[Frontiers in Bioscience 8, e410-419, May 1, 2003]

ROLE OF VASOACTIVE MEDIATORS IN THE PATHOGENESIS OF CHAGAS' DISEASE

Shankar Mukherjee [1], Huan Huang [1], Louis M. Weiss [1,2], Sylvia Costa [2], Julio Scharfstein [3], Herbert B. Tanowitz [1,2]

Departments of Pathology [1] and Medicine [2], Albert Einstein College of Medicine, Bronx, New York, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil [3]

.

TABLE OF CONTENTS

1. ABSTRACT

This review focuses on the vascular pathogenesis of Chagas' disease, the cardiomyopathy caused by infection with the parasitic protozoa *Trypanosoma cruzi*. Recent studies strongly suggests that *T. cruzi* infection is linked to functional changes in the activity of two potent vasoative peptidergic mediators, endothelin-1, a vasoconstrictor, and kinins, a group of vasodilator and pro-inflammatory peptides related to bradykinin. Understanding the molecular mechanisms underlying disturbances of vascular homoeostasis? induced by *T. cruzi* may provide opportunities for therapeutic intervention and amelioration of heart pathology.

2. INTRODUCTION

Chagas' disease is an important cause of acute myocarditis and chronic cardiomyopathy in endemic areas of Mexico, Central and South America. In recent years there has been a large influx of immigrants from these countries into non-endemic areas of North America and Europe. Some of these immigrants may have chronic Chagas' disease. In addition, Chagas' disease has now emerged as an important opportunistic infection in the setting of HIV/AIDS.

Recent observations underscore an overlooked aspect of the pathology and pathogenesis of chagasic cardiomyopathy: the role of the vasculature. Descriptions of microcirculatory abnormalities and altered patterns of extracellular matrix deposition in the myocardium of patients with chronic chagasic cardiomyopathy (1-3) have renewed interest in investigating the role of vascular dysfunction in the heart disease (1-6) (Figures 1 & 2). Evidence of reduced coronary blood flow in Chagas' disease derived from studies in experimental models (3,6) and in human Chagas' disease (7, 65). In addition, there is an intense vasculitis of the microvasculature of the coronary artery system, the endocardial endothelium as well as conduit vessels in infected mice (8,9). It has been difficult to assess the relationship between vascular lesions and the presence of residual myocardial parasitism in chronically infected patients or experimental animals, because intracellular amastigotes are not usually detected. However, amastigotes have been observed in endothelial cells lining the coronary microcirculation (14). The vasculitis is also found in other organs, such as the liver, indicating that *T. cruzi*-induced vasculopathy is not limited to the heart.

Infected endothelial cells exhibit perturbations in the host-cell signal transduction pathways that may be responsible for microvascular dysfunction (10,11). *T. cruzi*-infection of cultured endothelial cells resulted in the activation of the NF-κB pathway, which stimulates synthesis of proinflammatory cytokines and surface expression of vascular adhesion molecules (12). In addition, *T. cruzi*-infection of endothelial cells caused increased synthesis of the vasoactive peptide, endothelin-1 (ET-1) and activation of components of the mitogen activated protein kinase pathway (Tanowitz *et al*, unpublished observations). These and other infection-associated perturbations in endothelial-cell signal transduction mechanisms may contribute to focal pathology, including the coronary microvascular spasm.

Focusing on the early vascular changes elicited by infection, Scharfstein's group recently demonstrated that *T. cruzi* trypomastigotes, but not the non-infective forms (epimastigotes), stimulate edematogenic inflammation at early stages of infection in mice (13). Analysis of the temporal course of the vascular reaction indicated that it depends on sequential activation of B2 (constitutive) and B1 (inducible) kinin receptors. As the infection progresses, other evidences for vascular dysfunction emerged in studies of mouse, rat and hamster models. For example, Factor *et al* (1) and Tanowitz *et al* (3) observed that *T. cruzi* infection of mice caused segmental microvascular spasm

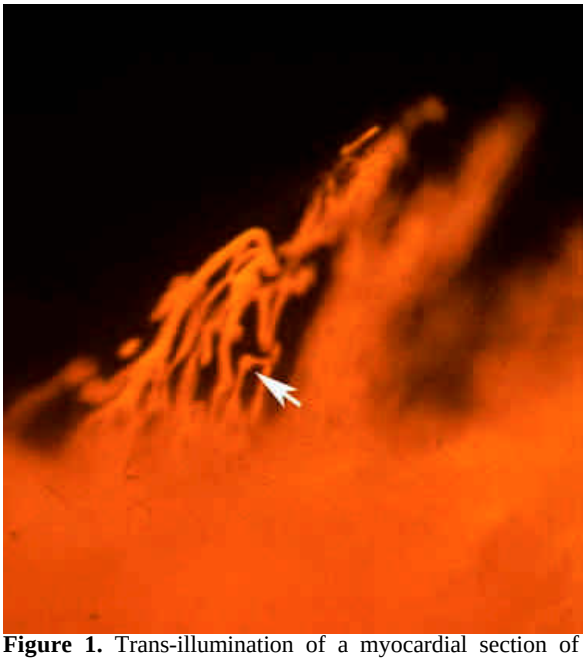

Figure 1. Trans-illumination of a myocardial section of *Trypanosoma cruzi* (Brazil strain) infected mouse during acute infection. Microfil injection was performed just prior to sacrifice. Note aneurysms are predominantly in the capillaries and focally associated with vascular constriction (reference 1, permission obtained from the *Am J Trop Med Hyg*)

and microaneurysm formation as well as reduced red blood cell velocity in vascular beds.

Platelet thrombi were found in the coronary microvasculature of acutely -infected mice (14). In other experiments increased platelet aggregation and elevated plasma levels of thromboxane A_2 were observed (14). Thromboxane A_2 promotes vasospasm as well as platelet aggregation. Notably, increased platelet adherence was observed in *T. cruzi* infected cultured endothelial cells. Therefore, elevated levels of plasma thromboxane A_2 may contribute to the pathogenesis of chagasic heart disease.

Evidence for the involvement of the microvasculature in the pathogenesis of chagasic heart disease was also reported by studies with the calcium channel blocker, verapamil (15-17). This drug is known to increase coronary blood flow and inhibit platelet aggregation. Verapamil was administered to *T. cruzi*-infected CD1 mice for 120 days beginning from the first day of infection. When the myocardium was evaluated at 120 –150 days post infection, there was a reduction in chronic myocardial inflammation and fibrosis. Echocardiography of infected untreated mice revealed severe myocardial dysfunction, which was not observed in infected, verapamil treated mice. However, when verapamil was administered from day 60 of infection there was no amelioration of pathology or function suggesting that the action of verapamil is optimal only when administered in the early stage of acute infection (unpublished). Although not excluding other physiological effects, these data also suggest that verapamil acts on vasculature. This idea was underscored by the observation that infection caused a reduction in blood flow in small arterioles and venules of the cremaster muscle of the male mouse that was reversed by the administration of verapamil. Moreover, *in vitro* studies have demonstrated that verapamil acts on vascular smooth muscle cells to diminish the effects of the vasoconstrictor, ET-1 (18).

Vasculitis of the microvasculature has been described in the acute dog model (19,20) and it has been suggested that the heart microangiopathy observed in early stages of infection (6) may regress as the disease enters the asymptomatic phase (20). However, it is also possible that the recovery of endothelial function at a later time may help to maintain the self-limited cycles of focal inflammatory changes that characterizes the indeterminate phase (21). Todorov *et al.* (13) have recently suggested that in the indeterminate stage, the presence of small numbers of parasites in the mildly inflamed cardiac tissues may induce low level release of kinin peptides (see below). The authors proposed that the vasodilating responses which kinins convey through their vascular receptors may lessen the myocardium from the detrimental effects of ischemia (22), while increasing parasite infectivity for cardiovascular cells due to activation of kinin-receptors (23). More recently, bradykinin did not reverse the infection-associated decrease in coronary blood flow in a rat model of *T. cruzi* infection (24) suggesting that either kinin receptor function is impaired or that such maneuvers are ineffective, once the damage is established.

3. ROLE OF KININ RECEPTORS AND KININASES IN CARDIOVASCULAR DISEASE AND INFECTION

Bradykinin (9 amino acid) and lysyl-bradykinin (10 amino acid) are potent vasoactive peptides that dilate vessels and increase vascular permeability. Thus they have been traditionally viewed as mediators of inflammation, pain, and hyperalgesia. It is now well accepted that these peptides significantly contribute to the homeostasis of the microcirculation (25). Recent studies have suggested a cardioprotective role for kinins in animal models of ischemia-reperfusion (22). These observations form the basis for the clinical benefits observed with inhibitors of the angiotensin-converting enzyme (ACE) in the treatment of myocardial infarction (26,27).

The biological effects of kinin peptides are exerted by two subtypes of G-protein coupled kinin receptors (B1 and B2). These receptors can be distinguished on the basis of DNA sequence, pharmacological specificity and tissue distribution. The kinin B2 receptors are constitutively expressed on many cell types such as endothelial cells and smooth muscle cells, and are responsible for most of the physiological effects of kinins in the heart (28,29). However, B1 receptor expression is upregulated by pro-inflammatory cytokines and likely accounts for some of the pathophysiological effects of kinins, particularly those induced during chronic inflammation (30). The B2 receptor agonists are

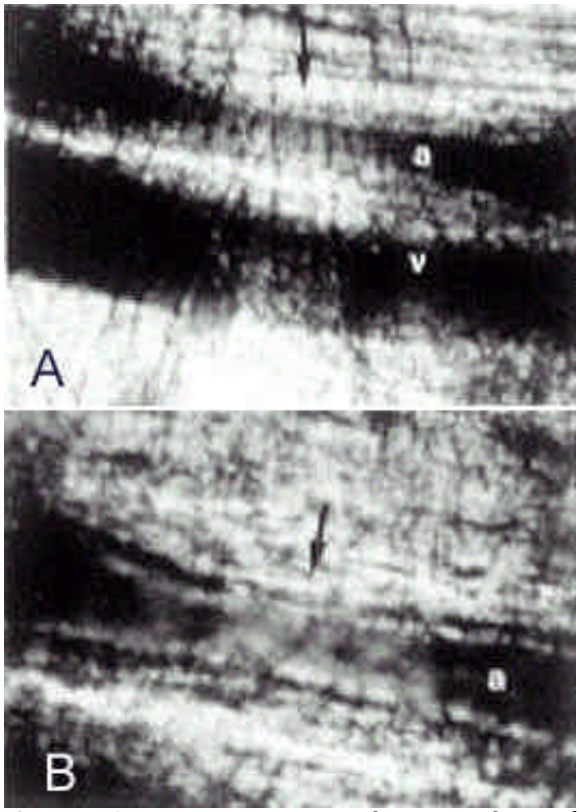

Figure 2. Representative videomicrographs of microvasculature of cremaster muscle obtained from *Trypanosoma cruzi* (Brazil strain) infected mouse. (a, arteriole; v, venule). Note areas of vasospasm (arrows) Reference 3 permission obtained from the *J Parasitol*)

bradykinin and lysyl-bradykinin, the excision products derived from an internal segment of high and low molecular weight kininogens (25), by the action of plasma or tissue kallikrein, respectively. Owing to the presence of kinin-degrading peptidases in the tissues (*eg*. ACE/Kininase II), these short-lived vasoactive kinin peptides must rapidly bind to high-affinity B2 receptors displayed by adjacent endothelial cells/smooth muscle (25). The B1 agonists, *i.e.*, [des-Arg9] bradykinin and [des-Arg10]lysyl-bradykinin, are the metabolites resulting from enzymatic removal of the C-terminal Arg residue from B2 agonists by Kininase I (CPM/N) (30).

Bradykinin has also been implicated in the pathogenesis of bacterial infections. For example, it is well known that gram-negative bacteria activate the release of bradykinin and may be involved in microbial dissemination (31). The B1 receptor is induced by infection through the pro-inflammatory activity of lipopolysacharide and cytokines such as TNF-α and IL-1β (31). In addition, there is evidence of bradykinin release in patients with streptococcal toxic shock syndrome and *Staphylococcus aureus* bacteremia (32). These observations suggest that bradykinin mediated responses may cause hypovolemia and hypotension associated with the sepsis syndrome, being perhaps also relevant in the context of lethal *T. cruzi* infection (66).

4. THE KININ SYSTEM AND EXPERIMENTAL CHAGAS' DISEASE

Involvement of *T. cruzi* proteases in vascular permeability changes was initially explored by the topical application of activated cruzipain, the parasite`s major cysteine proteinase in the hamster cheek pouch model (39). Active forms of cruzipain, but not the enzyme inactivated with the cysteine protease inhibitor E64, evoked a significant increase in microvascular leaks in post capillary venules. Of interest, this effect was partially inhibited by a histamine receptor (H1) blocker (39).

Evidence linking *T. cruzi* infectivity to activation of the kinin system has emerged from the molecular studies of Scharfstein *et al* (13,23), which focused on the activation of pathways involved in host cell invasion by trypomastigotes. Prior to these observations, biochemical studies identified kininogens as natural substrates of cruzipain (33), the major cysteine protease of *T. cruzi* (34-36). Further investigations of the molecular mechanisms underlying the kinin-release activity of cruzipain indicated that the processing of high molecular weight kininogen (HK) is modulated by heparan sulfate proteoglycans (37). This glycosaminoglycan is thought to serve as a dense platform for the docking of plasma borne-kininogens to endothelial cell surfaces (38). Once released from surface-bound HK, the short-lived kinin peptide agonist liberated by cruzipain swiftly binds to the constitutive B2 kinin-receptor of endothelial cells (23) and cardiac myocytes (13). By inducing vigorous increase in intracellular calcium concentration through this G-protein coupled receptor, the pathogen renders these cells increasingly susceptible to invasion (13,23). More recently, it has been shown that trypomastigotes were also able to invade endothelial cells more efficiently by stimulating B1, the receptor whose expression is up regulated during inflammation (13). Interestingly, engagement of the B1 upregulated pathway depends on the co-factor activity a host carboxypeptidase, Kininase I (CMP/N), because this is the enzyme that removes the C-terminal Arg residue from the liberated B2-agonists, bradykinin or lysyl-bradykinin, forming the B1 agonists, des-Arg-kinins (30). As previously mentioned, *in vivo* studies showed that host kininases such as the angiotensin-converting enzyme (ACE) and Kininase I likewise modulate the intensity of vascular responses which trypomastigotes stimulate through B2 and B1 kinin-receptors (13).

Given that kinin receptors are expressed in the normal human heart (28). Scharfstein *et al.* (13,23) have proposed that changes in kinin system homeostasis may alter the host-parasite relationship in the indeterminate stage of chagasic heart disease. Accordingly, dysfunctions and /or decreased expression levels of kinin-degrading enzymes in sites of infection may lead to excessive formation of kinins in cardiovascular tissues. This may in turn stimulate plasma leakage, hence intensifying inflammation and resulting in B1 kinin receptor upregulation by cardiovascular cells (13). Parasites may thus take advantage of the host inflammatory response to invade cardiovascular cells more efficiently at expense of activation of the upregulated B1 pathway (13) (Figure 3). It

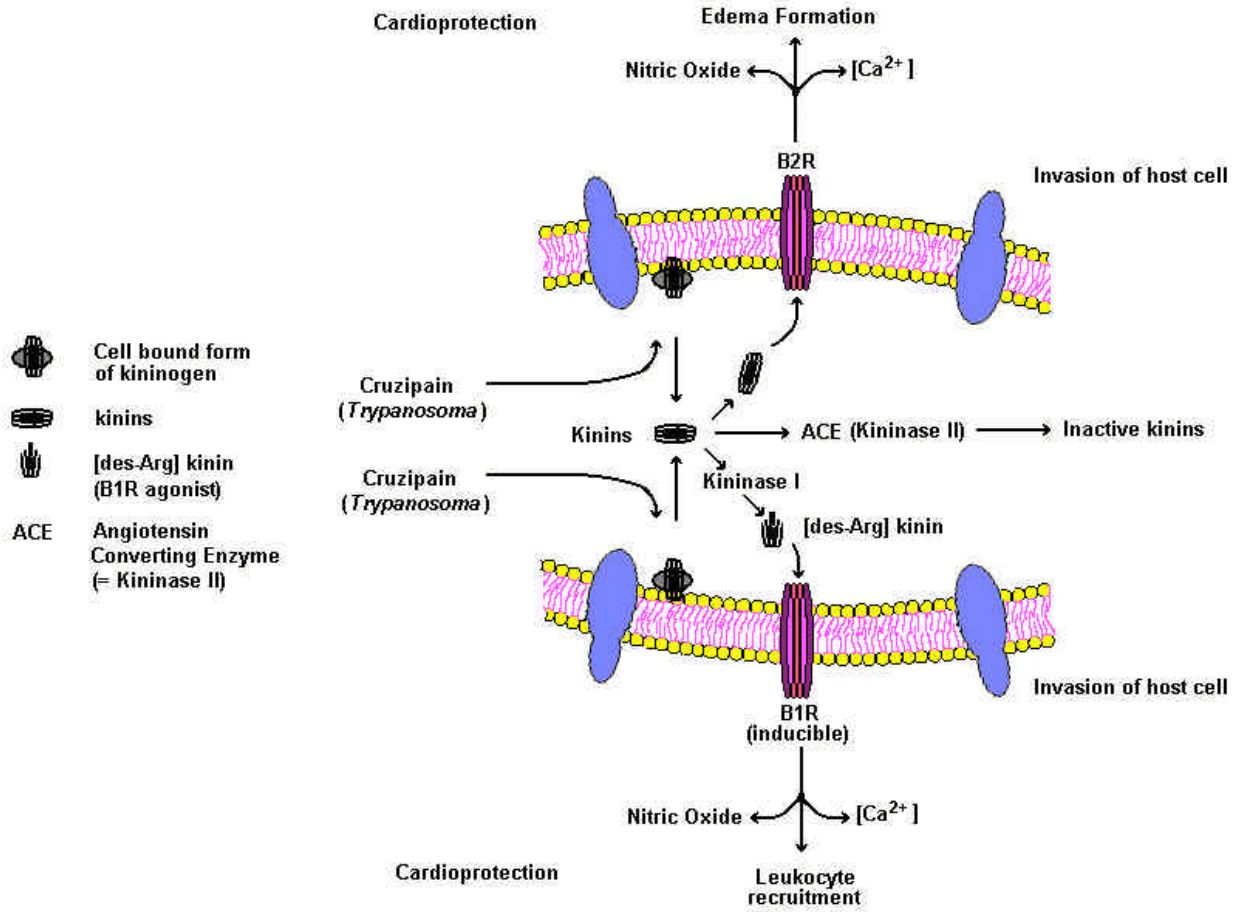

Figure 3. Proposed role of host kininases in the pathogenesis of Chagas' disease. The parasite liberates kinins from cell-bound forms of kininogens through cruzipain. Acting by the paracrine mode, the short-lived kinins (bradykinin or lysyl-bradykinin) exert their vasoactive activity by binding swiftly to the constitutive B2 receptors. Alternatively, the kinins are either degraded by kininase II (*eg.* ACE) or are metabolized by kininase I (carboxypeptidase M/N), generating [des-Arg] kinins, the agonists for the inducible B1 receptor subtype. The increase intracellular calcium concentration [Ca^{2+}] activates those responses mediated by B2 and/or B1 kinin receptors which increase the endocytic uptake of trypomastigotes by host cells (*eg*. endothelial cells and cardiac myocytes). In addition, the triggering of endothelial kinin receptors induces plasma leakage and other vascular responses. Alterations in the expression levels of the various kininases may alter kinin homeostasis, exacerbating pathology in chronically infected myocardial tissues (13,64).

was further envisaged that the increased intracellular parasite burden that results from earlier engagement of host cell kinin-receptors can only be efficiently reduced at expense of vigorous activation of CD8+ T cells directed against parasite antigens (40), a process that in itself may produce myocardial inflammation and fibrosis (5). Studies in mice with targeted deletion of B2 and B1 kinin receptor genes (41) are currently underway to evaluate if the kinin system may play a role in the pathogenesis of chronic Chagas' disease (Scharfstein and Tanowitz, unpublished observations).

5. ENDOTHELIN: GENERAL CONSIDERATIONS

The endothelin system is conserved in mammals, fish and invertebrates, indicating that it is important in different biological contexts. There are three isoforms of endothelin; endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) (42,43). ET-1 is a 21 amino acid peptide that has structural homology with sarafotoxin, a snake venom that induces myocardial infarction by exaggerated contraction of the cardiac vessels and interruption of the blood supply to the heart. The amino acid sequences of ET-1 from human, porcine, canine, rat, mouse and bovine are identical (42,43) Endothelins are synthesized as high molecular weight peptide, *e.g.*, prepro ET-1 is a 212-amino acid precursor molecule that is cleaved to form big ET-1, a 38-amino acid peptide (Figure 4). The conversion of big ET-1 to ET-1 is essential for biological activity. Cleavage is mediated by a family of membrane bound zinc metalloproteases known as endothelin converting enzymes (ECE). ECE mRNA is distributed in many tissues, but the highest expression is found in the endothelium (44). ECE can process big endothelin both intracellularly as well as on the cell surface. In several species, ECE exists in several isoforms (ECE-1, ECE-2).

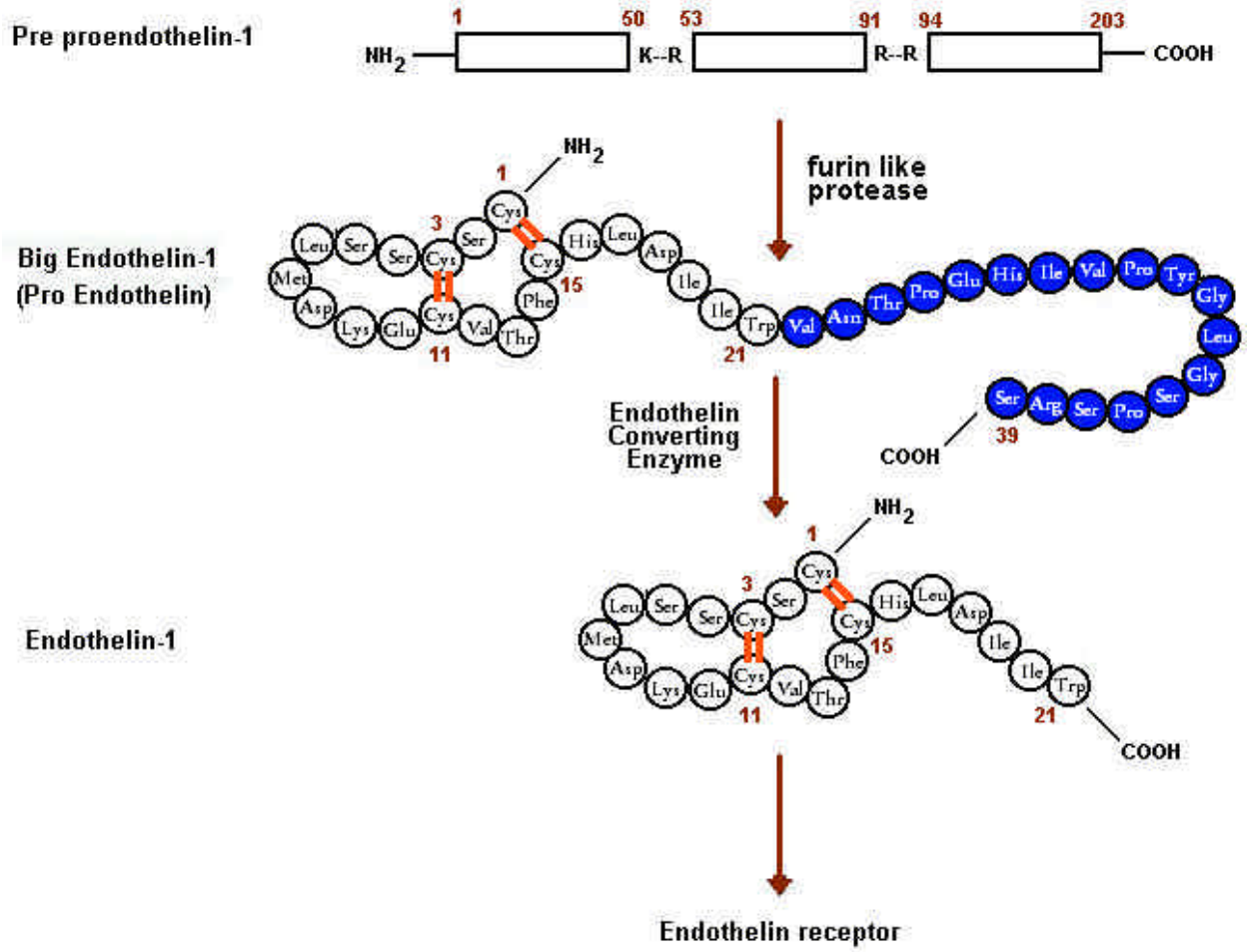

Figure 4. Proteolytic processing pathway for the conversion of preproendothelin to endothelin-1. The endothelin-1 system in mammalian cells. ET-1 is a 21 amino acid peptide formed by the action of endothelin converting enzyme (ECE) on big ET-1. We hypothesize, based on the current available data, that when the parasite infects a cell there is an activation of endothelin converting enzyme leading to an increase in the formation of ET-1. ET-1 in turn is bound to the ET_A receptor on the surface of the underlying smooth muscle cell resulting in an increase in intracellular calcium causing vasoconstriction of the vessel.

ET-1 is synthesized by a variety of cells (42,43) including cardiac myocytes, cardiac fibroblasts, neurons, endothelial cells, vascular smooth muscle cells, tracheal epithelium, hepatic sinusoids and Kupffer cells, mast cells, mucosal epithelium, macrophages, and inflammatory cells Secretion of ET-1 occurs in a polar fashion, with approximately 80% towards the abluminal side of the vessel (42,43). ET-2 is found in intestine and ET-3 in the lung, central nervous system and intestine. The generation of ET-1 is increased by changes in sheer stress of the vessel wall, hypoxia, and endotoxins. Secretion of ET-1 is stimulated by epinephrine angiotensin II, arginine vasopressin, TGF-β, thrombin, IL-1β and TNF-alpha. ET-1 mRNA expression is increased after the exposure of endothelial cells to growth factors, thrombin, cytokines, chemokines, norepinephrine and angiotensin II. The pulmonary vascular bed is an important location for plasma clearance of ET-1 (42,43), but both the kidney and the liver have been shown to participate in this process.

Although ET-1 was previously regarded solely as a vasoactive agent, it is now recognized that it is also involved in the inflammatory process. ET-1 primes neutrophils, stimulates neutrophils to release elastase and activates mast cells (45). In addition, ET-1 stimulates monocytes to produce cytokines and upregulate the expression of vascular adhesion molecules. High levels of ET-1 are found in alveolar macrophages, leukocytes and fibroblasts (42,43,45).

There are two G protein-coupled cell surface endothelin receptors that have been identified, ET_A and ET_B. As in the case of the kinin-receptors, they are members of the seven transmembrane helix family of proteins and are coupled to phospholipase-C. In vascular smooth muscle, an increase in intracellular calcium is a common feature occurring after activation of all receptor subtypes (Figure 4). Upon binding of ET-1 to ET_A, phospholipase-C is activated and inositol triphosphate is generated. Calcium is then released form intracellular

stores accompanied by the influx of extracellular calcium and activation of the contractile machinery. The precise mechanism by which ET-1 affects intracellular calcium regulation is not fully understood. In that regard, it has been reported that ET-1 induces mitogen-activated protein kinases and c-fos expression, thus promoting smooth muscle cell and cardiac fibroblast proliferation (42,43). The activation of the transcription factor activator protein-1 (AP-1) contributes to the regulation of ET-1 production (43). Moreover, components of the mitogen activated protein kinase pathway and ET-1 induce the expression of cyclin D1, which results in cellular proliferation (46).

The endothelin receptors have been cloned and characterized. ET_A is predominantly found on smooth muscle cells where it mediates vasoconstriction and proliferative responses, whereas, ET_B receptors predominate on the low-pressure side of the circulation. ET_B receptors are located on the surface of the endothelium, in fibroblasts and smooth muscle cells that are abundant in the brain and kidneys (41,42). Recently, it has been demonstrated that ET_B receptors are antiproliferative in the injured artery (47). The ET_A receptor shows a higher affinity for ET-1 than for ET-2 and the lowest affinity for ET-3. The ET_B receptor shows approximately equal affinity for each of the threeendothelin isoforms. ET-1 has several pharmacological actions in the cardiovascular system, which is mediated by the smooth muscle cell endothelin receptors.

The introduction of mice with various deletions of components of the endothelin system has provided important information regarding the pivotal role of ET-1 in normal embryonic development. For example, homozygous mice deficient in ET-1 (48), the ET_A receptor (49), and ECE (50) die soon after birth with craniofacial and cardiac abnormalities. ET_B receptor deficient mice exhibit aganglionic megacolon (associated with coat color spotting), that resembles human Hirschsprung's disease (51). Knockout of only one allele of the ET_A or ET_B gene significantly ameliorated the ET-1-mediated vasoconstriction in murine mesenteric and renal vascular bed (52).

6. ENDOTHELIN AND THE HEART

There is evidence that ET-1 contributes to local ischemia, hypertension, vasospasm and states of sustained vasoconstriction including malignant hypertension and congestive heart failure. Studies on the putative role for ET-1 in the modulation of cardiovascular structure have centered on the role of ET-1 in the induction of smooth muscle cell proliferation. For example, following balloon angioplasty, there is neointima formation with an increased tissue level of ET-1. This was reported to be associated with the induction of mRNAs for ET-1, ECE and the ET_A receptor (53). The administration of endothelin receptor antagonists or ECE inhibitors resulted in the reduction of neointima formation, suggesting a role for ET-1 in the pathogenesis of restenosis. ET-1 stimulates smooth muscle cell hypertrophy, protein synthesis and the incorporation of ^{3}[H] thymidine (54). These effects of ET-1 are mediated, in part, by activation of the smooth muscle cell ET_A receptor. Activation of this receptor results in the synthesis of types I and III collagen and a reduction in collagenase activity. Consequently, these observations could account for some of the vascular remodeling observed in cardiovascular diseases, perhaps including those observed in human Chagas` disease (2).

Both cardiac myocytes and endothelial cells synthesize ET-1. Locally produced ET-1 acts on cardiac myocytes in both an autocrine and/or paracrine manner, increasing the contractility of smooth muscle cells, and chronically induces myocardial hypertrophy and cardiac myocyte injury (42,43). ET-1 improves contractility in the failing heart, and upregulation of ET-1 may provide short-term inotropic support for the failing myocardium. ET-1 production was increased in the myocardium of rats with congestive heart failure and was associated with the expression of the c-jun. In the setting of myocardial infarction, increased plasma ET-1 levels correlated with infarct size. In addition, levels of ET-1 correlate with the severity of congestive heart failure. In the various disease states, the increased ET-1 levels reflect both the degree of endothelial and myocardial cell damage, as well as a possible mechanism of myocardial damage. Treatment with an ET_A receptor antagonist improved the survival of animals with congestive heart failure and was accompanied by improvement in both left ventricular dysfunction and ventricular remodeling suggesting that upregulation of ET-1 may be a potential target for therapeutic intervention in the treatment of congestive heart failure.

7. ENDOTHELIN IN OTHER DISEASE STATES

There is accumulating evidence that ET-1 contributes to the pathogenesis of bronchial asthma, pulmonary hypertension, diabetes mellitus, renal failure, gastric ulceration, cerebral vasospasm following subarachnoid hemorrhage, and migraine headaches (43,43). Elevated levels of ET-1 are observed in HIV infection and some cancers.

The role of ET-1 in the pathogenesis of infectious diseases has received increasing attention. For example, vascular endothelial cell damage is observed in bacterial sepsis that may be accompanied by elevated plasma levels of ET-1and resultant multi-organ failure. As a result of these observations, ET-1 has been implicated as a major contributor to the pathogenesis of septic shock (55,56). ET-1 has also been implicated in the pathogenesis of viral infections of the respiratory tract and the myocardium. In that regard, Ono *et al* (57) demonstrated that ET-1 contributed to myocardial injury in a murine model of viral myocarditis. They clearly demonstrated that infection with encephalomyocarditis virus resulted in elevated plasma levels of ET-1 and increased expression in the myocardium of mRNAs for prepro ET-1 and endothelin converting enzyme. *Rickesttia* infections, which are associated with a vasculitis, have also been reported to be associated with elevated ET-1 levels (58).

8. ENDOTHELIN IN CHAGAS' DISEASE

In order to provide a basis for the microvascular spasm observed in Chagas' disease the role of the potent vasoconstrictor was initially explored. *T. cruzi* infection of cultured endothelial cells resulted in an increased synthesis of ET-1 (18,59) (Figure 4). Bioassay studies were then performed using rat aortic rings that had been denuded of endothelial cells thus leaving smooth muscle cells and their ET_A receptors intact. Exposure of these rat aortic rings to supernatants of infected endothelial cells elicited significant contractile responses. The increased aortic contractility was significantly attenuated by pre-treatment with the ET_A subtype selective antagonist, BQ-123. In addition, incubation of endothelial cells with phosphoramidon, an inhibitor of ECE, prior to infection caused a significant decrease in ET-1 levels in the endothelial cell supernatant reduction in aortic contractility (18). Among the factors known to activate ET-1 synthesis is the transcription factor, AP-1. In this regard, it is of interest to note that *T. cruzi* infection causes activation of AP-1 in the myocardium (60).

The contribution of ET-1 in a model of acute murine *T. cruzi* infection was next examined. Petkova *et al* (8) found elevated plasma levels of ET-1, peaking at days 10–15 post infection. In addition, there was an increased expression of mRNAs for prepro ET-1, ECE and ET-1 in the myocardium at 15 and 30 days post infection. Immunohistochemistry studies employing an anti-ET-1 antibody revealed increased expression in vascular and endocardial endothelium in infected mice. Many of the parasitized and necrotic cardiac myocytes also stained with anti-ET-1 antibody. Cardiac myocytes, fibroblasts and inflammatory cells are capable of synthesizing ET-1. Therefore, the increase in ET-1 observed in the myocardium of infected mice is likely to have several sources. The increase in ET-1 may reflect damage to a variety of cell types found in the heart such as endothelial cells, cardiac myocytes and fibroblasts. In addition, an elevated ET-1 level provides a mechanism to explain, in part, the vascular spasm and ischemia observed as a consequence of this infection.

The role of ET-1 in *T. cruzi* infection was evaluated in mice in which the ET-1 gene was deleted from cardiac myocytes (KO). The extent of myocardial damage assessed during the chronic stage (150 days post infection) was similar in the KO mice and the wild type mice during the acute stage of infection. However, Trichrome staining revealed less fibrosis in the KO mice. In addition, there was significantly less remodeling in the KO mice as determined by cardiac MRI and echocardiography (61). These observations suggested that pharmacological intervention using drugs targeted at components of the endothelin system would ameliorate the consequences of *T. cruzi* infection. Therefore, Jelicks *et al* (62) treated mice with phosphoramidon, which is an inhibitor of ECE as well as an inhibitor of neutral endopeptidases. They observed that when phosphoramidon was administered to infected mice for the first 14 days of infection there was a significant reduction in myocardial inflammation and fibrosis when evaluated during the chronic stage. In addition, cardiac magnetic resonance and echocardiography revealed that treatment resulted in a significant reversal of the structural and functional alterations that accompanied this infection. For example, cardiac magnetic resonance imaging of infected mice revealed that right ventricular dilation that was less severe in infected-treated with phosphoramidon. Phosphoramidon-treated CD1 mice survived the acute infection. Transthoracic echocardiography demonstrated left ventricular dilation and reduced percent fractional shortening and relative wall thickness. These alterations were significantly attenuated as a result of phosphoramidon treatment. These data indicate that interference with ET-1 synthesis ameliorates *T. cruzi*-induced cardiac remodeling.

Recently, Camargos *et al* (24) treated *T. cruzi*-infected rats with an orally active ET_A antagonist, BSF-461314. Interestingly, treatment in this rat model resulted in increased parasitemia and increased nests of pseudocysts in the heart. Nonetheless, pretreatment with BSF-46134 attenuated the infection-associated reduction in coronary blood flow confirming a role for ET-1 in vascular dysfunction in Chagas' disease. More recently, Salomone *et al* (63) reported that elevated levels of plasma ET-1 in patients with chronic chagasic cardiomyopathy. It is unclear if these elevations were due to infection per se or non-specific elevations as observed in congestive heart failure of other etiologies.

9. CONCLUSIONS

The etiology of chagasic cardiomyopathy remains a subject of controversy. However, there are now substantial amounts of data demonstrating that chagasic heart disease is, at least in part, a vasculopathy. This is based on observations indicating that both human and experimental infections cause a reduction in blood flow and an intense vasculitis. Bradykinin, a potent vasodilator, and ET-1 and thromboxaneA2, two potent vasoconstrictors, have been identified as factors that may contribute to the vasculopathy of chagasic heart disease. Therefore, study of the mechanisms underlying regulation of ET-1 and kinin function may provide new targets for development of adjunctive therapy for Chagas' disease.

10. ACKNOWLEDGEMENTS

HBT was supported by NIH Grant AI-12770 and American Heart Association Grant (Heritage Branch). JS was supported by grants from WHO-TDR (# A10340), FAPERJ and CNPq.

11. REFERENCES

1. Factor, S.M., S. Cho, M. Wittner & H.B. Tanowitz: Abnormalities in the microcirculation in Chagas' disease. *Am J Trop Med Hyg* 34, 246-253 (1985)

2. Higuchi M.L., S. Fukasawa, T. De Brito, L.C. Parzianello, G. Bellotti & J.A. Ramires: Different microcirculatory and interstitial matrix patterns in idiopathic dilated cardiomyopathy and Chagas' disease: a three dimensional confocal microscopy study. *Heart* 82, 279-285 (1999)

3. Tanowitz H.B., D.K. Kaul, B. Chen, S.A.Morris, S.M. Factor, L.M. Weiss & M. Wittner: Compromised microcirculation in acute *Trypanosoma cruzi* infection. *J Parasit* 82,124-130 (1996)

4. Rossi, M.: Microvascular changes as a cause of chronic cardiomyopathy in cardiomyopathy in Chagas´ disease. *Am Heart J* 120, 233-236 (1990)

5. Palomino S.A., V.D. Aiello & M.L. Higuchi: Systematic mapping of hearts from chronic chagasic patients: the association between the occurrence of histopathological lesions and *Trypanosoma cruzi* antigens. *Ann Trop Med Parasitol* 94, 571-579 (2000)

6. Andrade Z.A., S.G. Andrade, R. Correa, M. Sadigursky & V.J. FerransJ: Myocardial changes in acute Trypanosoma cruzi infection. Ultrastructural evidence of immune damage and the role of microangiopathy. *Am J Pathol* 144, 1403-1411 (1994)

7. Tanowitz H.B., S.A. Morris, S.M. Factor, L.M. Weiss & M. Wittner: Parasitic disease of the heart I: Acute and chronic Chagas' disease. *Cardiovasc Pathol* 1:7-15 (1992)

8. Petkova S.B., H.B. Tanowitz, H.I. Magazine, S.M. Factor, J. Chan, R.G. Pestell, B. Bouzahzah, S.A. Douglas, V. Shtutin, S.A. Morris, E. Tsang, L.M. Weiss, G.J. Christ, M. Wittner & H. Huang: Myocardial expression of endothelin-1 in murine *Trypanosoma cruzi* infection. *Cardiovasc Pathol* 9, 257-265 (2000)

9. Petkova S.B., H. Huang, S.M. Factor R.G. Pestell, B. Bouzahzah, L.A. Jelicks, L.M. Weiss. S.A. Douglas, M. Wittner & H.B. Tanowitz: The role of endothelin in the pathogenesis of Chagas' disease. *Int J Parasitol.* 31, 499-511(2001)

10. Oz H.S., M. Wittner, H.B. Tanowitz, J.P. Bilezikian, M. Saxon & S.A. Morris: *Trypanosoma cruzi*: mechanisms of intracellular calcium homeostasis. *Exp Parasitol* 74, 390-399 (1992)

11. Morris S.A., H.B. Tanowitz, M. Makman, V. Hatcher, J.P. Bilezikian & M. Wittner: *Trypanosoma cruzi*: Infection of human umbilical vein endothelial cells alter cAMP metabolism. *Exp Parasitol* 74, 69-76 (1992)

12. Huang H., S.B. Petkova, A.W. Cohen, B. Bouzahzah, J. Chan, J-N Zhou, S.M. Factor, L.M. Weiss, M. Krishnamachary, S. Mukherjee, M. Wittner, R.N. Kitsis, R.G. Pestell, M.P. Lisanti, C. Albanese & H.B. Tanowitz: Activation of transcription factors (AP-1 and NF-κB) in murine chagasic myocarditis. *Infection & Immunity* In press.

13. Todorov A.G., D. Andrade, J.B. Pesquero, C. Araujo Rde, M. Bader, J. Stewart, L. Gera, W. Muller-Esterl, V. Morandi, R.C. Goldenberg, H.V. Neto & J. Scharfstein: *Trypanosoma cruzi* induces edematogenic responses in mice and invades cardiomyocytes and endothelial cells *in vitro* by activating distinct kinin receptor (B1/B2) subtypes. *FASEB J.* 17. 73-75 (2003)

14. Tanowitz H.B., E.R. Burns, A.K. Sinha, N.N. Kahn, S.A. Morris, S.M. Factor, V.B. Hatcher, J.P. Bilezikian, S.G. Baum & M. Wittner: Enhanced platelet adherence and aggregation in Chagas´disease: a potential pathogenic mechanism for cardiomyopathy. *Am J Trop Med Hyg* 43, 274-281 (1990)

15. Morris S.A., L.M. Weiss, S.M. Factor, J.P. Bilezikian., H.B. Tanowitz & M. Wittner: Verapamil ameliorates clinical pathological and biochemical manifestations of experimental chagasic cardiomyopathy in mice. *J Am Coll Cardiol* 14, 782-789(1989)

16. Chandra M., J. Shirani, V. Shtutin, L.M. Weiss, S.M. Factor, S.B. Petkova, M. Rojkind, J.A. Dominguez-Rosales, L.A. Jelicks, M. Wittner & H.B. Tanowitz: Cardioprotective effects of Verapamil on myocardial structure and function in a murine model of chronic *Trypanosoma cruzi* infection (Brazil strain): an echocardiographic study. *Intl J Parasitol* 32, 207-215 (2002)

17. Tanowitz H.B., M. Wittner, B. Chen, H. Huang, L.M. Weiss, G.J. Christ, V.L. Braunstein, J.P. Bilezikian, S.A. Morris: Effects of Verapamil on acute Chagas' disease. *J Parasitol* 82, 814-819 (1996)

18. Tanowitz H.B., M. Wittner, S.M. Morris, W. Zhao, L.M. Weiss, V.B. Hatcher, V.L. Braunstein, S.A. Douglas, M. Valcic, M Spektor & G.J. Christ: The putative mechanistic basis for the modulatory role of endothelin-1 in the altered vascular tone induced by *Trypanosoma cruzi. Endothelium.* 63, 217-230 (1999)

19. Morris S.A., S. Barr, L.M. Weiss, H.B. Tanowitz, M. Wittner & J.P. Bilezikian: Myocardial Beta-adrenergic adenylate cyclase complex in a canine model of chagasic cardiomyopathy. *Circ Res* 69, 185-195 (1991)

20. Andrade Z.A., S.G. Andrade, M. Sadigursky, R.J. Wenthold Jr., S.L. Hilbert & V.J. Ferrans: The indeterminate phase of Chagas' disease: ultrastructural characterization of cardiac changes in the canine model. *Am J Trop Med Hyg* 57, 328-336 (1997)

21. Andrade Z.A: Immunopathology of Chagas' disease. *Mem Inst Oswaldo Cruz* 94 (Suppl 1), 71-80 (1999)

22. Yang X.P., Y.H. Liu, D. Mehta, M.A. Cavasin, E. Shesely, J. Xu, F. Liu & O.A. Carretero: Diminished Cardioprotective Response to Inhibition of Angiotensin-Converting Enzyme and Angiotensin II Type 1 Receptor in B2 Kinin Receptor Gene Knockout Mice. *Circ Res* 88, 1072-1079 (2001)

23. Scharfstein J., V. Schmitz, V. Morandi, M.M. Capella, A.P. Lima, A. Morrot, L. Juliano & W. Muller-Esterl: Host cell invasion by *Trypanosoma cruzi* is potentiated by

activation of bradykinin B_2 receptors. *J Exp Med* 192, 1289-1300 (2000)

24. Camargos E.R.S., C.R.S. Machado, A.L. Texeira Jr., L.L.V. Rocha, A.J. Ferreira, A.P. Almeida, M. Barton & M.M. Teixeira. Role of endothelin during experimental *Trypanosoma cruzi* infection in rats. *Clin Sci* 103 (Suppl 48), 64S-67S (2001)

25. Bhoola K.D., C.D. Figueroa & K. Worthy: Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev* 44, 1-80 (1992)

26. Erdös E.G., P.A. Deddish & B.M: Marcic: Potentiation of Bradykinin Actions by ACE Inhibitors. *Trends Endocrinol Metab* 10, 223-229 (1999)

27. Erdös E.G: Kinins, the ling march-A personal view. *Cardiovasc Res* 54, 485-491 (2002)

28. Figueroa C.D., A. Marchant, U. Novoa**,** U. Forstermann, K. Jarnagin, B. Scholkens & W Muller-Esterl: Differential distribution of bradykinin B2 receptors in the rat and human cardiovascular system. *Hypertension* 37, 110-120 (2001)

29. Fritz H., M. Jochum & W. Muller-Esterl: Kinins 1925-2000. *Biol Chem* 382, 3-6 (2001)

30. Marceau F. Kinin B1 receptors: a review. *Immunopharmaco.* 30, 1-26.8. (1995.

31. Herwald H., M. Morgelin, A. Olsen, M. Rhen, B. Dahlback, W. Muller-Esterl, & L. Bjorck: Activation of the contact-phase system on bacterial surfaces-a clue to serious complications in infectious disease.
Nat Med 4, 298-302 (1998).

32. Mattsson E, H. Herwald, H. Cramer, K. Persson, U. Sjobring & L. Bjork: *Staphylococcus aureus* induces release of bradykinin in human plasma. *Infect Immun* 69, 3877-3882 (2001)

33. Del Nery E., M.A. Juliano, A.P. Lima, J. Scharfstein & L. Juliano: Kininogenase activity by the major cysteinyl proteinase (cruzipain) from *Trypanosoma cruzi. J Biol Chem* 272, 25713-2578 (1997)

34. Cazzulo J.J, V. Stoka & V. Turk: Cruzipain, the major cysteine proteinase from the protozoan parasite *Trypanosoma cruzi. J Biol Chem* 378, 1-10 (1997)

35. Murta A.C., P.M. Persechini, T.S. Padron, W. De Souza, J.A. Guimaraes,& J. Scharfstein: Structural and functional identification of GP57/51 antigen of *Trypanosoma cruzi* as a cysteine proteinase. *Mol Biochem Parasitol* 43, 27-38 (1990)

36. Eakin A.E., A.A. Mills, G. Harth, J.H. McKerrow & C.S. Craik: the sequence, organization, and expression of the major cysteine protease (cruzain) from *Trypanosoma cruzi. J Biol Chem* 267, 7411-7420 (1992)

37. Lima AP.C.A., P.C. Almeida, I.L.S. Tersariol, V. Schmitz, A.H. Schmaier, L. Juliano, I.Y. Hirata, W. Muller-Esterl, J.R. Chagas, J. Scharfstein J: Heparan sulfate modulates kinin-release by *Trypanosoma cruzi* through the activity of cruzipain. *J Biol Chem* 277, 5875-5881 (2000)

38. Renne T., J. Dedio, G. David & W. Muller-Esterl: High molecular weight kininogen utilizes heparan sulfate proteoglycans for accumulation on endothelial cells. *J Biol Chem* 275, 33688-33696 (2000)

39. Svensjo E, F. Z. Cyrino, L. Juliano & J. Scharfstein: Plasma leakage induced in postcapillary venules by the major cysteine-proteinase from *Trypanosoma cruzi* and its modulation by H1-blocker mepyramin. *Microvasc Res* 54, 93-97 (1997)

40. Leavey, J.K. & R.L. Tarleton: Cutting edge: Dysfuntional $CD8^+$ T cells reside in nonlymphoid tissues during chronic *Trypanosoma cruzi* infection. *J Immunol* 170, 2264-2268 (2003)

41. Pesquero J.B., R.C. Araujo , P.A. Heppenstall, C.L. Stucky, J.A. Silva Jr, T. Walther, S.M. Oliveira, J.L. Pesquero, A.C. Paiva, J.B. Calixto, G.R. Lewin & M. Bader: Hypoalgesia and altered inflammatory responses in mice lacking kinin B1 receptors. *Proc Natl Acad Sci U S A* 97, 8140-8145 (2000)

42. Kedzierski R.M. & M. Yanagisawa: Endothelin System: The double-edged sword in health and disease. *Ann Rev Pharmacol Toxicol* 4, 851-876 (2001)

43. Miyauchi T. & T. Masaki: Pathophysiology of endothelin in the cardiovascular system. *Annu Rev Physiol* 61:391-415 (1999)

44. Korth P, R.M. Bohle, P. Corvol & F. Pinet: Cellular distribution of endothelin converting enzyme-1 in human tissues. *J Histochem Cytochem* 47,447-461 (1999)

45. Teder P. & P.W. Noble: A cytokine reborn? Endothelin-1 in pulmonary inflammation and fibrosis. *Am J Respir Cell Mol* 23, 7–10 (2000)

46. Suzuki, E., N. Daisuke, M. Kakoki, H. Hayakawa, A. Goto, M. Omata & Y. Hirata: Molecular mechanisms of endothelin-1–induced cell-cycle progression: involvement of extracellular signal-regulated kinase, protein kinase C, and phosphatidylinositol 3-kinase at distinct points. *Circ Res* 84, 611-619 (1999)

47. Murakoshi N., T. Miyauchi, Y. Kakinuma, T. Ohuchi, K. Goto, M. Yanagisawa & I. Yamaguchi: Vascular endothelin-B receptor system *in vivo* plays a favorable inhibitory role in vascular remodeling after injury revealed by endothelin-B receptor knockout mice. *Circulation* 106, 1991-1998 (2002)

48. Kurihara Y., H. Kurihara, H. Suzuki, T. Kodama, K. Maemura, R. Nagai, H. Oda, T. Kuwaki, W.H. Cao, N. Kamada. Elevated blood pressure and craniofacial

abnormalities in mice deficient in endothelin-1. *Nature* 368:703-710 (1994)

49. Clouthier D.E., K. Hosoda, J.A Richardson, S.C Williams, H. Yanagisawa, T. Kuwaki, M. Kumada, R.E. Hammer & M. Yanagisawa: Cranial and cardiac neural crest defects in endothelin-A receptor deficient mice. *Development* 125, 813-824 (1998)

50. Yanagisawa H., M. Yanagisawa, R.P. Kapur, J.A. Richardson, S.C. Williams, D.E. Clouthier, D. de Wit, N. Emoto & R.E. Hammer: Duel genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme gene. *Development* 125, 825-836 (1998)

51 Puffenberger E.G. K. Hosoda, S.S. Washington, K. Nakao, D. deWit, M. Yanagisawa & A. Chakravart: A missense mutation of the endothelin B-receptor gene in multigenic Hirschprung's disease. *Cell* 79, 1257-1266 (1994)

52. Berthiaume N., M. Yanagisawa & P. D'Orleans-Juste: Contribution of endogenous endothelin-1 and endothelin-A-receptors to the hypertensive state of endothelin-B heterozygous (+/-) knockout mice. *J Cardiovasc Pharmacol* 36, S72-74 (2000)

53. Wang X., S.A. Douglas, C. Louden, L.M. Vickery-Clark, G.Z. Feuerstein & E.H. Ohlstein: Expression of endothelin-1. Endothelin-2, endothelin-3, endothelin converting enzyme-1. Endothelin-A and endothelin B receptor mRNA following angioplasty-induced neoinitma formation in the rat. *Circ Res* 78, 322-328 (1996)

54. Krum H., A. Gu, M. Wilshire-Clement, J. Sackner-Bernstein, R. Goldsmith, N. Medina, L. Yushak, Miller & M. Packer: Changes in plasma endothelin-1 levels reflect clinical response to ß-blockade in chronic heart failure. *Am Heart J* 131: 337-341 (1996)

55. Wanecek M., E. Weitzberg, A.K. Alving, A. Rudehill & A. Oldner: Effects of the endothelin receptor antagonist bosentan on cardiac performance during porcine endotoxin shock. *Acta Anaesthesiol Scand* 45, 1262-1270 (2001)

56. Brauner J.S., L.E. Rohde & N. Clausell: Circulating endothelin-1 and tumor necrosis factor-a; early predictors of mortality in patients with septic shock. *Intensive Care Med* 26, 305-313 (2000)

57. Davi G., C. Giammarresi, S. Vigneri, A. Ganci, C. Ferri, L. Di Francesco, G. Vitale & S. Mansueto: Demonstration of Rickettsia conorii-induced coagulative and platelet activation *in vivo* in patients with Mediterranean spotted fever. *Thrombosis and Haemostasis* 74, 631–634 (1995)

58. Ono K., A. Matsumori, T. Shioi, Y. Furukawa, & S. Sasayama: Contribution of endothelin-1 to myocardial injury in a murine model of myocarditis: acute effects of bosentan, an endothelin receptor antagonist. *Circulation* 100, 1823–1829 (1999)

59. Wittner M., G.J. Christ, H. Huang, L.M. Weiss, V.B. Hatcher, S.A. Morris, G.A. Orr, J.W. Berman, G.A. Zeballos, S.A Douglas & H.B. Tanowitz: *Trypanosoma cruzi* induces endothelin release from endothelial cells. *J Inf Dis* 171, 493-497 (1995)

60. Huang H., S.B. Petkova, A.W. Cohen, B. Bouzahzah, J. Chan, J-N Zhou, S.M. Factor, L.M. Weiss, M. Krishnamachary, S. Mukherjee, M. Wittner, R.N. Kitsis, R.G. Pestell, M.P. Lisanti, C. Albanese & H.B. Tanowitz: Activation of Transcription factors (AP-1 and NF-κB) in Murine Chagasic Myocarditis. *Inf Immun* In Press

61. Huang H., M. Yanagisawa, Y.K. Kisanuki, L.A. Jelicks, M. Chandra, S.M. Factor, M. Wittner, L.M. Weiss, R.G. Pestell, V. Shtutin, J. Shirani & H.B. Tanowitz: The role of cardiac myocyte-derived endothelin-1 on Chagasic cardiomyopathy: molecular genetic evidence. *Clin Sci* (London) 103, (Suppl) 48, 263S-236S (2002)

62. Jelicks L.A., M. Chandra, J. Shirani, V. Shtutin, B. Tang, G.J. Christ, S.M. Factor, M. Wittner, H. Huang, L.M. Weiss, S. Mukherjee, B. Bouzahzah, S.B. Petkova, M.M. Teixeira, S.A. Douglas, M.L. Loredo, P. D'Orleans-Juste & H.B. Tanowitz: Cardioprotective effects of phosphoramidon on myocardial structure and function in murine Chagas' disease. *Int J Parasitol* 32, 1497-1506 (2002)

63. Salomone O.A., T.F. Caeiro, R.J. Madoery, M. Amuchastegui, M. Omelinauk, D. Juri, & J. Kaski: High plasma immunoreactive endothelin levels in patients with Chagas' cardiomyopathy. *Am J Cardiol* 87, 1217-1220 (2001)

64. Scharfstein J. Activation of bradykinin-receptors by *Trypanosoma cruzi*: a role for cruzipain in microvascular pathology". In; *Molecular Pathogenesis of Chagas´ Disease* (Kelly, J.M. ed.) Landes Bioscience, Austin, Texas, (2002). http://www.eurekah.com.

65. Marin-Neto J.A., P. Marzullo, C. Marcassa, L. Gallo Junior, B.C. Maciel, C.R. Bellina & A. L'Abbate: Myocardial perfusion abnormalities in chronic Chagas' disease as detected by thallium-201 scintigraphy. *Am J Cardiol* 69, 780-784

66. Holscher C., M. Mohrs, W.J. Da, G. Kohler, B. Ryffel, G.A. Schaub, H. Mossmann & F. Brombacher: Tumor necrosis factor alpha-mediated toxic shock in *Trypanosoma cruzi*-infected interleukin 10-deficient mice. *Infect Immun* 68, 4075-4083 (2000)

Key Words: Chagas' disease, Trypanosoma cruzi, Endothelin, Kinins, Bradykinin, Review

Send correspondence to: Herbert B. Tanowitz MD, Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx NY 10461, Tel: 718-430-3342, Fax: 718-430-8543, E-mail: tanowitz@aecom.yu.edu