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# The Vasculature in Chagas Disease

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## Abstract

The cardiovascular manifestations of Chagas disease are well known. However, the contribution of the vasculature and specifically the microvasculature has received little attention. This chapter reviews the evidence supporting the notion that alterations in the microvasculature especially in the heart contribute to the pathogenesis of chagasic cardiomyopathy. These data may also be important in understanding the contributions of the microvasculature in the aetiologies of other cardiomyopathies. The role of endothelin-1 and of thromboxane $A_2$ vascular spasm and platelet aggregation is also discussed. Further, these observations may provide target(s) for intervention.

## 4.1. HISTORICAL ASPECTS

Chagas disease, caused by infection with *Trypanosoma cruzi*, is a cause of acute myocarditis and chronic cardiomyopathy and often associated with a vasculitis. The involvement of the vasculature in the pathogenesis of Chagas disease has not been generally appreciated. Although its involvement was described in the early years following the initial description of the parasite and the disease it caused, it remained for others to suggest an aetiologic role for the vasculature in the development of chagasic heart disease. The understanding of the contribution of vascular and, in particular, microvascular dysfunction in the pathogenesis of chagasic heart disease is important in understanding not only chagasic disease but also cardiomyopathies of other infectious and non-infectious aetiologies.

Vianna (1911) was the first to detail the pathology of Chagas disease. From the earliest description of the disease, there was a fascination with the heart so that Chagas disease became almost synonymous for chagasic heart disease or chronic chagasic cardiomyopathy. Vianna stated that the “heart is one of the viscera for which the Schizotrypanosome shows predilection both in man and in animals”. In addition, Vianna first reported vascular involvement in Chagas disease stating that “perivascular inflammations exist, some of them quite pronounced, and others barely incipient … (in the myocardium) … in many of the arterioles that irrigate the nervous substance, overt phenomena of periarteritis are found … (in the cerebellum)”. Subsequently, in autopsy specimens, Torres described alterations in the heart and considered them as unrelated to tissue parasitism but rather a result of the disruption of the coronary circulation. These alterations were also observed in experimental *T. cruzi* infection (Torres, 1917). In 1941, Torres also observed cardiovascular involvement and he defined these lesions as “the inflammatory cell infiltrate in the interstitial tissue of the myocardium starts at the level of and around the capillaries, and not near *S. cruzi*, whether the latter is exudative myocarditis related to early vascular lesions”. He also described

similar lesions in the coronary arterioles of *T. cruzi*-infected monkeys that he considered to be ischaemic alterations in the myocardium resulting from occlusion of vessels. In 1960, Torres (1960) examined chagasic and non-chagasic human hearts and, in the former, identified marked, constriction-type irregularities with extensive myocytolysis in the intramyocardial arterioles. He suggested that the diffuse myocytolysis was caused by metabolic changes in the myocytes resulting from circulatory disorders of low intensity or short duration. Further, he suggested that the requirement for arterial blood supply was reduced resulting in areas of marked diffuse myocytolysis and extensive destruction of myocardial cells. However, this does not completely account for collapse of small arterial branches occasionally observed as these changes could also be the result of a cycle that includes passive hyperaemia, local anaemia, metabolic disturbances in myocardial fibres, myocytolysis. Several other investigators of that era described vascular lesions in Chagas disease. For example, Mazza and Benitez (1937) demonstrated amastigotes in cells of the perivascular adventitia in the conjunctiva of patients during acute infection, and Couceiro (1943) reported vascular lesions in the sciatic nerve of infected dogs and Coelho (1944) observed coronary arteriole lesions in 19 cases of chronic Chagas disease. These lesions were also detected at autopsy in coronary circulation by Ramos and Tibiriça (1945), Dias et al. (1956) and Koberle (1958).

Andrade and Andrade (1955) observed that the inflammation observed in chronic chagasic cardiomyopathy could be “allergic”, provoking ischaemic lesions of myocardium by capillary involvement. The microscopic infarctions could be responsible for alterations in the conduction system, mainly in the right branch of bundle of His, due to their preferential intramyocardial localization. They also suggested that the fibrotic lesions frequently detected at apex of the left ventricle could originate from vascular obstructions due to subendocardial parietal thrombosis. Subsequently, the “allergic phenomena” became less emphasized and the vascular changes were considered to be only congestion and marked dilatation of venules and capillaries. Brito and Vasconcelos (1959), in a study of 19 cardiac biopsies from patients with megaesophagus, detected necrotizing arteritis in nine and identified the inflammation as an “allergic phenomenon”. Vascular lesions in hearts of infected mice were also observed by Macclure and Poche (1963) using electron microscopy, and by Lucena et al. (1962) and Alencar et al. (1968) using light microscopy. Okumura et al. (1962) observed necrotizing arteritis in the myocardium and digestive tract to which they attributed an “allergic” origin. This concept was expanded when these investigators detected a parasitized endothelial cell (EC). They reported that “during the acute phase the trypanosomes may cause a focal lesion with sensitization of the vessels by an allergic mechanism, triggering hypersensitivity phenomena reflected by necrotizing arteritis”.

Jörg (1974) compared histological images obtained from a healthy heart after vascular injection of an opaque substance with those obtained in an injected heart of a patient who died of chagasic cardiomyopathy. “Decapillarization” was observed in those zones where the mesenchymal reaction was more intense. He postulated that the “angioarchitectonic” anarchy was a result of intense mesenchymal reaction secondary to the parasitic infection which led to a progressive decapillarization and a destructive loss of many meshes of the capillary net resulting in myocytolysis and destruction of cardiac ganglia. Subsequently, Jörg (1991) described vascular lesions characterized by endothelial oedema, denudation, cell accumulation and platelet–fibrin aggregation in a collecting vein of the left ventricle in a pig model of Chagas disease.

The observation that in chronic chagasic heart disease there was chronic inflammation and fibrosis and a dearth of parasites led investigators to search for a cause of the progressive pathological changes. In an effort to explain the pathology, several avenues of research were developed. Microvascular lesions in chagasic heart disease were described in the 1980s by

Rossi et al. (1984) and Factor et al. (1985). The involvement of microvasculature in the pathogenesis of chronic chagasic heart disease was further underscored by Rossi (1990). It should be noted that at this time, autoimmunity and disturbances in the cardiac anatomic nervous system were being intensely investigated (Acosta and Santos-Buch, 1985; Koberle, 1968; Oliveira, 1985; Ribeiro-dos-Santos and Rossi, 1985). In this regard, a relationship between cardiac autonomic nervous system abnormalities and sudden cardiac death has been demonstrated (Rossi and Bestetti, 1995). A relationship between cardiac autonomic nervous system abnormities and sudden death has been demonstrated. Malignant ventricular tachyarrhythmias such as ventricular tachycardia and fibrillation are major causes of sudden death among patients with chronic chagasic cardiomyopathy. We are now aware that parasite persistence is present in the cardiovascular system and in other organs even though it is not obvious by histological examination (Combs et al., 2005; Zhang and Tarleton, 1999) and that this is a major contributor to the chronic disease.

## 4.2. SMALL ANIMAL STUDIES OF THE MICROCIRCULATION IN *TRYPANOSOMA CRUZI* INFECTION

BALB/c mice immunized with epimastigotes of the avirulent PF strain of *T. cruzi* and challenged with trypomastigotes of the virulent Colombian strain developed a cardiomyopathy similar to that observed in human chronic chagasic cardiomyopathy including the development of an apical aneurysm (Rossi et al., 1984). Histological examination revealed focal areas of myocytolysis, necrosis and myocardial degeneration associated with a lymphomononuclear inflammatory infiltrate accompanied by interstitial fibrosis and occasional parasite pseudocysts. Additionally, platelet aggregates forming transient occlusive thrombi were observed in small epicardial and intramyocardial vessels. The focal nature of the myocardial lesion and the type of myonecrosis indicated involvement of the microcirculation.

A/J mice infected withthe Brazil strain and perfused with silicone rubber (Microfil) 15–17 days post-infection revealed numerous areas of focal vascular constriction, microaneurysm formation, vascular dilatation and proliferation of microvessel (Factor et al., 1985) which is similar to the observations in the Syrian cardiomyopathic hamster and in human cardiomyopathies of other aetiologies (Sonnenblick et al., 1985). In that model, the administration of verapamil ameliorated the microvascular alterations and the myocardial pathology. Similarly, in the Brazil strain-infected CD-1 mouse, verapamil ameliorated the myocardial pathology when verapamil was administered early but not late infection (Chandra et al., 2002; De Souza et al., 2004; Morris et al., 1989). Verapamil increases coronary blood flow, inhibits platelet aggregation and contributes to the amelioration of the pathology. These observations were corroborated by direct *in vivo* visualization utilizing a surrogate murine model, that is, the cremaster microvascular bed (Tanowitz et al., 1996). Direct observation of the effects of *T. cruzi* infection on microcirculatory flow *in vivo* and quantitative measurement of parameters such as the velocity of red blood cell flow (Vrbc) and vessel diameter were provided. When the cremaster model was examined 20–25 days post-infection in male CD-1 mice infected with the Brazil strain, a significant decrease in Vrbc, reversed by verapamil treatment, was observed in the first- and third-order arterioles and venules accompanied by an attenuation of the inflammation. The arterioles of the infected mice exhibited segmental areas of vasospasm and dilatation, possibly the initiating event in microaneurysm formation (Tanowitz et al., 1996; Fig. 4.1).

The infection of mice with *T. cruzi* caused a vasculitis. There was a gradual reduction in coronary flow in infected mice over time giving further credence to the notion that there was vascular dysfunction in experimental Chagas disease (Tanowitz, 1992b). Importantly, amastigotes are evident in the coronary microvascular ECs early in infection before

parasitaemia can be detected, suggesting that the coronary endothelium could be an initial target of infection (Factor et al., 1985). Acutely infected rats developed changes in the endothelial layer characterized by EC swelling and a few points of cytoplasmic discontinuity that appeared as holes exposing the subendothelial collagen that is usually associated with platelet–fibrin aggregates, which might affect the generation of vasoactive substances, and impairs the equilibrium between opposing forces (Rossi, 1997). *In vitro* and *in vivo* studies indicate that infection of the endothelium results in expression of both pro-inflammatory cytokines and vascular adhesion molecules, which are important components of the inflammatory response (Huang et al., 1999a,b; Tanowitz et al., 1992a,b). Infection of ECs activates NF-κB and likely contributing to the induction of cytokine and adhesion molecular expression in the endothelium (Huang et al., 1999a). Further, in the myocardium obtained from *T. cruzi*-infected humans and experimental animals, increased expression of cytokines, nitric oxide synthases and adhesion molecules has been reported (Huang et al., 1999a; Laucella et al., 1996; Reis et al., 1993; Fig. 4.2).

Taken together, all of aforementioned studies in experimental animals strongly suggest that the vasospasm of the branches of the coronary microcirculation leads to a reduction in blood flow and ischaemia to a small area of the myocardium subserved by that microvessel which resulted in a microinfarct. When this process is repeated over a period of time in different areas of the heart, these areas may coalesce and lead to falling out of cardiac myocytes and replacement by fibrous tissue. The focal but widespread nature of the pathology supports, in part, this hypothesis.

## 4.3. STUDIES IN DOGS

Dogs have been used in investigations of Chagas disease because they are a larger animal than the standard mouse model and may better recapitulate the human disease. Hearts obtained from dogs sacrificed 18–26 days after intraperitoneal inoculation with the 12SF strain of *T. cruzi* demonstrated myocarditis characterized by small focal areas of lesion and myocytic necrosis associated with interstitial mononuclear infiltration. Electron microscopic studies revealed degenerative changes in the ECs in contact with T lymphocytes, as well as platelet aggregates and fibrin thrombi in the intramyocardial capillaries. These alterations suggested that a possible interaction between ECs and effector immune cells might play an important role in the pathogenesis of the myocellular lesion and of the observed microangiopathy (Andrade et al., 1994). More recently, Melo et al. (2011) demonstrated that the administration of simvastatin ameliorated the cardiac remodelling in a canine model of chronic chagasic heart disease by histological and functional criteria. Importantly, statins have been demonstrated to inhibit platelet aggregation (Lee et al., 2010) and reduce the inflammation in the vasculature (Liu et al., 2009), thus increasing coronary blood flow in some studies (Brands et al., 1991).

## 4.4. VASOACTIVE PEPTIDES AND EICOSANOIDS

Endothelin-1 (ET-1), a 21-amino acid peptide (Yanagisawa et al., 1988), was originally described as a powerful vasoconstrictor secreted by endothelial cells (ECs). *T. cruzi* infection of ECs results in a dramatic increase in biologically active ET-1. However, other cell types have found to be sources of ET-1 such as cardiac myocytes, fibroblasts, astrocytes and macrophages (Kedzierski and Yanagisawa, 2001). The synthesis of ET-1 is mediated by endothelin-converting enzyme (ECE) which converts Big ET-1 (31 amino acids) to ET-1. The actions of ET-1 are mediated by the G-protein-coupled endothelin receptors $ET_A$ and $ET_B$. Although ET-1 is constitutively expressed in many cells, increased synthesis has been associated with many disease states such as malignant hypertension, primary pulmonary hypertension, CHF, sepsis, meningitis, eclampsia and subarachnoid haemorrhage

(Kedzierski and Yanagisawa, 2001). Increased expression/synthesis of ET-1 has been implicated in the pathogenesis of cerebral malaria (Machado et al., 2006) and chagasic cardiomyopathy (Petkova et al., 2000, 2001; Tanowitz et al., 2005).

Eicosanoids are lipid mediators that participate in many biological activities including vascular tone, inflammation, ischaemia and tissue homeostasis (Haeggstrom et al., 2010). The biosynthetic pathways in mammals for these important biological mediators are dependent upon liberation of arachidonic acid for the inner leaflet of the plasma membrane. Thromboxane $A_2$ ($TXA_2$), an eicosanoid generated during arachidonic acid metabolism, is the most potent vasoconstrictor known and acts via its receptors TPα and its splice variant TPβ, both of which are expressed on human ECs. Several parasitic organisms produce eicosanoids which may modulate host response and the progress of an infection (Belley and Chadee, 1995; Kubata et al., 1998, 2000; Liu and Weller, 1990; Noverr et al., 2003).

Thus, the observation in experimental animals and humans regarding vasospasm and platelet aggregation and thrombi in the coronary microcirculation was reminiscent of the actions of $TXA_2$. Tanowitz et al. (1990) observed that there was increased platelet aggregation in infected mice accompanied by an increase in plasma $TXA_2$. The increased levels of $TXA_2$ could explain the vascular spasm and the platelet aggregation (Tanowitz et al., 1990). Ashton et al. (2007), 17 years later, demonstrated that *T. cruzi* was capable of synthesizing $TXA_2$. It was further demonstrated that the majority of $TXA_2$ detected in the blood of infected mice is parasite derived. These observations suggest that $TXA_2$ could contribute to the pathogenesis of chronic chagasic cardiomyopathy and its clinical manifestations. More recently, on the basis of these observations, Mukherjee et al. (2011) administered aspirin (ASA) to *T. cruzi* (Brazil strain)-infected mice. There was a reduction in the plasma levels of $TXA_2$. ASA inhibits the mammalian COX-1 enzyme thus reducing the levels of $PGH_2$ available for the synthesis of $TXA_2$. Thus, we believe that ASA treatment of the infected host decreases the ability of the parasite to scavenge $PGH_2$ from the host to synthesize $TXA_2$. In addition, ASA-treated infected mice suffer a high parasitaemia and mortality. This effect of ASA is a result of “off-target” factors unrelated to $TXA_2$. It may suggest that caution should be used in the treatment of fever and pain with ASA during acute infection.

$TXA_2$ and ET-1 share several important properties important in the pathogenesis of Chagas disease. They both cause vasoconstriction and platelet aggregation. Additionally, they are both pro-inflammatory. Mice infected with *T. cruzi* display an increased expression of ET-1 protein and mRNA in the myocardium and an increase in plasma ET-1 levels (Petkova et al., 2000). Treatment of infected mice with phosphoramidon, an inhibitor of ECE, reduced *T. cruzi*-infection-induced right ventricular dilation (Tanowitz et al., 2005). *T. cruzi* infection of mice in which the gene for ET-1 is deleted either in cardiac myocytes or in ECs ameliorated cardiac remodelling as demonstrated by histopathology, echocardiography and cardiac MRI (Tanowitz et al., 2005). Elevated plasma levels of ET-1 have been demonstrated in patients with chronic chagasic cardiomyopathy (Salomone et al., 2001). However, it is unclear if this is a result of congestive heart failure in general or chagasic cardiomyopathy in particular. It is important to note that Hassan et al. (2006) found increased expression of ET-1 in the carotid arteries of infected mice. This observation clearly demonstrated the importance of ET-1 in the vasculature of infected mice and by implication in infected humans. The release of platelet-activating factor by macrophages in this infection causes transient ischaemia and myocytolytic necrosis (Talvani et al., 2003; see Chapter 1 for a discussion of eicosanoids and Chapter 5 for a discussion of the role of bradykinin and bradykinin receptors).

## 4.5. *IN VITRO* STUDIES

Direct infection of human ECs in culture with *T. cruzi* resulted in the alteration of various critical biochemical processes responsible for the maintenance of microvascular perfusion, such as calcium homeostasis and generation of inositol trisphosphate ($IP_3$), ET-1, $TXA_2$ and prostacyclin (PG12) which is a vasodilator and inhibits platelet aggregation (Morris et al., 1988). EC infection also resulted in alterations of cyclic AMP metabolism, which plays a protective role against the direct and/or indirect lesion caused by the adhesion and aggregation of circulating platelets to ECs (Morris et al., 1992).

Inflammatory cells contribute to microvascular hypoperfusion by secreting cytokines and other factors known to affect platelets and ECs. Infection of cultured ECs results in increased synthesis of interleukin-1β (IL-1β), IL-6 and colony-stimulating factor 1 (CSF-1) which may result in altered function (Tanowitz et al., 1992a). IL-1β is elaborated by activated macrophages and by peripheral blood mononuclear cells, including those infected with *T. cruzi*, and by a variety of other cell types, such as ECs (Van Voorhis, 1992). The antithrombotic properties of ECs may be altered by IL-1β. This cytokine may reduce tissue production of the plasminogen activator and increase production of the inhibitor of this activator, which may result in thrombus formation (Bevilacqua et al., 1984; Nachman et al., 1986). CSF-1 is an important growth factor needed for the proliferation and maturation of cells of the mononuclear lineage (Mantovani et al., 1990). It is also important in recruitment, possibly acting in conjunction with IL-1β. High CSF-1 levels have been detected in infected cultured ECs. These observations may reflect the growth of the monocyte population in the microvasculature resulting in the synthesis of pro-inflammatory cytokines (Mantovani et al., 1990; Tanowitz et al., 1992a). In addition, trypomastigotes have been demonstrated to produce neuraminidase (*trans*-sialidase) that may be involved in the removal of sialic acid from the surface of mammalian myocardial cells and ECs, facilitating thrombin binding. The loss of this endothelial surface protector molecule could contribute to platelet aggregation and thrombosis within the small coronary vessels (Libby et al., 1986). These factors acting together may ultimately result in spasm and thrombosis in the small coronary vessels, inducing focal myocardial damage.

Mukherjee et al. (2004) examined infected human ECs which resulted in activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) but not c-Jun N-terminal kinase or p38 MAPK. Treatment of these cells with the MAPK kinase inhibitor PD98059 prior to infection blocked the increase in phosphorylated ERK1/2 observed with infection. Transfection with dominant-negative Raf(301) or Ras(N17) constructs reduced the infection-associated levels of phospho-ERK1/2, indicating that the activation of ERK1/2 involved the Ras–Raf–ERK pathway. Infection also resulted in an increase in activator protein 1 (AP-1) activity, which was inhibited by transfection with a dominant-negative Raf(301) construct. Infected ECs were found to synthesize ET-1 and IL-1β, which activated ERK1/2 and induced cyclin D1 expression in uninfected smooth muscle cells. More recently, Tonelli et al. (2010) demonstrated that *T. cruzi* gp85/*trans*-sialidase surface protein family is important in the attachment of the parasite to the host cells.

Taken together, these data suggest a possible molecular paradigm for the pathogenesis of the vasculopathy in this infection.

## 4.6. STUDIES IN HUMANS

Anatomical studies have shown structural derangement and rarefied microvasculature in the left ventricular myocardium. A histotopographical study comparing the microcirculatory system after injection of an opaque medium into chagasic and control human hearts demonstrated focal decapillarization in chronic Chagas disease due to extraluminal

compression, suggesting that this might be the cause of focal myocytolytic necrosis ( Jörg, 1974). Similarly, a post-mortem radiological study of chagasic hearts revealed vascular changes at the heart apex characterized by distorted and/or scarce vessels associated with decreased arterial density, presumably related to the pathogenesis of apical aneurysm (Ferreira et al., 1980).

Patients with Chagas disease may exhibit symptoms that are atypical for classic angina pectoris. Although symptoms suggestive of myocardial ischaemia are present, coronary angiographic studies show normal or nearly normal coronary arteries in more than 90% of patients studied (Marin-Neto et al., 1992). Patients specifically selected on the basis of chest pain did show perfusion abnormalities detectable by thallium-201 scintigraphy, suggesting that myocardial ischaemia may be due to alterations in the microvasculature. Abnormal perfusion in different groups of chagasic patients has been confirmed using isonitrile-99m-technetium (Castro et al., 1988) or thallium-201 (Hagar and Rahimtoola, 1991; Marin-Neto et al., 1992). Myocardial capillary blood flow in chronic chagasic patients with no significant clinical or electrocardiographic manifestations proved to be markedly reduced when evaluated with rubidium-86, while the major coronary vessels appeared normal. The reduction observed is comparable to that exhibited by a group of non-chagasic patients with obstructive coronary disease (Kuschnir et al., 1974a,b). Vasospasm has been proposed in the genesis of myocardial ischaemia in patients with chronic chagasic cardiomyopathy (Vianna et al., 1979). For example, it was demonstrated that in patients with chagasic cardiomyopathy, there is an abnormal, endothelium-dependent, coronary-vasodilating mechanism as demonstrated by acetylcholine and adenosine infusion into the left coronary artery, suggesting that epicardial and microvascular coronary reactivity may be altered in these patients. The clinical importance of this alteration awaits elucidation. However, this abnormality of the coronary microvasculature may contribute to the genesis of the symptoms related to the ischaemic processes observed in chronic chagasic patients and to acute myocardial infarction in the absence of significant coronary damage (Torres et al., 1995).

Biopsies of chronic chagasic hearts revealed a marked thickening of the basement membrane in most myocytes and capillaries (Ferrans et al., 1988). These alterations are similar to the thickening reported for the basement membranes of myocardial capillaries in other cardiomyopathies (Factor et al., 1983). A very well developed capillary network has been observed in chagasic human hearts using a cell-maceration scanning electron microscopic method (Higuchi et al., 1999). This network may result in reduced flow of blood thus contributing to the hypoxic changes observed in chronic chagasic cardiomyopathy. Significant dilatations of arterioles and capillaries in ventricular areas of chagasic hearts compared to hearts with dilated cardiomyopathy were described. These microcirculatory dilatations could be responsible for a reduction in blood flow distribution in the watershed area lying between the two main coronary flow sources (the anterior- and posterior-descending arteries, and the right and circumflex coronary arteries). These findings could result in ischaemia and extensive fibrosis within the left ventricle apical and posterior regions (Higuchi et al., 1999).

The relation of regional sympathetic denervation and myocardial perfusion disturbance to wall motion impairment was described in patients with chronic chagasic cardiomyopathy. Global left ventricular function, segmental wall motion analysis and myocardial perfusion were evaluated in 58 patients. There were myocardial perfusion defects in the absence of epicardial coronary artery disease, and the extension and severity of perfusion abnormalities paralleled the progression of myocardial damage. These observations support the notion that perfusion disturbances in chronic chagasic cardiomyopathy may be caused by transient disturbances of coronary blood flow regulation at the microvascular level (Simoes et al.,

2000). The same group correlated the clinical, electrocardiographic, angiographic, electrophysiologic and wall motion/myocardial perfusion disturbances in chronic chagasic patients with either sustained or non-sustained ventricular tachycardia. The fact that both fixed perfusion defects (which reflect local fibrosis) and reversible and paradoxical defects predominate in the arrhythmias in the left ventricular region is also compatible with the hypothesis that microvascular ischaemia is aetiologic. Thus, several observations suggest that in human chagasic heart disease, transient disturbances of coronary blood flow regulation at the level of the microvasculature may result in regional myocardial degeneration, with a consequent reparative fibrosis that ultimately constitutes the substrate for re-entrant circuits and the appearance of both sustained and non-sustained ventricular tachycardia (Sarabanda et al., 2005).

## 4.7. CONCLUSIONS

Abnormalities in the coronary circulations were observed since the earliest studies by Vianna and Torres conducted soon after the discovery by Carlos Chagas of the disease that bears his name. Since then, much information has accumulated from attempts to define the physiopathology of chagasic heart disease. The changes observed both on humans and in experimental models of *T. cruzi* infection suggest that myocardial lesions are multifactorial including parasite persistence, autoimmunity and microvascular involvement. Importantly, they are not mutually exclusive.

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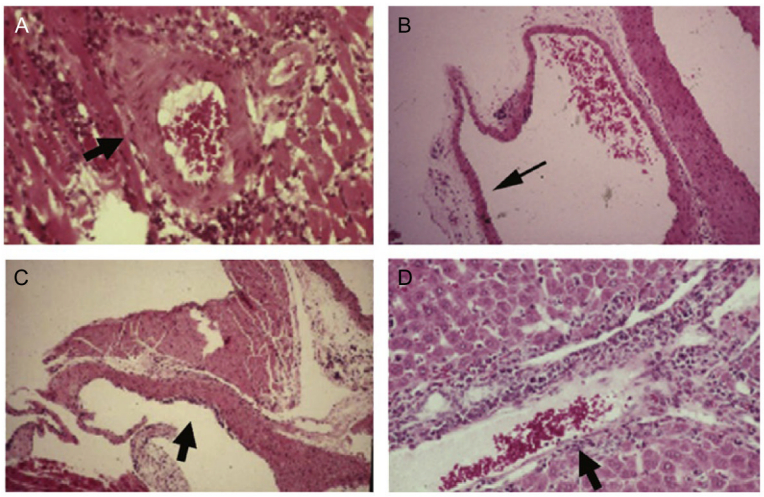


**FIGURE 4.1.**
(A) Images obtained from *T. cruzi*-infected mouse. Perivascular inflammation. (B) Vasculitis of the pulmonary vasculature. (C) Endothelialitis of the subendocardium. (D) Vasculitis of a blood vessel in the liver (images from Petkova et al., 2001).

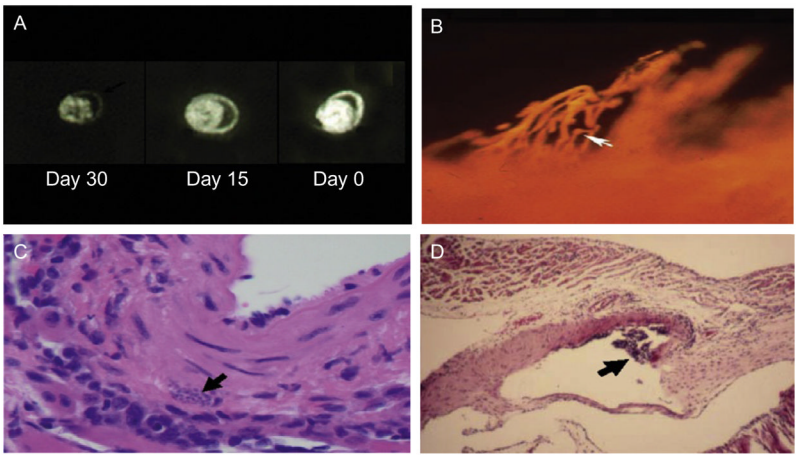


**FIGURE 4.2.**

(A) Coronary perfusion of mouse hearts as determined by autoradiographic imaging utilizing the fatty acid analog 19-iodo-3,3,-dimethyl-18 nonadecenoic acid (DMIVM). A: uninfected normal mouse with normal perfusion. B: perfusion in a mouse infected for 15 days. Note the reduced perfusion C: perfusion in a mouse infected for 30 days demonstrating a marked reduction in perfusion (taken from Tanowitz, 1992). (B) Microfil injection of the coronary vasculature of an A/J mice 15 days post-infection with the Tulahuen strain of *T. cruzi* demonstrating a section through the subendocardium of the atrium showing saccular microaneurysms and vasospasm (Rossi et al., 2010). (C) Pseudocyst in the wall of a blood vessel (Tanowitz et al., 2009). (D) Vasculitis of a large blood vessel obtained from an infected mouse (Tanowitz et al., 2009).