant differences to mammalian terminal enzymes (29, 30). Moreover, the *T. cruzi* enzymes are resistant to pharmacological inhibitors of mammalian terminal synthases indicating that the active sites are also topographically or structurally different (28). Thus, parasite-derived eicosanoids manipulate host responses during infection but are intractable to current therapies.

Most interestingly, the terminal synthase for the primary *T. cruzi* prostaonoid TXA_2 has eluded genomic efforts to identify it prompting suggestions that the parasite may co-opt host synthetic pathways by the process of trogocytosis (31). Similarly, the 15LO and 5LO activities needed for D and E series resolvin biosynthesis are not predicted from the *T. cruzi* genome. It therefore remains to be determined whether the biosynthetic pathways exist in *T. cruzi* or are serendipitously stolen from infected host cells. Only the examination of chronically infected null mice, once parasite numbers have declined, is likely to yield these answers.

Previously, we reported that *T. cruzi* produces the pro-inflammatory and pro-thrombotic lipid TXA_2. Herein we now demonstrate that this parasite also produces several pro-resolving lipids. Taken together, this parasite may be able to modulate its microenvironment and enhance

persistence. On the other hand, the administration of RvD1 may ameliorate the inflammation and fibrosis in the heart. These experiments are currently underway.

331 **ACKNOWLEDGEMENTS**

332 This work was supported by NIH Grants PO1 GM095467 (CNS) and AI-214000 (HBT)

333

REFERENCES

1. Nunes MC, Dones W, Morillo CA, Encina JJ, Ribeiro AL, Council on Chagas Disease of the Interamerican Society of C. 2013. Chagas disease: an overview of clinical and epidemiological aspects. J Am Coll Cardiol 62:767-76.
2. Tanowitz HB, Weiss LM, Montgomery SP. 2011. Chagas disease has now gone global. PLoS Negl Trop Dis 5:e1136.
3. Requena-Mendez A, Moore DA, Subira C, Munoz J. 2016. Addressing the neglect: Chagas disease in London, UK. Lancet Glob Health 4:e231-3.
4. Perez N, Lasarte P, Bibiloni N, Dejean CB. 1989. Alpha/beta interferon in serum from children with the hemolytic uremic syndrome. Acta Paediatr Scand 78:637-8.
5. Garcia MN, Aguilar D, Gorchakov R, Rossmann SN, Montgomery SP, Rivera H, Woc-Colburn L, Hotez PJ, Murray KO. 2015. Evidence of autochthonous Chagas disease in southeastern Texas. Am J Trop Med Hyg 92:325-30.
6. Tanowitz HB, Machado FS, Spray DC, Friedman JM, Weiss OS, Lora JN, Nagajyothi J, Moraes DN, Garg NJ, Nunes MC, Ribeiro AL. 2015. Developments in the management of Chagas cardiomyopathy. Expert Rev Cardiovasc Ther 13:1393-409.
7. Ashton AW, Mukherjee S, Nagajyothi FN, Huang H, Braunstein VL, Desruisseaux MS, Factor SM, Lopez L, Berman JW, Wittner M, Scherer PE, Capra V, Coffman TM, Serhan CN, Gotlinger K, Wu KK, Weiss LM, Tanowitz HB. 2007. Thromboxane A2 is a key regulator of pathogenesis during Trypanosoma cruzi infection. J Exp Med 204:929-40.
8. Hata AN, Breyer RM. 2004. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. Pharmacol Ther 103:147-66.

9. Armstrong RA, Jones RL, Wilson NH. 1985. Mechanism of the inhibition of platelet aggregation produced by prostaglandin F2 alpha. Prostaglandins 29:601-10.

10. Arakawa H, Lotvall J, Kawikova I, Lofdahl CG, Skoogh BE. 1993. Leukotriene D4- and prostaglandin F2 alpha-induced airflow obstruction and airway plasma exudation in guinea-pig: role of thromboxane and its receptor. Br J Pharmacol 110:127-32.

11. Anonymous. 1999. Robbins Pathologic Basis of Disease, 6 ed.

12. Tabas I, Glass CK. 2013. Anti-inflammatory therapy in chronic disease: challenges and opportunities. Science 339:166-72.

13. Serhan CN. 2014. Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92-101.

14. Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN. 2003. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. J Biol Chem 278:14677-87.

15. Sun YP, Oh SF, Uddin J, Yang R, Gotlinger K, Campbell E, Colgan SP, Petasis NA, Serhan CN. 2007. Resolvin D1 and its aspirin-triggered 17R epimer. Stereochemical assignments, anti-inflammatory properties, and enzymatic inactivation. J Biol Chem 282:9323-34.

16. Chiang N, Fredman G, Backhed F, Oh SF, Vickery T, Schmidt BA, Serhan CN. 2012. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature 484:524-8.

17. Ogata H, Teixeira MM, Sousa RC, Silva MV, Correia D, Rodrigues Junior V, Levy BD, Rogerio Ade P. 2016. Effects of aspirin-triggered resolvin D1 on peripheral blood mononuclear cells from patients with Chagas' heart disease. Eur J Pharmacol 777:26-32.

18. Trischmann T, Tanowitz H, Wittner M, Bloom B. 1978. Trypanosoma cruzi: role of the immune response in the natural resistance of inbred strains of mice. Exp Parasitol 45:160-8.

19. Rowin KS, Tanowitz HB, Wittner M, Nguyen HT, Nadal-Ginard B. 1983. Inhibition of muscle differentiation by trypanosoma cruzi. Proc Natl Acad Sci U S A 80:6390-4.

20. Prado CM, Fine EJ, Koba W, Zhao D, Rossi MA, Tanowitz HB, Jelicks LA. 2009. Micro-positron emission tomography in the evaluation of Trypanosoma cruzi-induced heart disease: Comparison with other modalities. Am J Trop Med Hyg 81:900-5.

21. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. 2014. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol 307:39-54.

22. Machado FS, Mukherjee S, Weiss LM, Tanowitz HB, Ashton AW. 2011. Bioactive lipids in Trypanosoma cruzi infection. Adv Parasitol 76:1-31.

23. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. 2012. Resolvin E2 formation and impact in inflammation resolution. J Immunol 188:4527-34.

24. Bannenberg GL, Aliberti J, Hong S, Sher A, Serhan C. 2004. Exogenous pathogen and plant 15-lipoxygenase initiate endogenous lipoxin A4 biosynthesis. J Exp Med 199:515-23.

25. Borges CL, Cecchini R, Tatakihara VL, Malvezi AD, Yamada-Ogatta SF, Rizzo LV, Pinge-Filho P. 2009. 5-Lipoxygenase plays a role in the control of parasite burden and contributes to oxidative damage of erythrocytes in murine Chagas' disease. Immunol Lett 123:38-45.

401 26. Titos E, Rius B, Lopez-Vicario C, Alcaraz-Quiles J, Garcia-Alonso V, Lopategi A, Dalli
402 J, Lozano JJ, Arroyo V, Delgado S, Serhan CN, Claria J. 2016. Signaling and
403 Immunoresolving Actions of Resolvin D1 in Inflamed Human Visceral Adipose Tissue. J
404 Immunol 197:3360-3370.
405 27. Basil MC, Levy BD. 2016. Specialized pro-resolving mediators: endogenous regulators
406 of infection and inflammation. Nature Reviews Immunology 16:51-67.
407 28. Kabututu Z, Martin SK, Nozaki T, Kawazu S, Okada T, Munday CJ, Duszenko M,
408 Lazarus M, Thuita LW, Urade Y, Kubata BK. 2003. Prostaglandin production from
409 arachidonic acid and evidence for a 9,11-endoperoxide prostaglandin H2 reductase in
410 Leishmania. Int J Parasitol 33:221-8.
411 29. Kubata BK, Duszenko M, Kabututu Z, Rawer M, Szallies A, Fujimori K, Inui T, Nozaki
412 T, Yamashita K, Horii T, Urade Y, Hayaishi O. 2000. Identification of a novel
413 prostaglandin f(2alpha) synthase in Trypanosoma brucei. J Exp Med 192:1327-38.
414 30. Okano Y, Inoue T, Kubata BK, Kabututu Z, Urade Y, Matsumura H, Kai Y. 2002.
415 Crystallization and preliminary X-ray crystallographic studies of Trypanosoma brucei
416 prostaglandin F(2 alpha) synthase. J Biochem 132:859-61.
417 31. Mukherjee S, Mukhopadhyay A, Andriani G, Machado FS, Ashton AW, Huang H, Weiss
418 LM, Tanowitz HB. 2015. Trypanosoma cruzi invasion is associated with trogocytosis.
419 Microbes Infect 17:62-70.

420

421

FFA Precursor			Trypomastigotes of *T. cruzi* strains (range, pg/mg protein)	
Species	Q1	Q3	Tulahuen	Brazil
EPA	301	257	1.6---1194^	2.9---1937^
DHA	327	283	113---15111^	62.1---3841^
4S,14S-diHDHA	359	221	*	*
17-HDHA	343	245	9.7---199.3	6.4---242.4
14-HDHA	343	205	15.7---86.8	14.9--141.6
7-HDHA	343	141	16.9--262.4	11.3---88.2
4-HDHA	343	101	94.5---427.4	70.0--471.3
AA	303	259	15.5---2529^	32---5766^

Table 1: Quantitation of free fatty acid (FFA) precursors in lysates of trypomastigotes by LC-MS/MS. Expression of lipid species are quantified from peak height and expressed a pg/mg protein. Data represent the range of samples documented from 3 independent lysates for each strain. * represents samples with values below the detection limit, ^ = samples are in ng/mg protein. EPA,; DHA,; HDHA, hydroxy Docosahexaenoic Acid; AA, .arachidonic acid

Prostaglandin Lipid Species	Q1	Q3	Trypomastigotes of *T. cruzi* strains (range, pg/mg protein) Tulahuen strain	Brazil strain
PGD_2	351	189	1.4-3.9	2.7-23
PGE_2	351	189	2.0-21.4	6.0-48.6
$PGF_{2\alpha}$	353	193	2.6-38.9	2.7-2.6

Table 2: Quantitation of prostaglandin species in lysates of trypomastigotes of *T. cruzi* by LC-MS/MS. Expression of lipid species are quantified from peak height and expressed a pg/mg protein. Data represent the range of samples documented from 3 independent lysates for each strain. PG, prostaglandin.

Lipoxygenase-derived Lipid Species	Q1	Q3	Trypomastigotes of *T. cruzi* strains (range, pg/mg protein) Tulahuen strain	Brazil strain
LTB_4	335	195	*	*
20-OH-LTB_4	351	195	*	*
20-COOH-LTB_4	365	195	*	*
5-HETE	319	115	54.6-101.6	26.3-122.1
12-HETE	319	179	11.1-46.9	15.3-143.3
15-HETE	319	219	24.8-166.4	39.7-328.1
5S,15S-diHETE	335	235	2.3-5.2	5.7-8.0
5-HEPE	317	115	5.4-12.9	2.5-31.0
12-HEPE	317	179	0.7-6.1	0.6-1.0
15-HEPE	317	219	2.5-3.3	2.3-3.4
18-HEPE	317	259	3.8-5.4	1.7-2.2
5S,15S-diHEPE	333	115	1.3-27.7	1.7-13.6

435 **Table 3: Quantitation of lipoxgenase-derived lipid species in lysates of *T. cruzi***
436 **trypomastigotes by LC-MS/MS.** Expression of lipid species are quantified from peak height
437 and expressed a pg/mg protein. Data represent the range of samples documented from 3
438 independent lysates for each strain. * represents samples with values below the detection limit.
439 LT, leukotriene; HETE,; hydroxy-eicosatetraenoic acid; diHETE, di-hydroxy-eicosatetraenoic
440 acid; HEPE, hydroxy-eicosapentaenoic acid; diHEPE, di-hydroxy- eicosapentaenoic acid

Pro-Resolving Lipid Species	Q1	Q3	Trypomastigotes of *T .cruzi* (range, pg/mg protein) Tulahuen strain	Brazil strain
RvD1	375	141	1.2-1.4	1.8-7.0
RvD2	375	215	*	*
RvD3	375	147	*	*
RvD5	359	199	1.4-1.6	0.7-1.9
RvD6	359	159	*	*
RvE1	349	161	*	*
RvE2	333	253	*	9.5-23.6
RvE3	333	201	*	*
PD1	359	153	*	*
22-OH-PD1	375	153	*	*
22-COOH-PD1	389	153	*	*
MaR1	359	250	*	*
LXA_4	351	115	*	*
LXB_4	351	115	*	*
LXA_5	349	215	*	*
LXB_5	349	221	*	*

441 **Table 4: Quantitation of pro-resolving lipid mediators in lysates of trypomastigotes of *T.***
442 ***cruzi* by LC-MS/MS.** Expression of lipid species are quantified from peak height and expressed
443 a pg/mg protein. Data represent the range of samples documented from 3 independent lysates for
444 each strain.
445 * represents samples with values below the detection limit. RvD, resolvins derived from
446 docosahexenoic acid; RvE, resolvins derived from eicosapentaenoic acid; PD, protectin; MaR,
447 maresin; LX, lipoxin.
448

449 **FIGURES**

450 **Figure 1**. **Kinetics of cardiomyopathy in T. cruzi infection.** Parasitemia (**A**) and mortality (**B**)
451 in CD-1 mice infected with $5x10^4$ trypomastigotes of the Brazil strain of *T. cruzi*. **C-D**.
452 Representative cardiac MRI of control (**C**) and infected (**D**) mouse heart revealing an enlarged
453 right ventricle. **E-F**. Representative histopathology of the heart during the acute and chronic
454 infection showing pseudocysts of amastigotes (⬅) and inflammation. Images are representative
455 of n=5 mice in each group.

456 **Figure 2**. **T-cruzi are a source of RvD1 during infection. A**. Serum RvD1 levels in mice
457 inoculated with $5x10^4$ Brazil strain trypomastigotes. Data are mean±SD (n=5). ** represents
458 significance ($p \leq 0.05$) from uninfected mice. **B**. Release of RvD1 from *T cruzi*, related protists,
459 and infected L_6E_9 cells. RvD1 release was measured by ELISA (pg/mL) from conditioned
460 media. Data are mean±SD (n=3). ** represents significance ($p \leq 0.05$) from epimastigotes and
461 uninfected L6E9 cells. epis= epimastigotes forms of Tulahuen strain, trypos=trypomastigotes of
462 the Brazil and Tulahuen strains, Toxo= lysates of the RH strain of *Toxoplasma gondii*, T.
463 *brucei= Trypanosma. bruce*i. L_6E_9 myoblasts infected (Inf) or uninfected (Uninf) with
464 trypomastigotes of the Tulahuen strain of *T. cruzi*.

465 **Figure 3. Representative spectra used to identify lipid mediators of the resolving class.** LC-
466 MS-MS fragmentation spectra employed for the identification of RvD1, RvD5 and RVE2 in
467 lysates of trypomastigotes of *T. cruzi* from Brazil and Tulahuen strains.

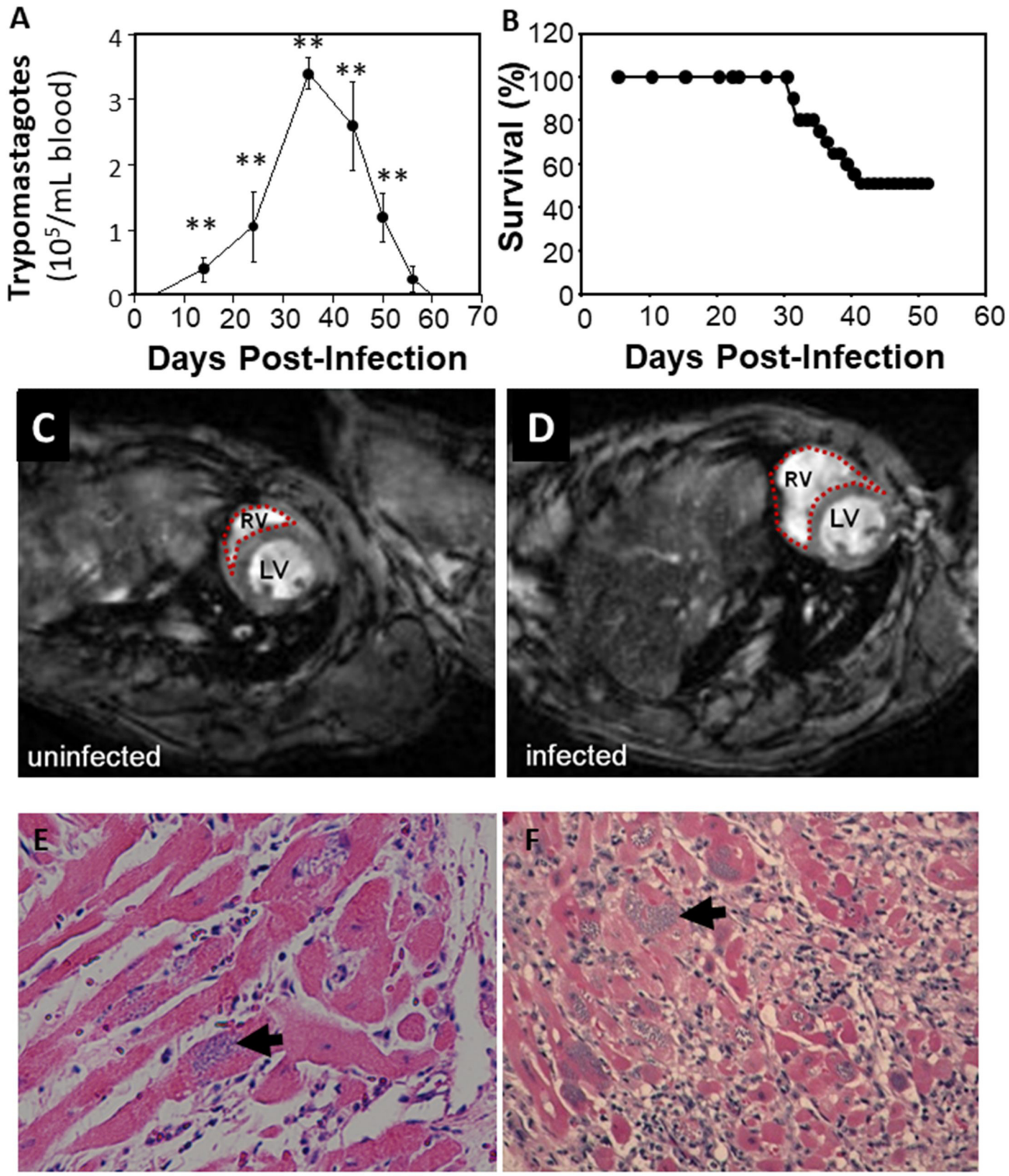


Figure 1

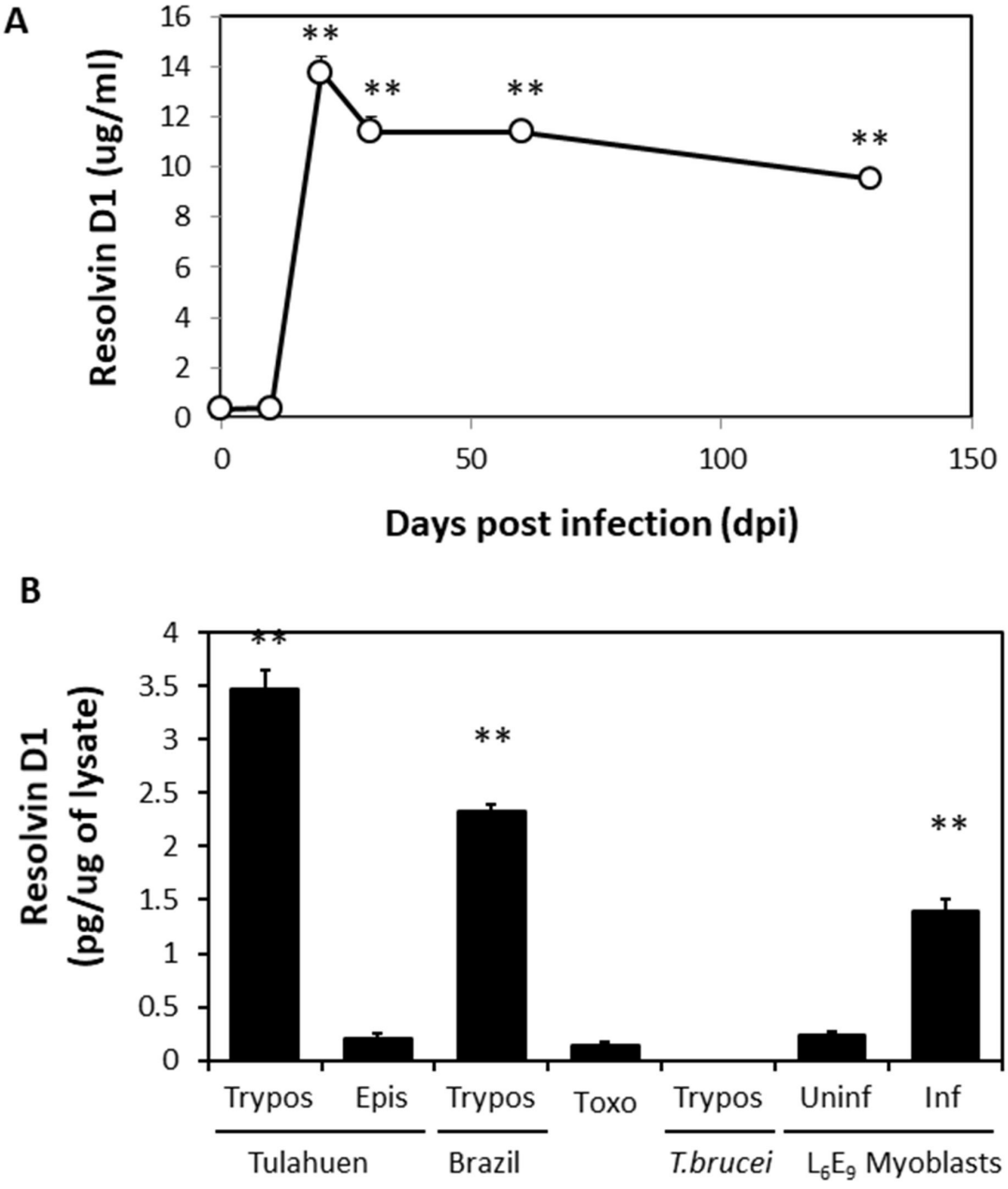
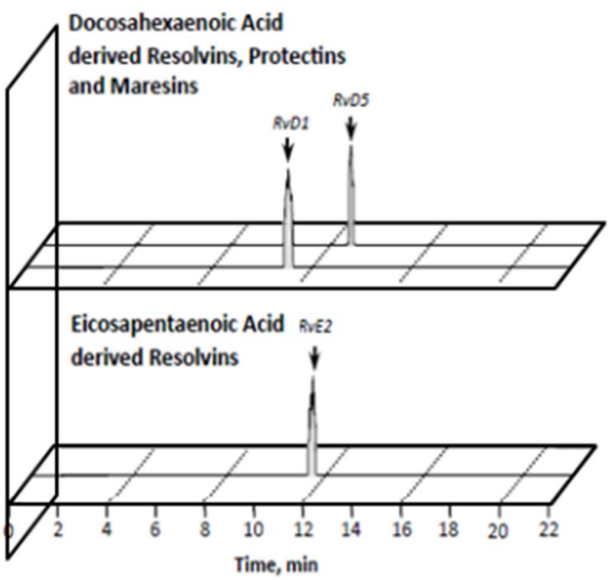


Figure 2



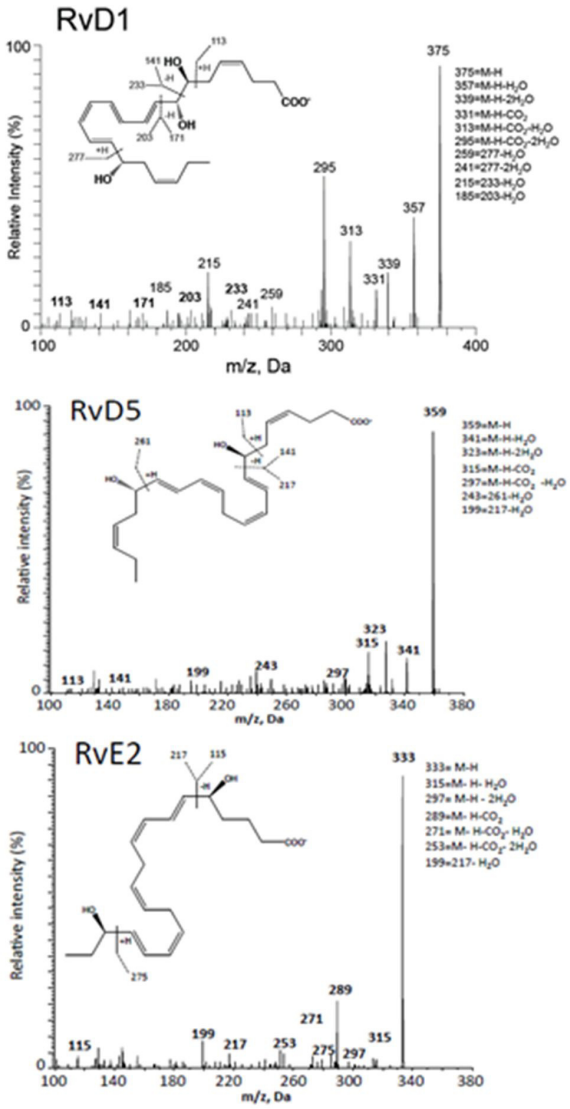


Figure 3