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Endothelin-1 and its role in the pathogenesis of infectious diseases

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## ABSTRACT

Endothelins are potent regulators of vascular tone, which also have mitogenic, apoptotic, and immunomodulatory properties [1-3]. Three isoforms of endothelin have been identified to date, with endothelin-1 (ET-1) being the best studied. ET-1 is classically considered a potent vasoconstrictor. However, in addition to the effects of ET-1 on vascular smooth muscle cells, the peptide is increasingly recognized as a pro-inflammatory cytokine [4, 5]. ET-1 causes platelet aggregation and plays a role in the increased expression of leukocyte adhesion molecules, the synthesis of inflammatory mediators contributing to vascular dysfunction. High levels of ET-1 are found in alveolar macrophages, leukocytes [5] and fibroblasts [6]. Clinical and experimental data indicate that ET-1 is involved in the pathogenesis of sepsis [7, 8], viral and bacterial pneumonia [9, 10], *Rickettsia conorii* infections [11], Chagas disease [12, 13], and severe malaria [14-17]. In this minireview, we will discuss the role of endothelin in the pathogenesis of infectious processes.

## INTRODUCTION

Since its discovery, endothelin-1 (ET-1) has been shown to exhibit mitogenic properties and to regulate several physiologic functions, including salt and water homeostasis, vascular tone, and inflammation [18-20]. ET-1 is one of three known isoforms of endothelin, each encoded by a distinct peptide, but produced via a similar two-step metabolic pathway. Endothelins act through two seven transmembrane G-protein coupled receptors, endothelin receptor A (ET<sub>A</sub>) [21] and endothelin receptor B (ET<sub>B</sub>) [22, 23], to

exert their effects on physiological and pathological processes. ET-1 is the most abundant isoform *in vivo*. It is formed by conversion of pre-pro-ET-1 into the intermediate precursor big ET-1, which is then cleaved by the ET converting enzyme (ECE) to form the active 21-amino acid peptide. ET-1 is synthesized by a variety of cells including endothelial cells, macrophages, cardiomyocytes, and neurons (*table 1*) [24-38].

ET-1 is constitutively synthesized and acts in an autocrine and paracrine manner in tissues throughout the body; its physiological plasma concentration is ~1 pM [26]. Binding of ET-1 to the ET<sub>A</sub> receptor triggers an increase in intracellular concentrations of calcium, resulting in very potent vasoconstriction and smooth muscle contraction [20, 39]. ET-1 can also promote vasodilation by inducing the production of NO via its interaction with the ET<sub>B</sub> receptor on endothelial cells [20, 40]. Under normal physiological conditions ET-1 effects are controlled by these different mechanisms; however, abnormal activation of these cellular signaling pathways can play a role in the progression of disease.

#### The pro-inflammatory cytokine ET-1

Although typically regarded as a smooth muscle spasmogen, ET-1 has also been shown to participate in many inflammatory processes. While constitutively expressed throughout the body in healthy individuals, expression of ET-1 is increased with stress to the endothelium in response to cytokines, reactive oxygen species, angiotensin II, and thrombin [26]. High levels of ET-1 have been found in alveolar macrophages, leukocytes [5] and fibroblasts [41], and it has been demonstrated that TNF- $\alpha$  facilitates the release of ET-1 by endothelial and epithelial cells [42]. In addition, there is ample evidence which

suggests that ET-1 regulates leukocyte trafficking and cytokine production. Studies have shown that ET-1 stimulates monocyte production of IL-8 and MCP-1, known neutrophil and monocyte chemoattractants [43]. Moreover, ET-1 can act as a mast cell activator, and lead to degranulation and release of inflammatory cytokines such as TNF- $\alpha$  and IL-6 [44]. The mechanisms by which these cells enter targeted tissue are also under the control of the ET system. shRNA knockdown of different components of the ET system have demonstrated ET-1, ET<sub>B</sub> receptor, and ECE involvement in monocyte diapedesis [45]. Cellular adhesion molecules like ICAM-1, VCAM-1, and e-selectin facilitate the leukocyte recruitment, binding, and infiltration. Human brain microvascular endothelial cells exposed to ET-1 upregulate the expression of ICAM-1, VCAM-1, and e-selectin [46]. This cascade of ET-1 mediated inflammatory events potentiates inflammation and the subsequent trafficking of immunocompetent cells into injured tissue.

Increased levels of ET-1 in response to stress have been implicated in a variety of infectious processes [17, 47-49]. Clinical and experimental data indicate that ET-1 is involved in the pathogenesis of sepsis [8, 47, 50], viral and bacterial pneumonia [9, 10], *Rickettsia conorii* infections [11], Chagas disease [13, 48], and severe malaria [15, 17, 51, 52]. ET-1 is associated with vasospasms, vascular damage, blood brain barrier (BBB) permeability, cardiovascular remodeling, and inflammation [19, 20, 46, 53, 54]. Septic patients have increased ET-1 plasma concentrations, which correlate with renal dysfunction and disease severity [55]. Levels of cerebral spinal fluid (CSF) ET-1 are significantly elevated in individuals with bacterial meningitis, which is associated with abnormalities in cerebral blood flow (CBF) [49]. Additionally, ET-1 has been shown to

play a major role in the development of vascular disruption caused by infection. In this review we will summarize the role of the ET in infectious diseases.

## **CNS INFECTIONS**

The endothelin system is widely distributed throughout the central nervous system (CNS). Brain microvascular endothelial cells, neurons, and glial cells synthesize ET-1 and express receptors for the ligand [24, 56]. During pathological processes of the CNS, in addition to its vasoactive actions, upregulated ET-1 has been shown to cause an increase in BBB permeability, activate astrocytes, enhance cell adhesion molecule expression, as well as act as a neurotransmitter [46, 57-61]. Infections are most often associated with the release of pyrogens, which disrupt the function of the hypothalamus. In this regard, Fabricio et al have demonstrated that ET-1 both independently causes fever and participates in fever induced by LPS [62]. ET-1 is associated with several CNS disease processes, including Alzheimer's disease, multiple sclerosis, subarachnoid hemorrhage, stroke, and impairment in spatial learning and reference memory [53, 63-65]. This section will explore the role of ET-1 in bacterial meningitis, cerebral malaria and HIV-associated neurological disorders.

### **Bacterial Meningitis**

Bacterial meningitis is characterized by inflammation of the subarachnoid space, which can lead to focal ischemia and necrosis in the cortex. Pathogenesis of this disease follows invasion of the bloodstream by bacterial organisms, which can develop from hematogenous spread or by direct extension of infection from a contiguous site [66, 67]. Despite current treatment, bacterial meningitis has a mortality rate of up to 30%, and

approximately 33% of survivors sustain residual neurological deficits [68-70]. Infection can cause tissue around the brain to swell, disrupting cerebral blood flow (CBF) and result in ischemia or even stroke and paralysis. Unfavorable outcomes of bacterial meningitis are usually due to cerebrovascular complications that develop during the acute phases of disease [71].

During bacterial meningitis, bacterial organisms penetrate the BBB either by transcellular, paracellular, or Trojan horse mechanisms (via infected phagocytes) [71]. Once in the brain, bacteria and bacterial products activate glial cells and vascular endothelial cells which go on to produce cytokines like TNF $\alpha$  and IL-1[66]. Release of these pro-inflammatory cytokines activates adhesion promoting receptors on the brain endothelia, which bind and recruit immune cells to the site of insult. In the brain, infiltrated leukocytes release a variety of proteolytic products and toxins permeating the BBB and further potentiating inflammation and injury to the vascular endothelium [66]. Interestingly, CSF levels of ET are elevated in patients with bacterial meningitis, where it exerts both vasoactive and pro-inflammatory activity [49]. Animal models have been very useful in elucidating the mechanism by which ET mediates the CNS pathology during bacterial meningitis.

Rats challenged with heat killed *Streptococcus pneumoniae* (HKP) developed meningitis approximately 18h post infection [72]. Infected animals demonstrated increased CBF, intracranial pressure, brain water content and CSF white blood cell count [73]. ET was found to play an important role in these cerebrovascular changes via stimulation of the ET<sub>B</sub> receptor. Infected animals pretreated with an ET<sub>B</sub> receptor antagonist, BQ-788, displayed significant reduction in CBF, intracranial pressure, brain

water content and CSF pleocytosis [73]. Animals with bacterial meningitis exhibited extensive damage in the cortex and dentate gyrus [72]. However, HKP challenged rats treated with the non-selective ET receptor antagonist, bosentan, displayed reduced cortical brain injury and CBF was restored to that of the uninfected controls [74]. This highlights a role for ET-1 in neuronal damage in bacterial meningitis.

The trafficking of immune cells into the brain are key mediators in bacterial meningitis. Monocyte diapedesis involves different components of the ET system. ET-1, ET<sub>B</sub> receptor, and ECE contribute to this process and inflammation at the BBB [45]. Interestingly, ET-1 is known to induce the production of inflammatory cytokines like MCP-1 and IL-8, which are elevated in bacterial meningitis [75-77]. Thus, it is probable that ET-1 enhances white blood cell infiltration into the CSF by inducing chemokine production. The combination of BBB disruption and infiltration of immune cells leads to inflammation, unbalanced brain homeostasis and neuronal damage.

Though CSF ET-1 levels are increased in both experimental animal models and human cases, the cells responsible for this increased production remain a mystery. Experiments with various cells of the cerebrovasculature exposed to HKP have been used to create an *in vitro* setting of bacterial meningitis. In this regard, *in vitro* studies using rat astrocyte cultures challenged with HKP induced a three-fold increase in ET levels [49]. Treatment with phosphoramidon, an ECE inhibitor, prevented the increase in ET, suggesting astrocytes as a possible source of ET production during infection [49]. Brain microvascular endothelial cells are another source of increased ET-1 production during bacterial meningitis. Brain endothelial cells stimulated with HKP result in an increase in



both NO and ET-1 release, from activation of ET<sub>B</sub> receptors, supporting the hypothesis that alterations in the cerebrovasculature may be due to ET<sub>B</sub> mediated release of NO [73].

ET-1 plays a pivotal role in the host response during the acute phase of bacterial meningitis. An imbalance in ET production causes abnormalities in CBF, CSF white blood cell count, brain water content, and cortical injuries. Studies using ET receptor antagonists have provided data corroborating that these abnormalities may be due to ET<sub>B</sub> receptor mediated production in NO, a key player in pneumococcal meningitis [78]. Though, there are still gaps in determining the precise cells responsible for the increased production of ET during infection, astrocytes and/or endothelial cells are likely candidates.

### Cerebral Malaria

Malaria is a potentially life threatening disease with approximately 1 million deaths annually. Half of the world's population is at risk of acquiring malaria. Cerebral malaria (CM) is the most severe and potentially fatal neurological complication with *Plasmodium* infection. Children younger than 5 years old are the most susceptible victims of CM and account for 90% of the malaria related deaths each year [79]. Despite extensive research CM has a mortality rate of 20%, and approximately 25% of survivors develop long-term neurological deficits. These long-term deficits have created a social and economical burden in malaria-endemic countries [79, 80].

CM is characterized by the binding of infected red blood cells to the brain endothelium, ischemia, inflammation, BBB disruption, impaired perfusion, and cognitive and motor dysfunction [81-89]. The study of human CM is restricted to post-mortem

histological analysis; however animal models have contributed immensely to a better understanding of the disease. Experimental CM such as the *Plasmodium berghei* ANKA (PbA) model has demonstrated similar pathological and behavior changes as in the human disease [90, 91]. As with humans, experimental models demonstrate persistent neurocognitive deficits even after antimalarial treatment [89].

The role of ET-1 during CM is not completely understood. It has been shown that big ET-1 and ET-1 are increased in the plasma of *P. falciparum* infected patients [17, 51, 92]. Moreover, experimental CM displayed significant increases in ET-1 mRNA expression in the brain correlating to human findings [15]. This increase in the ET system was associated with glial activation, neuronal damage, and a reduction in CBF [15, 84]. Interestingly, ET-1 administration in the left middle cerebral artery in rats results in a dose dependent reduction in CBF and in ischemic brain damage [93]. In addition, intraventricular injection of ET-1 results in behavioral changes, including barrel rolling, body tilting, nystagmus, facial clonus, forelimb clonus, and tail extension in rats, even at doses that do not cause any changes in CBF [94]. By virtue of its vasoactive effects and adverse neurological effects, increased levels of ET-1 during CM may be partly responsible for the reduced CBF, vasospasms, and vascular collapse observed in human cases and animal models of CM [86], and for the cognitive and motor deficits observed in CM [88, 89, 95].

CM is associated with vascular damage, vasospasms, and changes in levels of vasoregulatory molecules such as ET-1 [86]. In response to stress ET-1 is upregulated, consequently increasing the expression of cellular adhesion molecules including ICAM-1 and LAM-1 and inflammatory cytokines like TNF $\alpha$  and IL-1 [19, 58]. Conversely, TNF $\alpha$

is known to enhance the expression of ET-1. TNF $\alpha$  is a key player in CM pathology, and elevated levels are found in the brain and plasma of patients with CM [51, 96]. Focal injections of TNF $\alpha$  induce a dose-dependent reduction in cerebral blood volume, thought to be ET-1 mediated, as this reduction in CBF was reversible after treatment with endothelin receptor blockers [42]. The abnormal increase in cellular adhesion molecules in response to ET-1 suggests that the peptide may be involved in adhesion of infected red blood cells, leukocytes, and platelets to the brain endothelium, potentiating the vascular obstruction observed in CM.

Recent murine CM studies revealed that pharmacologic blockade of ET<sub>A</sub> receptors decreased the incidence of hemorrhages in the brain and seemed to increase survival when used in conjunction with artemether (an anti-malarial artemisinin derivative) in the treatment of experimental CM [52]. These results suggest that ET-1 might cause cerebrovascular disruption via ET<sub>A</sub> receptor activation during CM. It is possible that ET-1-mediated BBB disruption during CM plays an important role in the brain hemorrhage observed during the disease. Blocking the ET<sub>A</sub> receptor may provide vascular and subsequent neuronal protection during treatment of CM.

It is likely that ET-1 contributes to the deleterious effects of CM via abnormal regulation of mitogen-activated protein kinase (MAPK) signaling. ET-1 activates various MAPK families including ERK, JNK, and p38, all of which cause induction of pro-inflammatory and apoptotic genes. Stimulation of the stress activated protein kinase JNK has been shown to contribute to inflammation, neuronal cell death, and survivability during experimental CM [97, 98]. The JNK/c-Jun signaling pathway also participates in astrocyte proliferation and reactive gliosis in an ET-1 dependent manner [99]. Lu *et al*

using JNK2  $-/-$  deficient mice demonstrated that JNK2 controls cytokine production and development of experimental CM [100]. ET<sub>A</sub> receptor blockade may attenuate the inflammation and cellular death attributed to JNK signaling in experimental CM [20].

Although present data indicate that ET-1 is involved in the pathogenesis of CM, the precise mechanisms by which this molecule acts to induce or prevent physiological alterations which precede neurologic signs during malaria infections remain unknown. Studies to determine the cells responsible for the increased levels of ET-1 and the cells which are the main targets of its action during malaria infection are needed, as are examinations of ET-2 and ET-3 to determine their role during infection. ET-3 is of particular interest as it is produced in the brain by endothelial cells and astrocytes and has been shown to cause brain ischemia and inflammation [101-103].

### HIV Encephalitis

HIV-associated neurological disorders, known as HAND, are a spectrum of neurological disorders associated with HIV infection of the CNS [104]. These classifications of HAND range from asymptomatic neurocognitive impairment to severe HIV associated dementia [104]. Despite dramatic advances in antiretroviral therapy, aging of HIV+ patients and the inability of drugs to enter the CNS HAND occurs in 40-60% of HIV infected individuals [105].

Early after peripheral infection, HIV enters the brain [106]. Monocytes carrying virus transmigrate across the BBB into the CNS [107, 108]. Once in the brain infected monocytes accumulate in the CNS parenchyma releasing virus and viral proteins.

Resident microglia, macrophages, and a small population of astrocytes become infected

with virus [109]. Infection of these cells leads to further release of virus, viral proteins, and the secretion of neurotoxins.

Monocytes/macrophages are crucial in the pathogenesis of HIV. They are the source of viral replication, CNS entry, and cytokine production [110-113]. As ET-1 is key in monocyte diapedesis across the BBB, it is interesting to note that cerebral macrophages in patients with HIV encephalopathy are strongly positive for ET-1 [114]. ET-1 may facilitate monocyte transmigration into the brain, a step which is critical to the neuropathology of HIV. Increased levels of ET-1 have been observed in the CSF of HIV+ patients with encephalopathy [115]. These concentrations of ET-1 correlated with the degree of HIV encephalopathy corroborating Ehrenreich and colleagues' findings [114].

ET-1 levels are also significantly elevated, in an *in vitro* human BBB model, when brain endothelial cells and astrocytes are exposed to HIV-1 or gp120 [116]. Tat, an HIV viral protein, enhances ET-1 production in astrocytes, implicating astrocytes as a potential source of increased ET-1 synthesis during infection [117]. Post-mortem analysis demonstrates increased ET-1 immunoreactivity in neuroglia cells of HIV infected individuals [117]. ET-1 staining is prominent in the frontal lobe and basal ganglia in the brains of HIV infected patients, in a pattern analogous to Alzheimer's disease [118]. Similar to Alzheimer's disease, increased amyloid plaques have been reported in the cortex of AIDS patients [119]. Accumulation of amyloid beta has also been observed in HIV encephalitic brains [120]. A recent study treated animals with amyloid beta, which increased the expression of the ET system, oxidative stress, and induced cognitive impairments [121]. However, treatment with a selective ET<sub>A</sub> receptor antagonist

ameliorated these changes. These findings may support the ET system as a potential target for cognitive protection in HAND.

## **ENDOTHELIN AND PNEUMONIA**

ET-1 is a potent vasoconstrictor and in the endothelium its synthesis is induced by hypoxia and pulmonary infection [122]. In the infected lung ET-1 may be synthesized by a variety of cell types including bronchial epithelial, smooth muscle cells, endothelial cells, and inflammatory cells such as monocytes and macrophages.

Community-acquired pneumonia (CAP) is an important cause of morbidity and mortality world-wide and the precise microbial etiology of an episode of CAP is only made in a minority of cases. There has been a continuing effort to examine potential biomarkers that could predict outcome in CAP. The white blood cell count, C-reactive protein and procalcitonin have been used to predict severity and outcome [123]. In recent years there has also been a focus on the possibility that ET-1 or its precursors could be employed as biomarkers to aid in the diagnosis and predict outcomes in CAP, especially those due to bacterial etiologies. In this regard, it has long been recognized that many diseases of the lung diseases are associated with an increase in ET-1. For example, in addition to pneumonia, pulmonary hypertension, interstitial fibrosis and acute respiratory distress syndrome (ARDS) have been associated with increased expression of ET-1 [124, 125].

Scheutz et al [9] in a study of 281 consecutive patients with CAP demonstrated that levels of the ET-1 precursor peptide, proET-1 were significantly increased. The

levels of proET-1 on admission were increased in patients with adverse outcomes including the need for ICU admission and death and correlated well with other laboratory and clinical criteria. Using a multivariate logistic regression model, only proET-1 and the clinical severity scores were independent predictors for death and the need for admission to the ICU. ProET-1 was a superior prognostic tool to predict disease severity. In a study of an additional 728 patients with CAP proET-1 levels were found to be among other biomarkers to be strong predictors of mortality at 28 and 180 days [126].

In addition to the endothelin peptide, proET-1 as a biomarker, endothelin has also been considered to contribute to the pathogenesis of pulmonary infection. Since endothelins are activated during lung injury and endothelin blockers reduce the inflammation it seems reasonable that endothelins participate in the pathogenesis of lung injury [124, 125, 127]. Experimental evidence suggests that this indeed, might be the case. For example, Gamze et al [128] reported that the ET<sub>A</sub>/ET<sub>B</sub> blocker bosentan significantly reduced the concentration of pro-inflammatory cytokines in a rat model of emphysema. More recently, Trabold et al [129] reported that bosentan reduced the oxidative burst in the lung in an acid-aspiration rat model. These limited studies suggest the possibility that either endothelin receptor blockers or inhibition of the endothelin pathway might serve as adjunctive therapy of severe pneumonia. This is an area of research that deserves examination.

## **SEPSIS AND ENDOTHELIN**

Despite the advances in our understanding of the mechanisms and therapeutic strategies in managing this disease complex, bacterial sepsis and septic shock continue to be important contributors to morbidity and death in the industrialized world, especially in the hospital setting, [130]. The administration of antibiotics within the early hours of the recognition with broad-spectrum antibiotics is a "goal" of therapy and is considered good medical practice. Nevertheless, even when antibiotics are administered early and the offending bacteria eradicated, the pro-inflammatory cascade initiated may remain unfettered long after the eradication of the organism [131].

It is now appreciated that the etiology of bacterial sepsis and the ensuing septic shock is multi-factorial and that the associated multi-organ failure is usually attributed to profound vasodilation and pooling of blood secondary to increased production of nitric oxide. However, end-organ failure may also be the result of vasoconstriction such as that afforded by the increased synthesis of ET-1.

Soon after the discovery of endothelin by Yanagisawa in 1988 [31] it was implicated in the pathogenesis of sepsis [132, 133]. Indeed, blood levels of ET-1 are increased in the setting of sepsis and may serve as a biomarker of severity. However, as with the case in pneumonia, ET-1 may also contribute to the pathogenesis of sepsis and septic shock. It is beyond this paper to discuss the mechanism of sepsis but rather to focus on the role of endothelin. Bacterial toxins such as lipopolysaccharide (LPS) of Gram-negative bacteria and lipoteichoic acid (LTA) from Gram positive bacteria act on macrophages to synthesize pro-inflammatory cytokines (TNF- $\alpha$  and IL-1- $\beta$ ). These cytokines mediate the production of ET-1 from various sources such as endothelial cells, cardiac myocytes and cardiac fibroblasts. However, there is a consensus that the increase



in plasma levels of ET-1 in the setting of sepsis is mainly due to endothelial cell damage. The plasma levels of ET-1 in the setting of severe sepsis in adults and neonates are significantly increased and correlate with renal and myocardial dysfunction [50, 55, 133-135]. The ET-1 levels correlate with Sepsis-related Organ Failure Assessment (SOFA) scores and correlate with other parameters of severe sepsis such as C-reactive protein, procalcitonin and natriuretic propeptide (NT-proBNP) [55]. Brauner et al [136] demonstrated that increased levels of TNF- $\alpha$  were higher at all time-points in non-survivors with septic shock while ET-1 levels were an early predictor of mortality. Together measurement of both may identify patients at a higher risk for adverse outcome.

Since ET-1 is a locally active peptide it is unclear if plasma levels accurately reflect its role in pathogenesis. Therefore, in order to fully evaluate the role of ET-1 in the pathogenesis of bacterial sepsis animal models are employed. The early studies centered on the administration of anti-endothelin antibodies in rat models of bacterial sepsis. For example, in a rat model of endotoxin-associated shock, anti-ET-1 antibody improved renal function [137]. Additionally, anti-ET anti-sera improved blood flow in a rat model of *E. coli* bacteremia [138]. There was a reduction in LPS-induced increase in coronary vascular resistance as a result of treatment with a specific ET<sub>A</sub> receptor blocker, F139317 [139]. Similarly, the ET<sub>A</sub>/ET<sub>B</sub> receptor blocker restored systemic and gut oxygen delivery in a porcine model of endotoxin shock [140] while administration of BQ123, a selective ET<sub>A</sub> blocker attenuated endothelin-induced contraction of pulmonary artery rings from endotoxemic rats [141]. The cecal-ligation and puncture model of septic shock is widely used to mimic septic shock in humans. In this mouse model, bosentan improved survival [142]. In a more recent study blockade of the ET<sub>B</sub> receptor in the LPS-induced

endotoxemic mouse improved blood hemodynamics whereas blockade of the ET<sub>A</sub> receptor caused a deterioration of the blood hemodynamics [143]. Interestingly, in an animal model of LPS-induced shock inhibition of metalloendopeptidases by phosphoramidon reduced the synthesis of ET-1 in heart presumably by inhibition of endothelin converting enzyme, but also down-regulated myocardial protein expression of iNOS and p38-MAPK phosphorylation [144]. It is clear that ET-1 contributes to the pathogenesis of sepsis. However, whether the observations that the inhibition of the endothelin pathway can lead to effective therapy in humans is unclear at this time [145].

## CHAGAS DISEASE AND ENDOTHELIN

Chagas disease is a neglected tropical disease that is a life-long persistent infection. It is caused by the protozoan parasite, *Trypanosoma cruzi*, and is a major cause of morbidity and mortality in endemic areas of Latin America stretching from the Texas-Mexican border to almost the tip of the South American continent. Due to increased immigration to the non-endemic areas of North America, Europe, Asia and Australia this disease can no longer be considered an esoteric disease or a medical curiosity confined only to isolated rural areas of Latin America [146]. It has been estimated that there may be 300,000 individuals in the United States that are seropositive [147]. Up to 30% of seropositive individuals ultimately develop clinically relevant Chagas disease. Chagas disease is now being diagnosed with increasing frequency among immigrant populations due in part to the mandatory screening of potential blood donors as well as random screening of immigrants from endemic areas that are hospitalized for a variety of

diseases. In Barcelona, Spain there is a prevalence of approximately 16% of Chagas disease among the Bolivian immigrant population [148]. There are significant clinical manifestations in the seropositive groups in Spain [149].

The most important manifestations of Chagas disease are cardiomyopathy and megasyndromes of the gastrointestinal tract. Infection results in an upregulation of the pro-inflammatory pathway associated with upregulation of pro-inflammatory cytokines, chemokines, Toll-like receptors (TLRs), components of the mitogen-activated protein (MAP) kinase pathway, ET-1 [48] and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) [150, 151]. The intense inflammatory response of the host to this parasite is a double-edged sword. Although required for killing of the parasite, the increase in inflammation damages the host tissues. In the heart, inflammation is replaced by fibrosis leading to cardiac remodeling, arrhythmias, congestive heart failure and stroke. In the gastrointestinal tract infection results in alterations in structure and function. In the setting of immune suppression there may be a reactivation of this chronic infection resulting in a recurrent acute myocarditis, encephalitis and other syndromes [152, 153]. The parasite lives in many cell types notably cells of the cardiovascular, reticulo-endothelial and autonomic nervous systems as well as cells that comprise the adipose tissue.

Chagas disease also has a vascular component. Microvascular lesions in Chagasic heart disease were described in detail by Rossi et al. [154]. At that time there was a new appreciation that microvascular compromise was an important contributing factor in the pathogenesis of experimental and human cardiomyopathies of diverse etiologies and that treatment with verapamil improved the coronary blood flow and outcome. In that regard, Factor et al [155], employing a mouse model of acute *T. cruzi* infection, demonstrated

vasospasm and saccular aneurysms in the subendocardial microvasculature in this murine model of Chagas disease similar to that described in other cardiomyopathies. They suggested that these alterations might contribute to the development of the typical dilated cardiomyopathy observed in chronic Chagasic cardiomyopathy. Subsequently, it was demonstrated that *T. cruzi* infection caused a reduction in blood flow in the microvascular bed which could be reversed by treatment with verapamil [156]. The early, but not late administration of verapamil ameliorated Chagasic cardiomyopathy in a murine model [157]. This is consistent with the effect of verapamil being due to an amelioration of *T. cruzi*-induced microvascular spasm by increasing coronary blood flow thereby preventing myocardial dysfunction. *T. cruzi*-associated microvascular spasm probably involves the vasoconstrictor agents ET-1 and TXA<sub>2</sub>. Importantly, ET-1 and TXA<sub>2</sub> share several important properties; they both enhance platelet aggregation, inflammation and vasoconstriction, all of which have been associated with *T. cruzi* infection.

In the 1990s studies linked ET-1 to the vasculopathy of Chagas disease [13, 48, 158-161]. Wittner et al [162] and Tanowitz et al [160] demonstrated that *T. cruzi* infection of cultured endothelial cells resulted in an increased synthesis of biologically active ET-1 when tested in the setting of isolated aortic rings. The addition of verapamil ameliorated the action of ET-1 likely at a post endothelin receptor site [160]. Petkova et al [13] subsequently reported that *T. cruzi*-infected mice displayed an increase in plasma ET-1 levels and that there was increased expression of ET-1 in the vasculature of infected mice. This was accompanied by an increased expression of endothelin converting enzyme and of preproET-1 in the myocardium. An increased level of ET-1 was observed in the carotid arteries during experimental *T. cruzi* infection [163]. Overall this suggests

that ET-1 participates in the vasculature changes during this infection [163]. Of note, chronic Chagasic patients with cardiomyopathy display increased plasma levels of ET-1 but since individuals with cardiomyopathy of various etiologies also have increased levels of plasma ET-1, the specificity of this observation for Chagas disease is unclear [164].

In the cardiovascular system in addition to endothelial cells several other cell types, i.e. cardiomyocytes, fibroblasts and macrophages, also synthesize ET-1 [165]. Locally produced ET-1 increases smooth muscle contractility and induces cardiomyocyte hypertrophy and injury [26, 166, 167]. In various disease states, increased ET-1 levels may reflect the degree of endothelial and myocardial cell damage. An induction of the expression of vascular adhesion molecules and proinflammatory cytokines was demonstrated to occur in cultured endothelial cells as a result of *T. cruzi* infection in an NF- $\kappa$ B pathway-dependent manner [168]. Infection also resulted in the activation of ERK and AP-1 [169], both of which are important in the activation of ET-1 and enhanced synthesis of ET-1 [162]. It is important to note that ET-1 regulates ERK and that both ET-1 and ERK regulate cyclin D1, which in turn mediates smooth muscle cell proliferation [169, 170] and likely cardiomyocyte hypertrophy [171]. Furthermore, ET-1 induces myocardial fibrosis by enhancing collagen deposition [169, 172] and such collagen deposition is observed in Chagasic cardiomyopathy. Therefore, the increased expression of ET-1 in *T. cruzi* infection results in both focal vasospasm and the myocardial pathology seen in Chagas disease [173-175].

The cytokines produced in the myocardium of *T. cruzi*-infected animals and humans such as IL-1 $\beta$ , TNF- $\alpha$  and IFN- $\gamma$ , are potent inducers of ET-1 [48, 176, 177]. IL-

IL-1 $\beta$  was demonstrated to induce cardiomyocyte hypertrophy during *T. cruzi* infection *in vitro* [178], and infected endothelial cells produce high levels of IL-1 $\beta$  [179]. Thus, in endothelial cells ET-1 may be stimulated by cytokines produced in adjacent smooth muscle cells and ET-1, in turn, may stimulate cytokine production and the expression of vascular adhesion molecules. IL-1 $\beta$  is probably important in the initiation of cardiomyocyte hypertrophy and ET-1 contributes to the pathology of infection at a later time point of infection. ET-1 induces vasospasm which results in focal myocardial ischemia followed by myonecrosis, which increases ET-1 production that results in cardiomyocyte hypertrophy and enhanced fibrosis [160]. *T. cruzi*-infected cultured endothelial cells display increased levels of biologically active ET-1 that results in the up-regulation of the pro-inflammatory pathway, vascular injury and cardiac remodeling [180]. ET-1 causes rat cardiomyocytes hypertrophy through a calcineurin/nuclear factor of activated T cells (NFAT)-dependent mechanism [181, 182]. Different signaling pathways triggered by ET-1 are associated with increasing intracellular calcium levels and ERK1/2 activation leading to enhanced levels of inflammatory mediators that contribute to Chagasic cardiomyopathy [163, 174].

To more directly ascertain the role of ET-1 in the development of *T. cruzi*-induced cardiomyopathy, Tanowitz et al [160], infected mice in which the ET-1 gene was deleted from cardiomyocytes and mice in which the ET-1 gene was deleted from endothelial cells. Mice were evaluated 130 days post infection. Inflammation and fibrosis were observed in all infected mice; however, fibrosis was reduced in mice in which the ET-1 gene was deleted from cardiomyocytes. Cardiac magnetic resonance imaging revealed that infection resulted in a significant increase in right ventricular internal diameter

except in mice devoid of the ET-1 gene in cardiomyocytes. Echocardiography of the left ventricle demonstrated increased left ventricular end-diastolic diameter, reduced fractional shortening, and decreased relative wall thickness in all of the infected mice. The magnitude, however, of the changes was significantly less in mice in which the ET-1 gene was deleted from cardiomyocytes. Treatment of *T. cruzi*-infected mice with phosphoramidon resulted in reduction of right ventricular enlargement caused by the infection [160]. Collectively, these observations provide direct evidence to implicate ET-1 as a major contributing factor in the pathogenesis of Chagasic cardiovascular disease.

In recent years there has been increased interest in the role of eicosanoids in the pathogenesis of Chagas disease [183]. There has also been an examination examining a link between ET-1 and eicosanoids in the development of Chagasic cardiomyopathy. Cyclooxygenase-2 (COX-2), which catalyzes the rate-limiting step in prostanoid biosynthesis expression, is induced by ET-1 [184-186]. This induction requires activation of the  $\text{Ca}^{2+}$ /Cn/NFAT pathway, the last translocated to the nucleus upon stimulation with the peptide and subsequent infection where it binds to NFAT response elements in the promoter region of COX-2 that are essential for transcriptional induction of the gene [187]. Cardiomyocytes pre-treated with ET-1 followed by infection with *T. cruzi*, displayed enhanced production of eicosanoids and atrial natriuretic peptide (ANP), suggesting the participation of NFAT in the ET-1 stimulated *T. cruzi*-mediated induction of mediators involved in the pathogenesis of chronic Chagas heart disease [187]. The finding that plasma levels of ET-1 are increased both in Chagasic patients and mice [13, 164] leads to the speculation that ET-1-driven vasospasm causes myocardial ischemia and myonecrosis [160].

Another factor involved in the pathophysiology of Chagas disease is plasma leakage, a neutrophil-driven inflammatory response induced by *T. cruzi* via the kinin/ET pathways. This mechanism can enhance parasite invasion of cardiovascular cells [188, 189]. TLR2, the B2 kinin and CXCR2 receptors trigger intracellular inflammatory pathways induced by *T. cruzi* and directly interact with endogenous ET-1 [188, 189]. Andrade et al demonstrated that HOE-140, a B2 kinin receptor (BK2R) antagonist can reduce the ET-1-induced increase in *T. cruzi* invasion seen in human smooth muscle cells in vitro [188, 189]. An interaction between the kinin and endothelin pathways at the early stages of *T. cruzi* infection has therefore been postulated [188]. Using antagonists of ET<sub>A</sub> or ET<sub>B</sub> receptors and/or the BK2R antagonist (HOE-140) prior to *T. cruzi* infection there was a reduction in leukocyte accumulation in microvascular beds. In addition, these endothelin receptor antagonists reduced plasma leakage in the hamster cheek pouch and inhibited the inflammatory edema in *T. cruzi*-infected mice. These studies indicate that endothelin and bradykinin receptors-induced inflammatory cascade are triggered via TLR2/CXCR2 through the proteolytic activation of the kallikrein-kinin-system [190]. Taken together, these studies, demonstrate that the inflammatory cascade pathways that participate in cardiovascular remodeling during *T. cruzi* infection are both complex and interactive.

## CONCLUDING REMARKS

The role of endothelins in the pathogenesis of infectious diseases has been an important field of study almost immediately since the discovery of the peptide. Though ET-1 is implicated in the pathogenesis of several processes with infectious diseases, there is no unifying hypothesis as to how ET-1 effects its actions, rendering the precise elucidation



of this role difficult; particularly since the signaling pathways involved are complex, interrelated and redundant. Several of these disease processes are accompanied by neurological sequelae which appear to be in part mediated by ET-1, and further study is needed to determine both the cells responsible for the increased synthesis of ET-1 and the cells which are the main targets of ET-1 effects. ET-1 is able to affect several host responses upon activation of its receptors, and targeting these receptors might be useful as adjunctive therapy for preservation of host organ function during disease caused by infectious organisms.

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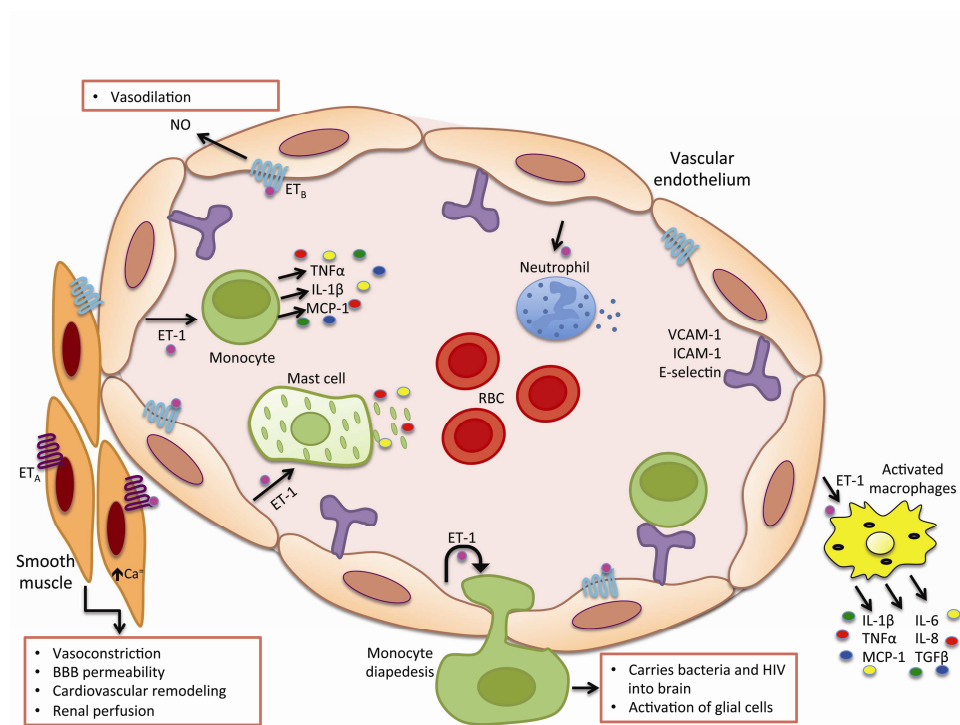
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## LEGENDS

**Table 1.** Expression pattern of the endothelin system. ET-1, endothelin-1; ET<sub>A</sub>, endothelin type A receptor; ET<sub>B</sub>, endothelin type B receptor.

**Figure 1. A schematic illustration of ET-1 effects on different cell types.** Binding of ET-1 to its cognate receptors causes activation of monocytes, neutrophils, mast cells, and endothelial cells. ET-1 contributes to cytokine production, enhanced cellular adhesion molecule expression, as well as monocyte diapedesis. Additionally, ET receptor activation on vascular smooth muscle cells results in vasoconstriction, vascular permeability, and tissue remodeling.



**Figure 1**

Table 1

Cell Type	ET-1	ET <sub>A</sub>	ET <sub>B</sub>	References
<b>Adipocytes</b>		+	+	[10]
<b>Astrocytes</b>	+		+	[11-12]
<b>Cardiomyocytes</b>	+	+	+	[13]
<b>Endothelial cells</b>	+		+	[14]
<b>Hepatocytes</b>	+	+	+	[15]
<b>Keratinocytes</b>	+	+	+	[16]
<b>Mast cells</b>	+	+	+	[17-18]
<b>Neurons</b>	+	+	+	[19-20]
<b>Smooth muscle cells</b>		+	+	[21]