Microvascular Research 110 (2017) 56–63

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Expression and production of cardiac angiogenic mediators depend on the *Trypanosoma cruzi*-genetic population in experimental C57BL/6 mice infection☆

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ARTICLE INFO

Article history:
Received 1 September 2016
Revised 5 December 2016
Accepted 6 December 2016
Available online 9 December 2016

Keywords:
Inflammation
Trypanosoma cruzi
Angiogenic factors, chemokines
VEGF

ABSTRACT

Mammalian cardiac cells are important targets to the protozoan *Trypanosoma cruzi*. The inflammatory reaction in the host aims at eliminating this parasite, can lead to cell destruction, fibrosis and hypoxia. Local hypoxia is well-defined stimulus to the production of angiogenesis mediators. Assuming that different genetic *T. cruzi* populations induce distinct inflammation and disease patterns, the current study aims to investigate whether the production of inflammatory and angiogenic mediators is a parasite strain-dependent condition. C57BL/6 mice were infected with the Y and Colombian strains of *T. cruzi* and euthanized at the 12th and 32nd days, respectively. The blood and heart tissue were processed in immune assays and/or qPCR (TNF, IL-17, IL-10, CCL2, CCL3, CCL5, CCR2, CCR5 and angiogenic factors VEGF, Ang-1, Ang-2) and in histological assays. The *T. cruzi* increased the inflammatory and angiogenic mediators in the infected mice when they were compared to non-infected animals. However, the Colombian strain has led to higher (i) leukocyte infiltration, (ii) cardiac TNF and CCL5 production/expression, (iii) cardiac tissue parasitism, and to higher (iv) ratio between heart/body weights. On the other hand, the Colombian strain has caused lower production and expression VEGF, Ang-1 and Ang-2, when it was compared to the Y strain of the parasite. The present study highlights that the *T. cruzi*-genetic population defines the pattern of angiogenic/inflammatory mediators in the heart tissue, and that it may contribute to the magnitude of the cardiac pathogenesis. Besides, such assumption opens windows to the understanding of the angiogenic mediator's role in association with the experimental *T. cruzi* infection.

1. Introduction

Inflammation is a multifactorial process involving cellular activation and migration, inflammatory mediators' production, vascular responses and local and/or systemic reactions. Angiogenesis emerges as an event cross-linked to inflammation and it aims at keeping homeostasis and repairing the tissue through the formation of new capillary networks from a preexisting vasculature. These networks are tightly regulated by the production and release of angiogenic factors (Medzhitov, 2008; Folkman, 2006).

The protozoan *Trypanosoma cruzi* triggers acute and chronic inflammatory processes in smooth, skeletal and cardiac muscles and promotes a progressive damage that causes local fibrosis prevalence and functionality deficit (Lannes-Vieira et al., 2009; Talvani and Teixeira, 2011; Penitente et al., 2015). This parasite has a well-adapted mechanism that favors its survival in mammalian host cells and drives an inflammatory response in the host according to the *T. cruzi* high genetic variability, which is evidenced through six distinct Discrete Typing Units—DTU's expressed as *T. cruzi* I (TcI) to *T. cruzi* VI (TcVI). Each of these units present a biological feature based on their geographic distribution, and eco-epidemiological and clinical associations (Zingales et al., 2012). The inflammatory response aims at eliminating the parasites in the acute phase; whereas, in the chronic phase, such response is rather kept under control in order to keep the parasites and the immune response under control as a way to prevent excessive tissue damage (Nagajyothi et al., 2012; Cardoso Reis-Cunha and Bartholomeu, 2015). When these protective strategies fail, the pathophysiological

☆ Angiogenic mediators in *T. cruzi* infection

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http://dx.doi.org/10.1016/j.mvr.2016.12.002
0026-2862/

manifestations and the disease emerge in the host, in part, in a close-dependence of the parasite DTUs.

It is likely that the immune response and the *T. cruzi* molecules trigger the angiogenic factors since they are required to tissue repair (Ferreira et al., 2005, Guedes-da-Silva et al., 2015). In turn, inflammation may lead to conditions favorable for angiogenesis, partly due to the hypoxia that emerges after fibrosis generation and, partly, to the inflammatory mediators that are further amplified by pro-angiogenic factors released around the inflammatory site (Yamakawa et al., 2003, Van Nieuwenhoven and Turner, 2013). Besides the conventional roles played by cytokines and chemokines in the activation and recruitment of leukocytes to the inflammatory foci, some other studies have shown that these soluble mediators can directly or indirectly increase the vascular growth by stimulating the production of the vascular endothelial growth factor (VEGF) (Barcelos et al., 2009; Pickens et al., 2010; Maloney and Gao, 2015). The VEGF is an essential factor responsible for angiogenesis after its binding in the VEGF receptor, which is found in many cells. It leads to the proliferation, migration and survival of new endothelial cells (Leung et al., 1989; Bao et al., 2009; Zhang et al., 2012; Wang et al., 2013). Furthermore, the angiopoietins can inhibit the tumor necrosis factor (TNF)-stimulated leukocyte transmigration and act as an important angiogenesis and inflammation regulator (Gamble et al., 2000; Seok et al., 2013).

Since distinct genetic *T. cruzi* populations may cause different inflammation patterns in experimental models, the aim of the present study is to show, for the first time, that the Y (DTU II) and Colombian (DTU I) strains of *T. cruzi* perform different interactions with angiogenic and inflammatory mediators in the plasma and cardiac tissue of infected C57BL/6 mice.

2. Materials and methods

2.1. Ethics statement

All procedures in the current study meet the guidelines issued by the Brazilian College of Animal Experimentation (COBEA); the research was previously approved by the Ethics Committee on Animal Research of UFOP (Protocol number 043/2010).

2.2. Experimental animals, parasites and infection

Ten week-old male C57BL/6 mice were bred and housed at the Animal Sciences Center at Universidade Federal de Ouro Preto—UFOP, Brazil. The mice (n = 10) were infected with 100 blood trypomastigote forms of *T. cruzi* using the following strains: (i) Y and (ii) Colombian. The Y strain of *T. cruzi*, classified as DTU II, is characterized by a rapid parasite multiplication with very high peaks between 9 and 10 days of infection, presenting high virulence and mortality between 10 and 14 days of infection. The Colombian strain, classified as DTU I, presents a very slow parasitic multiplication, reaching very high parasitemic peaks between 20 and 30 days of infection, low virulence with no mortality up to 50 days (Andrade and Magalhães, 1996; Zingales et al., 2012; Zingales et al., 2009). Parasitemia was daily determined according to the method described by Brener (1962). The mice were euthanized after the parasitemia peak, at the 12th and 32nd days of infection in the Y and Colombian strains, respectively. Blood was collected for immunoassay; half heart (i) was fixed in 10% formalin for histological analysis, the other half (ii) was used in molecular and immunoassay parameter assessments.

2.3. Heart mass measurement

The heart from each animal was carefully excised after blood collection. The vessels and heart chambers were washed in phosphate buffer solution. The wet organ was weighed and the relative heart weight was calculated using the heart weight in milligram/body weight in gram (mg/g). This mg/g value was used to determine the cardiac mass measurement at the time of the euthanasia. In addition, both ventricles were split in two fragments: the upper part was used in the homogenate (immune and molecular assays); and the lower (apex) one, in the histopathological analysis.

2.4. Immunoassay

The circulating levels of TNF, VEGF, IL-10, IL-17, the macrophage inflammatory protein alpha (MIP-alpha/CCL3) regulated upon activation, normal T cell expressed and secreted normal T cells (CCL5/RANTES), and the monocyte chemoattractant proteins (CCL2/MCP-1) were detected in plasma. Blood from the orbital venous sinus (0.5 mL) was collected during euthanasia and centrifuged (1500 g for 15 min at 4 °C). The plasma was stored at −80 °C. In parallel, a 10 mg cardiac tissue fragment collected from each animal was homogenized in cold radioimmunoprecipitation assay (RIPA) buffer using a protease inhibitor; the supernatant was stored at −80 °C. Next, these samples were used to measure TNF, CCL2, CCL3, CCL5, IL-10, IL-17 and the angiogenic factor VEGF (Peprotech, Ribeirão Preto, Brazil), according to the protocol recommended by the manufacturer. The samples were simultaneously measured in duplicates.

2.5. Morphometric and histopathological analysis

Cardiac tissue fragments were fixed in 10% buffered-formalin solution; then, dehydrated, cleared and embedded in paraffin in order to analyze and quantify the inflammatory infiltration and the amastigote nests. Blocks were cut in 4 mm-thick sections and stained in hematoxylin and eosin (HE). Twenty fields from each HE stained section were randomly chosen at 40× magnification, thus totaling 74,931 μm^2—the equivalent area of 50 fields of the analyzed myocardium. Images were obtained in a Leica DM 5000 B micro chamber (Leica Application Suite, UK, version 2.4.0 R1) and processed in the Leica Quinn (V3) image analyzer software. The inflammatory process was assessed through the number of cellular nuclei found in the infected heart tissue and compared to the background of the cardiac cellular nuclei found in the non-infected mice. Amastigote nests were quantified in the Image J 1.45s software, at the National Institute of Health, USA (www.imagej.nih.gov/ij). The area occupied by parasites was assumed

Table 1
Primer sequences according to the GenBank database.

	Sequences (forward and reverse)
VEGF	5′AAAAACGAAAGCGCAAGAAA 3′
	5′TTTCTCCGCTCTGAACAAGG 3′
ANG-1	5′GGGGGAGGTTGGACAGTAA 3′
	5′CATCAGCTCAATCCTCAGC 3′
ANG-2	5′GATCTTCCTCCAGCCCCTAC 3′
	5′TTTGTGCTGCTGTCTGGTTC 3′
TNF	5′TGAGTGACCAAGGGACAGAACC 3′
	5′AGCCAGGAGGGAGAACAG 3′
IL-10	5′ACTACCAAAGCCACAAGG 3′
	5′AAGAGCAGGCAGCATAG 3′
CCL2	5′AACTGCATCTGGCTGAGC 3′
	5′CAGCACCAGCCAACTCTC 3′
CCL5	5′ACCCTCTATCCTAGCTCATC 3′
	5′CGTGTTTGTCACTCGAAG 3′
CCR2	5′CCTGTCCACTAATGCGTTTC 3′
	5′GCAAAGCCAGACCACAATG 3′
CCR5	5′CCCTGTCATCTATGCCTTTG 3′
	5′GCTTGCACGATCAGGATTG 3′
β-actin	5′CCACTTTCCTGTCTTACCCAA 3′
	5′AATTAACCACCCACGGTGTT 3′

VEGF-A (Vascular Endothelial Growth factor A); ANG-1 (Angiopoietin 1); ANG-2 (Angiopoietin 2); TNF (Tumor Necrosis Factor); IL-10 (Interleukin-10); CCL-2/MCP-1 (Monocyte Chemoattractant Protein); CCL5/RANTES (Regulated upon activation, normal T cell expressed and secreted); CCR2 (CCL2 receptor); CCR5 (CCL5 receptor).

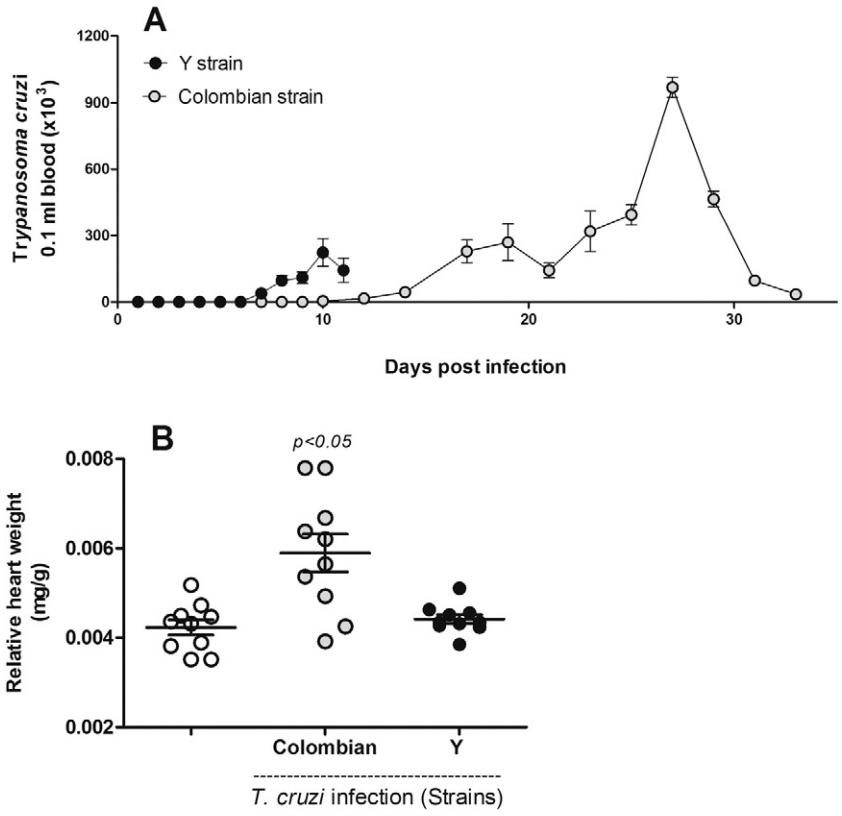

Fig. 1. Parasitemia and relative heart weight of animals infected with different *Trypanosoma cruzi* strains. C57BL/6 mice were infected with Colombian and Y strains of *T. cruzi* (100 parasites/mouse) and (A) the parasitemia was assessed up to 12 days, in the Y strain, and up to 32 days, in the Colombian strain. (B) Animals were weighed before euthanasia and the relative heart weight was expressed in (mg/g) 12 and 32 days after infection. The results are representative of 10 animals/group and expressed in median ± SEM. The $p < 0.05$ indicates difference between the uninfected animals and the Y strain-infected group.

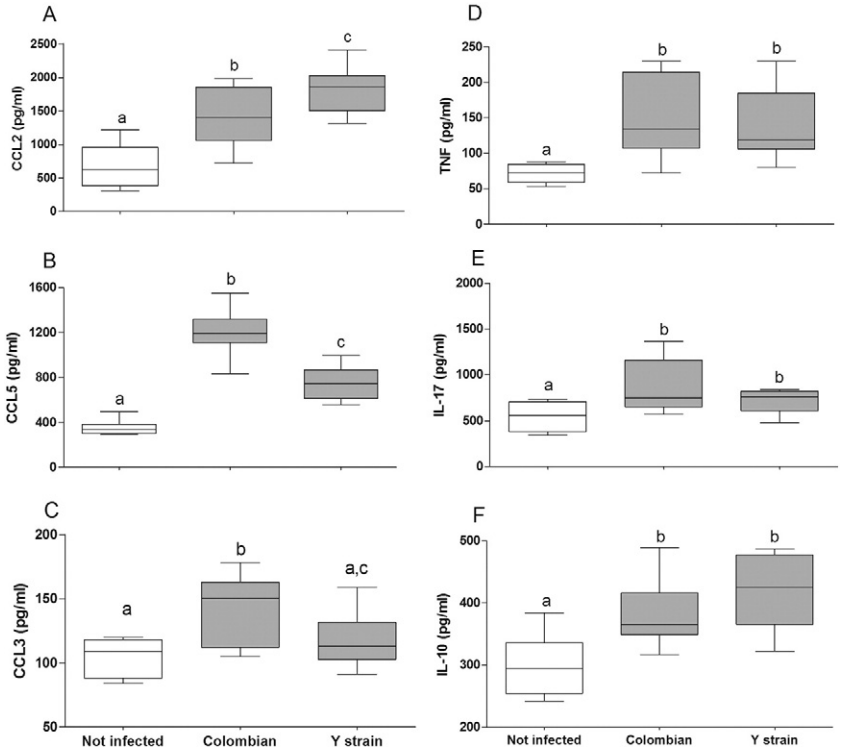

Fig. 2. Concentrations of inflammatory mediators in the plasma. CCL2/MCP-1 (A), CCL5/RANTES (B) and CCL3/MIP-1 alpha (C) TNF (D), IL-17 (E) and IL-10 (F) were measured through immunoassay (ELISA) in the plasma of C57BL/6 mice infected with the Colombian and Y strains of *T. cruzi*. Data are shown as the mean of 10 animals ± SEM and different letters denote difference ($p < 0.05$).

to be the same area previously used to quantify the inflammatory process.

2.6. Expression of the inflammatory and angiogenic mediators

We quantified the expression of inflammatory and angiogenic genes. Thus, we extracted the total RNA from the cardiac tissue of the animals with the *SV Total RNA Isolation System Kit* (Promega, USA) following the manufacturer's protocol. Subsequently, we made the complementary DNA (cDNA) using the *high-capacity cdna reverse transcription kit* (Applied Biosystems, USA). Then, for the angiogenic (VEGF, Ang-1, Ang-2) and inflammatory (CCL2, CCL5, CCR2, CCR5, TNF and IL-10) genes, a standard curve from serial dilutions of a known concentration of purified DNA was achieved. This quantified DNA consisted of the target PCR product prepared by conventional PCR from cDNA positive for the corresponding target mRNA. Threefold measurement for each standard dilution point over the whole standard curve range was produced to generate a reliable standard curve. Then, real-time PCR quantitative mRNA analyses were performed using an ABI Prism 7000 SDS unit (Applied Biosystems) through the Platinum® SYBR® Green qPCR SuperMix UDG with ROX reagent (Invitrogen) for quantification of amplicons. The standard PCR conditions were as follows: 50 °C (2 min), 95 °C (10 min); 40 cycles of 94 °C (30 s), 58 °C (30 s), and 72 °C (1 min); followed by the standard denaturation curve. The sequences of the primers were designed using the Primer Express software (Applied Biosystems) assuming the nucleotide sequences available in the GenBank database (Table 1). In each reaction the Platinum® SYBR® Green qPCR SuperMix UDG with ROX reagent (Invitrogen), 1 μg/μL of each specific primer, and cDNA diluted 20 times were used. In this study, all data were normalized to beta-actin mRNA. Relative increase in CK and CKR were plotted in comparison to the non-infected control group using $2 - \Delta/\Delta$ CT method.

2.7. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM) and were analyzed using the *Kolmogorov-Smirnov* normality test and One-Way analysis of variance. All analyses were performed using PRISM 5.01 software (GraphPad, San Diego, CA, USA) and the level of significance was accepted at $p < 0.05$.

3. Results

3.1. Parasitemia curve and the relative heart weight

The parasitemia curves are represented in Fig. 1A and show the biological feature of the Y and Colombian strains of *T. cruzi* used in the present study. The pre-patent period ranged from 6 days, in the Y strain, to 10 days, in the Colombian strain of *T. cruzi*. Throughout the current study, both strains have led to high parasite load in the infected C57BL/6 mice. However, in the end of the acute phase, only mice infected with the Colombian strain of *T. cruzi* have presented increased heart/body weight ratios. The animals infected with the Y strain kept heart/body weight ratio similar to that of the non-infected animals (Fig. 2B).

3.2. The production and expression of plasma and heart inflammatory, regulatory and angiogenic mediators

The plasma cytokines (IL-10, IL-17 and TNF) and chemokines (CCL2, CCL3, CCL5) production was quantified in order to be associated with the inflammatory pattern of each *T. cruzi* strain, since the *T. cruzi* infection develops systemic inflammatory mediators and since these soluble factors play a potential role in the release of angiogenic factors. All infected animals have presented plasma elevation in the production of inflammatory/regulatory cytokines and chemokines when they were compared to the uninfected mice (Fig. 2C). There was inverse relation between CCL2 and CCL5 production; the Y and Colombian strains have induced more CCL2 (Fig. 2A) and CCL5 (Fig. 2B) in infected animals, respectively. Interestingly, besides the pattern observed to the chemokines, no differences were observed between both parasite strains in TNF (Fig. 2D), IL-17 (Fig. 2E) and IL-10 (Fig. 2F) production after the day of their respective parasitemia peaks.

In parallel, the vascular endothelial growth factor (VEGF), the main representative among the angiogenic mediators, increased in the plasma when it was associated with both *T. cruzi* strains (Fig. 3A). However, the DTU I, Colombian strain, was able to inhibit the VEGF production in the heart tissue, which was measured in the tissue homogenate (Fig. 3B).

By following the plasma cytokines and chemokines production, the cardiac expression of the CCL2 (Fig. 4A), CCL5 (Fig. 4B), TNF (Fig. 4C), IL-10 (Fig. 4D), and the CCL2 and CCL5 receptors, respectively, CCR2 (Fig. 4E) and CCR5 (Fig. 4F) also increased in the presence of *T. cruzi*. However, the Colombian strain was able to increase by 200 and by 6 times the expression of CCL5 (Fig. 4B) and TNF (Fig. 4C), respectively, in the heart tissue of infected mice when it was compared to the Y strain.

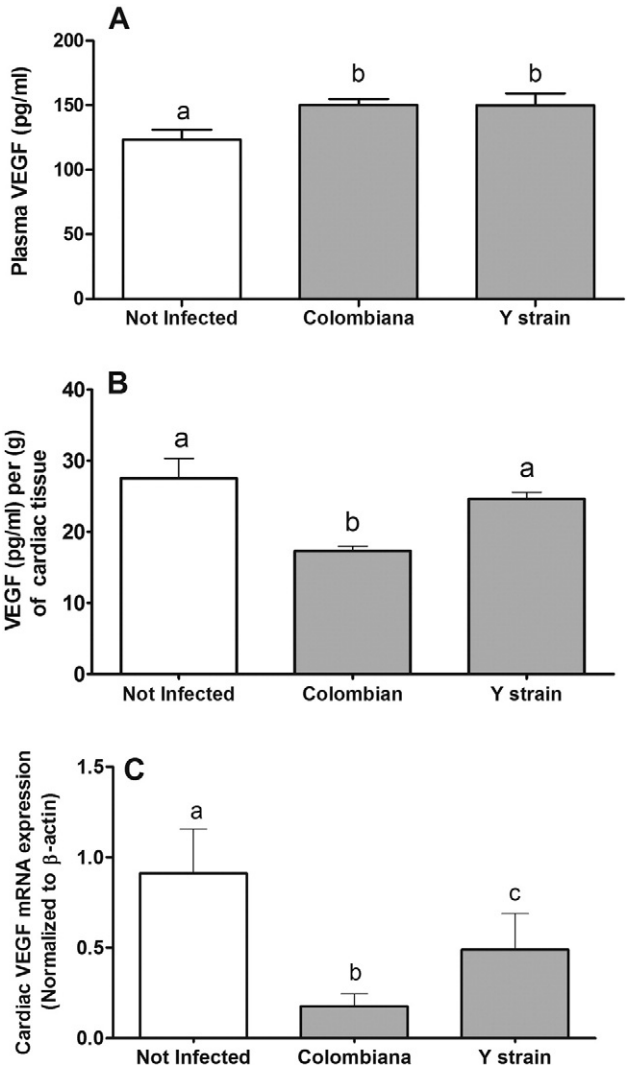

Fig. 3. Plasma production and cardiac expression of the vascular endothelial growth factor (VEGF) in *Trypanosoma cruzi* infected animals. The VEGF was measured through ELISA in (A) the plasma and in (B) the macerated cardiac tissue of C57BL/6 mice infected with the Colombian and Y strains of *T. cruzi*. In parallel, (C) the cardiac VEGF was also measured through quantitative-PCR. Data are shown as the mean ± SEM of 10 animals and the expression of mRNA expression was normalized to the constitutive beta-actin gene. Different letters denote difference in production and expression ($p < 0.05$).

In parallel, the VEGF expression in cardiac tissue was also measured through qPCR (Fig. 3C). The Colombian strain was, once more, capable of inhibiting this angiogenic factor more intensely at molecular level when it was compared to the Y strain of the parasite. Moreover, the expression of angiopoetin-1 (Ang-1) (Fig. 5A) and angiopoetin-2 (Ang-2) (Fig. 5B), which are other angiogenic mediators, just decreased in animals infected with the Colombian strain of *T. cruzi*.

3.3. Inflammatory infiltration and parasitism in cardiac tissue

Although all animals have received the same load of parasites during the infection, each DTU has promoted specificities in the inflamed cardiac tissue. With regard to the present study, the Colombian strain was associated with higher leukocytes influx, followed by the Y strain (Fig. 6A). Fig. 6 (left side) shows a representative photomicrography of the non-infected cardiac tissue and the presence of amastigote nests associated with the *T. cruzi* (Colombian strain). These images are reinforced by the quantification of the amastigote nest area (Fig. 6B). Tissue parasites associated with the Y strain were not detected in the current study.

4. Discussion

There is no way to escape from the close relation between the genetic variability of the *T. cruzi*, currently classified as DTU's, and the generation of the pathology and of cardiac diseases in humans and in experimental animals (Zingales et al., 2012, 2009). However, the distinct genetic variability between mammalians is also another highlight that must be considered in the *T. cruzi* pathogenic puzzle. The parasite/host interaction dictates the immune balance or imbalance and leads to serious disturbances in the affected organs (Penitente et al., 2015; Guedes et al., 2010; Oliveira et al., 2012; Martins et al., 2013; Bryan et al., 2016; de Oliveira et al., 2016). Some studies have proposed that a panel of inflammatory mediators is related to the protection against circulating or tissue infecting *T. cruzi* (Lannes-Vieira et al., 2009; Talvani and Teixeira, 2011; Penitente et al., 2015; Guedes et al., 2010; Gomes et al., 2003). Soluble mediators such as IFN-gamma, IL-12, TNF and IL-17 were previously described to activate macrophages and to circulate mononuclear cells in reactive oxygen species—ROS (Guedes et al., 2010; Gupta et al., 2011; Magalhães et al., 2013; Costa et al., 2006; Machado et al., 2000) in order to eliminate parasites. Some other regulatory proteins are also released in order to control the immune response intensity (e.g. IL-10, IL-4, IL-22) and, consequently, to contribute to pathogenic process decay of Chagas disease (Gomes et al., 2003; Flórez et al., 2011; Poveda et al., 2014) in humans and in experimental animals infected with *T. cruzi* (Abrahamsohn, 1998; Hiyama et al., 2001). Part of this pathogenic process is driven by the chemokines (e.g. CCL2, CCL3, CCL5 and others) and was previously evidenced in human and experimental *T. cruzi* infection (Talvani et al., 2000; Teixeira et al., 2002; Talvani et al., 2004; Nogueira et al., 2012). These small and soluble molecules are capable of recruiting leukocytes in the bone marrow and of sending them to the blood, and/or of sending them from the blood to the tissue, thus intensifying local inflammation and parasite elimination.

This parasite-dependent inflammatory process is usually persistent and contributes to tissue destruction, to toxic products release and to local tissue hypoxia, thus culminating in a heart repair or remodeling process (Rossi and Carobrez, 1985; Melo et al., 2011). This hypoxic environment is a required stimulus to the release of a new set of angiogenic mediators such as VEGF, angiopoietin (Ang)-1 and Ang-2. These mediators are involved in the angiogenesis process and in the modulation of inflammatory activities (McCarter et al., 2006; Scholz et al., 2015). The VEGF acts through tyrosine kinase receptors (VEGFRs) expressed in myelomonocytic inflammatory cells, as well as in vascular endothelial cells. The inhibition of the VEGF signaling has been assumed to induce anti-inflammatory properties through the blockage of STAT-3 (Wang et al., 2013; Waldner et al., 2010). The angiopoietins are oligomeric-secreted glycoprotein ligands that bind to a Tie family of receptors primarily expressed in the vascular endothelium. The expression of the leukocyte adhesion molecules E-selectin, ICAM1, and VCAM1 is usually suppressed through Ang-1; whereas, Ang-2 appears to be a

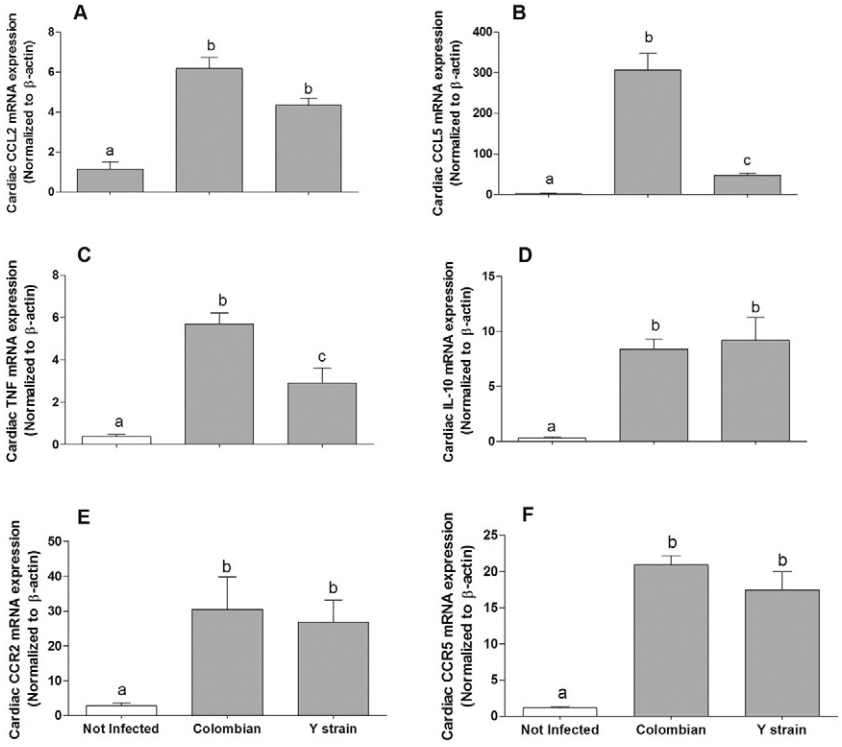

Fig. 4. Expression of cytokines, chemokines and chemokine receptors in the heart. CCL2/MCP-1 (A), CCL5/RANTES (B), TNF (C), IL-10 (D), CCR2 (E) and CCR5 (F) expressions were measured through quantitative-PCR in the hearts of mice infected with the Colombian and Y strains of *T. cruzi*. Data represent fold expression of the mean from 10 animals ± SEM and different letters denote difference in expression ($p < 0.05$). The expression of mRNA expression was normalized to the constitutive beta-actin gene.

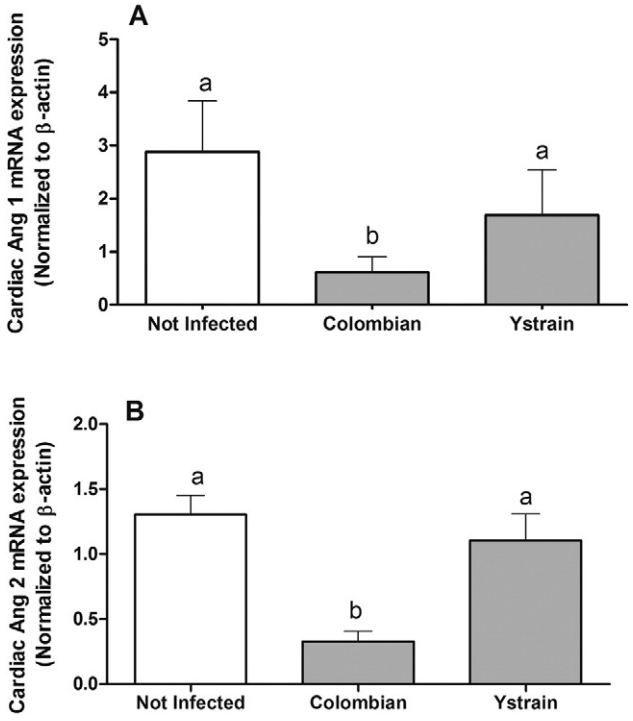

Fig. 5. Expression of Angiopoietin-1 and -2 in infected cardiac tissue. Angiopoietin-1 (A) and Angiopoietin-2 (B) expressions were measured through quantitative-PCR in the hearts of mice infected with the Colombian and Y strains of *T. cruzi*. Data represent the fold expression of the mean from 10 animals ± SEM and the expression of mRNA expression was normalized to the constitutive beta-actin gene. Different letters denote difference in expression ($p < 0.05$).

key regulator in vascular inflammations. The signaling mechanism in these immune regulatory mechanisms remains unclear, although some studies point out PI3K and Akt as required in this pathway (Brindle et al., 2006).

The magnitude of the regulatory, inflammatory or angiogenic mediators appears to depend on the DTU's of the protozoan, which demands care at the interpretation of the data on experimental and human infections. Indeed, according to the literature, the presence of the parasite or of its antigen molecules (e.g. mucin-glycosylphosphatidylinositol) in experimental animals is sufficient to trigger inflammatory protein/lipid mediators (Talvani and Teixeira, 2011; Golgher and Gazzinelli, 2004; Rodrigues et al., 2012). However, it is now suggested that the magnitude depends on the parasite strain.

The Colombian strain of *T. cruzi* is an inducer of high murine TNF levels and, consequently, it is responsible for releasing high levels of distinct chemokines and of their receptors in the immune and cardiac cells (Medeiros et al., 2009). The DTU I is an adaptive strain to infected heart tissue (Oliveira et al., 2012) and it also presents biological features concerning the resistance against available nitro derivative drugs anti-*T. cruzi* such as benznidazole (Romanha et al., 2010; Gruendling et al., 2015). This genetic parasite population led to reduction in the expression of the following angiogenic factors in the present study: VEGF, Ang-1 and Ang-2; however, in the cardiac tissue only, not in the plasma context. On the other hand, the Colombian strain promoted TNF, CCL5 and CCL3 increase in the plasma and/or in the cardiac tissue. The persistence of the tissue amastigote forms, the increase of other inflammation mediators (TNF, IL-17, chemokines) and the reduction of Ang-1 and Ang-2 may have reinforced the magnitude of the local inflammatory response due to the presence of greater leukocyte infiltration.

On the other hand, the Y strain of *T. cruzi* has also induced TNF and other inflammatory mediators, but it was less intense than in the Colombian strain. This genetic population of the parasite was homogeneous throughout different organs, including the heart (Oliveira et al., 2012). It is partially resistant against derivative drugs anti-*T. cruzi* (Romanha et al., 2010; Gruendling et al., 2015). The Y strain of *T. cruzi* in the current study has induced higher expression of cardiac VEGF, Ang-1 and Ang-2 in comparison to the lower production and/or expression of TNF, CCL5, amastigotes and leukocytes infiltration, which were observed in the Colombian strain. The chemokine receptors were herein higher expressed during the expression of both *T. cruzi* strains, as well as

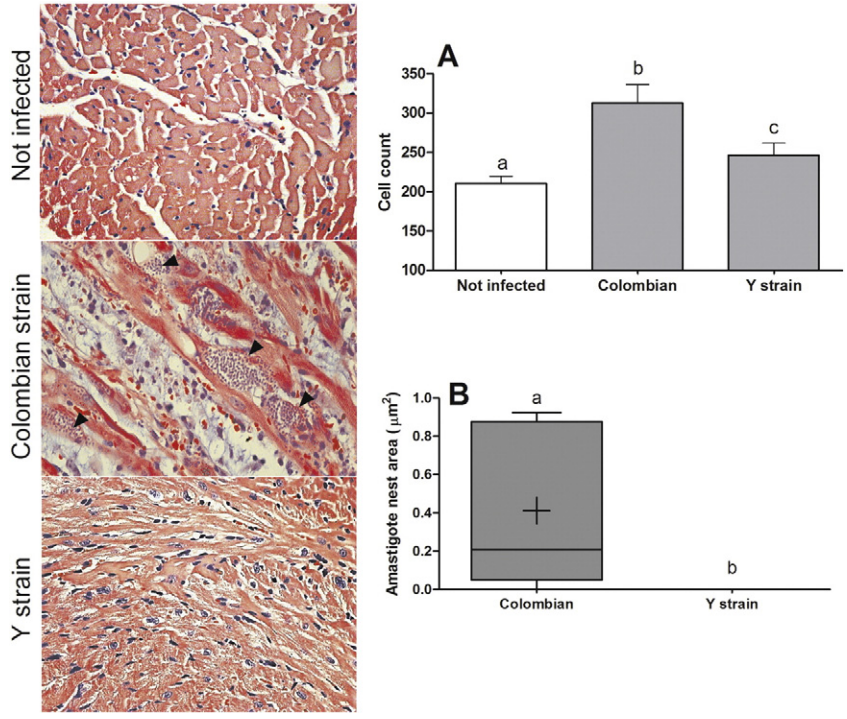

Fig. 6. Cardiac inflammatory cells and amastigote nests. Cellular infiltration in the cardiac tissue of C57BL/6 mice infected with the Colombian and Y strains of *T. cruzi* was quantified in (A), and the area of amastigote nests in (B). Data are shown as the mean from 10 animals ± SEM. Different letters denote difference in expression ($p < 0.05$). Illustrations from the uninfected cardiac tissue or of tissue infected by *T. cruzi* (left) present 40× magnification. Black arrows = amastigote nests.

of other soluble mediators such as IL-17 and CCL2, when they were compared to animals who had no contact with the protozoan.

It is plausible that both polar populations of *T. cruzi* have led to increased, but distinct, patterns of inflammatory and angiogenic mediators in the experimental infection model. The persistence of parasites triggering inflammation in the cardiac tissue may cause local oxygen reduction, hypoxia, and it may trigger favorable conditions to VEGF secretion (Liu et al., 1995; Krock et al., 2011). The local inflammatory process and the new tissue repair environment activate the platelet used as the first vascular component to release VEGF after thrombin stimulation (Möhle et al., 1995). The monocytes expressing the Flt-1 receptor can be attracted by this VEGF, which, in part, is activated by TNF. In turn, the TNF may induce more VEGF expression through the local cells (Clauss et al., 2001; Lu et al., 2012). As for the present study, VEGF, Ang-1 and Ang-2 have presented low expression in the heart tissue of animals infected with the Colombian strain and presented higher amastigote nests. Accordingly, the proinflammatory activity of VEGF seems to have been balanced by the low activity of Ang-1 and Ang-2 during the infection with both genetic populations of *T. cruzi*.

5. Conclusion

The present study has demonstrated that DTU I (Y strain) and DTU II (Colombian strain) *T. cruzi* parasites promote the release and the expression of different levels of angiogenic and inflammatory mediators in the acute phase of the experimental infection. In this particular case, the angiogenic mediators may work side by side with chemokines and inflammatory cytokines in order to switch "on/off" the cardiac tissue pathogenesis development, depending on the features dictated by each DTU of the parasite.

Funding sources

The present study was granted by Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq (#476229/2009-0), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG APQ-01698-13), International Society for Infectious Diseases (ISID/EUA—Small Grant/2009) and Universidade Federal de Ouro Preto/UFOP (PROPP # 67/2016). AT thanks CNPq for the fellowship granted to the research in productivity and for the PhD fellowships of DS and BB (both CNPq/TWAS Program).

Disclosures

Authors declare no conflict of interest regarding the publication.

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