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Role of Endothelin 1 in the Pathogenesis of Chronic Chagasic Heart Disease

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On the basis of previous observations, endothelin 1 (ET-1) has been suggested as contributing to the pathogenesis of Chagasic cardiomyopathy. Therefore, ET-1$^{flox/flox}$;α-MHC-Cre(+) mice in which the ET-1 gene was deleted from cardiac myocytes and ET-1$^{flox/flox}$;Tie 2 Cre(+) mice in which the ET-1 gene was deleted from endothelial cells were infected with *Trypanosoma cruzi*. Genetic controls for these cell-specific ET-1 knockout mice were used. Ninety percentage of all mice survived acute infection with the Brazil strain and were evaluated 130 days postinfection. Inflammation and fibrosis were observed in all infected mice; however, fibrosis was reduced in ET-1$^{flox/flox}$;α-MHC-Cre(+) mice. Cardiac magnetic resonance imaging revealed that infection resulted in a significant increase in right ventricular internal diameter (RVID) in all mice except ET-1$^{flox/flox}$; α-MHC-Cre(+) mice; i.e., RVID was not changed in infected ET-1$^{flox/flox}$;α-MHC-Cre(+) mice. Echocardiography of the left ventricle demonstrated increased left ventricular end-diastolic diameter, reduced fractional shortening, and decreased relative wall thickness in infected mice. However, the magnitude of the changes was significantly less in ET-1$^{flox/flox}$;α-MHC-Cre(+) mice compared to other groups. These data provide further evidence of a role for ET-1, particularly cardiac myocyte-derived ET-1, in the pathogenesis of chronic Chagasic cardiomyopathy.

Chagas' disease caused by infection with the protozoan parasite, *Trypanosoma cruzi*, is a major cause of acute myocarditis and chronic cardiomyopathy in areas of endemicity in Latin America (51). Chronic Chagasic cardiomyopathy has also been reported among immigrants in North America and Europe (21) and is found as an opportunistic infection in immunosuppressed individuals, including those with human immunodeficiency virus infection/AIDS (54).

Chronic Chagasic heart disease manifests itself as a dilated cardiomyopathy. The precise etiology of Chagasic cardiomyopathy is not yet entirely understood, but it is generally believed to be multifactorial. Parasite persistence (53), autoimmunity (26) and vascular compromise (49) have all been suggested as possible contributory factors. Limited studies in individuals with Chagas' disease and in experimental animals have demonstrated that injury to the vascular endothelium is associated with an increase in inflammatory markers (11, 32, 50), vasospasm, and a reduction in blood flow (49).

Endothelin 1 (ET-1) is a 21-amino-acid vasoactive peptide synthesized by many cell types. In the normal, uninjured vasculature, endothelial cells synthesize endothelin. ET-1 interacts with the ET_A receptor on the underlying smooth muscle cell, causing an increase in intracellular calcium leading to vessel constriction. ET-1 can also interact with the endothelial cell ET_B receptor in an autocrine or paracrine fashion, causing an increase in cyclic GMP, which diffuses, to the underlying smooth muscle cell layer leading to vasodilation. Injury to the endothelial cells during disease states leads to an increase in levels of ET-1. This peptide is also synthesized by cardiac myocytes and cardiac fibroblasts (8, 19). Importantly, ET-1 has been associated with vasospasm, vascular damage, cardiovascular remodeling and inflammation (9, 19, 33).

ET-1 is involved in many cell signaling pathways that include calcium mobilization and activation of proinflammatory cytokines, ERK1/2, and cyclin D1 (12, 19, 29, 48). All of these pathways are crucial in the development of cardiovascular dysfunction. Previous observations from our laboratory and others have implicated ET-1 in the vasculopathy associated with *T. cruzi* infection (2, 31, 32, 52, 57).

T. cruzi infection of mice exhibit increased levels of ET-1 in plasma and an increased expression of myocardial mRNA for the precursor molecule preproET-1, ET-1, and endothelin converting enzyme (ECE), the enzyme responsible for the conversion of the precursor to ET-1 (35, 36). In addition, immunohistochemical analysis revealed increased ET-1 expression in the vasculature and in *T. cruzi*-infected myocardial cells (36). Interestingly, phosphoramidon, an inhibitor of ECE, ameliorated the pathology and reduced the cardiac remodeling in *T.*

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cruzi-infected mice (15). Finally, recent studies demonstrated that individuals with chronic Chagasic cardiomyopathy had increased levels of ET-1 in plasma (42).

The possible role of ET-1 in the pathogenesis of Chagasic cardiomyopathy was studied in mice in which the gene for ET-1 was deleted in either cardiac myocytes (46) or endothelial cells (22). When mice in which the ET-1 gene was deleted from cardiac myocytes were infected with the Brazil strain of *T. cruzi*, there was a remarkable amelioration of cardiac remodeling, as determined by histopathology, echocardiography, and cardiac magnetic resonance imaging (MRI). These observations suggest that drugs targeted at the ET-1 system could be used as adjunctive therapy in Chagasic heart disease. In addition, it suggests a role for cardiac myocyte derived ET-1 in the pathogenesis of Chagasic cardiomyopathy.

(These data were presented in part at the Seventh International Endothelin Conference, Edinburgh, United Kingdom, in September 2001.)

MATERIALS AND METHODS

Infection of mice and pathology. The Brazil strain of *T. cruzi* was maintained by passage in C3H/HeJ mice, and the Tulahuen strain was maintained by passage in A/J mice (Jackson Laboratories, Bar Harbor, Maine). We used predominantly male mice. In a few experiments female mice were also used but, since we did not find any significant difference between the sexes, all of the data were pooled. Mice were infected at 6 weeks of age, and parasitemia was determined in a hemocytometer. Hearts were fixed in 10% buffered formalin and were stained with hematoxylin and eosin or Trichrome.

Mouse strains. We used two transgenic mouse strains: ET-$1^{flox/flox}$;α-MHC-Cre(+) mice in which the ET-1 gene has been deleted from cardiac myocytes (46) and ET-$1^{flox/flox}$;Tie 2 Cre(+) mice in which the ET-1 gene has been deleted from endothelial cells (Tie 2) (22). A total of 70 to 90% of cells showed Cre-mediated deletion of the ET-1 gene, as demonstrated by Southern blotting and PCR (data not shown). As controls we used mice with the following genotypes: ET-$1^{+/+}$;Cre(−), ET-$1^{+/+}$;α-MHC-Cre(+), and ET-$1^{+/+}$;Cre(−) (wild type, C57BL/6). Since a recent report indicated that mice in which the ET-1 gene has been deleted from the cardiac myocytes may develop a dilated cardiomyopathy at >8 months (240 days) of age, we took care to study mice at earlier time points in order to remove this potential variable (62).

Noninvasive cardiac gated MRI. Cardiac MRI experiments were performed by using a GE Omega 9.4T vertical-bore MRI system equipped with a microimaging accessory and custom-built coils designed specifically for mice, as described previously (15, 16). Just prior to each image acquisition, the heart rate was determined from the electrocardiogram, and the spectrometer gating delay was set to acquire data in diastole. Multislice spin-echo imaging with an echo time of 18 ms and a repetition time of ca. 100 to 200 ms was performed. A 35-mm field of view with a 128 × 256 matrix size (interpolated to 256 × 256) was used. Short-axis images of the heart were acquired, and MRI data were processed off-line with MATLAB-based custom-designed software.

Transthoracic echocardiography. Echocardiography was performed with mice in a supine position on a heating pad set at 38°C. Light anesthesia was achieved by using isoflurane inhalation as previously described (3, 4). Continuous, standard electrocardiograms were taken from electrodes placed on the extremities. Echocardiographic images were obtained by using an annular array, broadband, 10/5-MHz transducer attached to an HDI 5000 CV ultrasound system (Advanced Technology Laboratories, Bethel, Wash.). A small gel standoff was placed between the probe and chest. Two-dimensional and M-mode images of the heart were obtained from the basal short-axis view of the heart and stored on SVHS tapes for off-line measurements by using the Nova-Microsonic (Kodak) Imagevue DCR workstation (Indianapolis, Ind.). All measurements were made in three to six consecutive cardiac cycles, and the averaged values were used for analysis.

Left ventricular end-diastolic and end-systolic diameters, as well as diastolic ventricular septal and posterior wall thickness, were measured from M-mode tracings. Diastolic measurements were performed at the point of greatest cavity dimension, and systolic measurements were made at the point of minimal cavity dimension by using the leading edge method of the American Society of Echocardiography (39, 44). In addition, the following parameters were calculated by using the above-mentioned measurements: left ventricular diastolic wall thickness was calculated as the average of ventricular septal and left ventricular posterior wall thickness; left ventricular percent fractional shortening was calculated as 100 × [(end-diastolic diameter minus end-systolic diameter)/end-diastolic diameter]; and relative wall thickness was calculated as 2 × (left ventricular diastolic wall thickness)/end-diastolic diameter. Echocardiograms were performed in all mice at baseline. Repeat echocardiograms were performed in surviving infected mice.

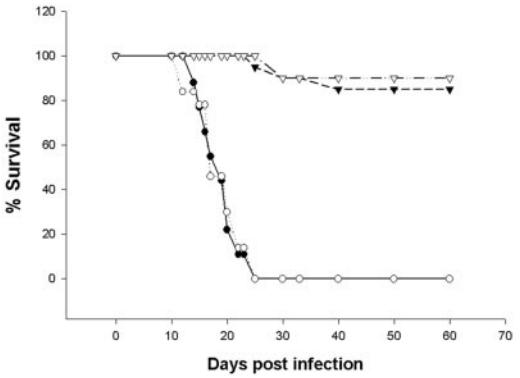

FIG. 1. Survival curves of ET-$1^{flox/flox}$;α-MHC-Cre(+) mice infected with the 10^3 trypomastigotes of the Tulahuen strain (○) and ET-$1^{flox/flox}$;α-MHC-Cre(−) (●) and ET-$1^{flox/flox}$;α-MHC-Cre(+) mice infected with 10^4 trypomastigotes of the Brazil strain (△) and ET-$1^{flox/flox}$;Cre(−) (▲). Mice infected with the Tulahuen strain were killed rapidly, whereas those infected with the Brazil strain survived the acute phase and became chronically infected.

Statistical analysis. Statistical analysis was performed by using SigmaStat 2.0 (SPSS, Chicago, Ill.). For parametric data the Student *t* test was used, and for nonparametric data the Wilcoxon signed-rank test was used. The results are presented as group means ± the standard error of the mean for the MRI and echocardiographic data. A *P* value of <0.05 was considered significant.

RESULTS

Parasitology and mortality. We found that there was 100% mortality in mice infected with 10^3 trypomastigotes of the Tulahuen strain by day 25 postinfection (p.i.) (Fig. 1). By day 17 postinfection (p.i.) the mean parasitemia was 6×10^6 trypomastigotes/ml and by day 21 p.i. it was $>10^7$/ml. There was minimal mortality in mice infected with either 10^3 or 10^4 trypomastigotes of the Brazil strain (Fig. 1). The mean parasitemia of all mice infected with 10^4 trypomastigotes of the Brazil was between 8×10^4 and 10^5 trypomastigotes/ml at days 33 to 35 p.i. The parasitemia waned by day 60. These findings are consistent with our observations that, in general, mice bred on a C57BL/6 background are highly susceptible to death during acute infection with the Tulahuen strain but that these mice, when infected with the Brazil strain, do not usually die during the acute stage and gradually develop a dilated cardiomyopathy by 100 days p.i. (3, 4, 12).

Pathology. During the acute stage of infection with either the Tulahuen or the Brazil strain there was an intense myocarditis, myocardial necrosis, and vasculitis accompanied by numerous pseudocysts (Fig. 2A). In the acute stage there were no significant differences in pathology among the different groups of infected mice. However, in mice infected with the Brazil strain >120 days there was chronic inflammation, myocytolysis, and both interstitial and replacement interstitial fibrosis (Fig. 2B to F).

In infected mice in which the ET-1 gene was deleted from cardiac myocytes there was interstitial fibrosis, but the amount

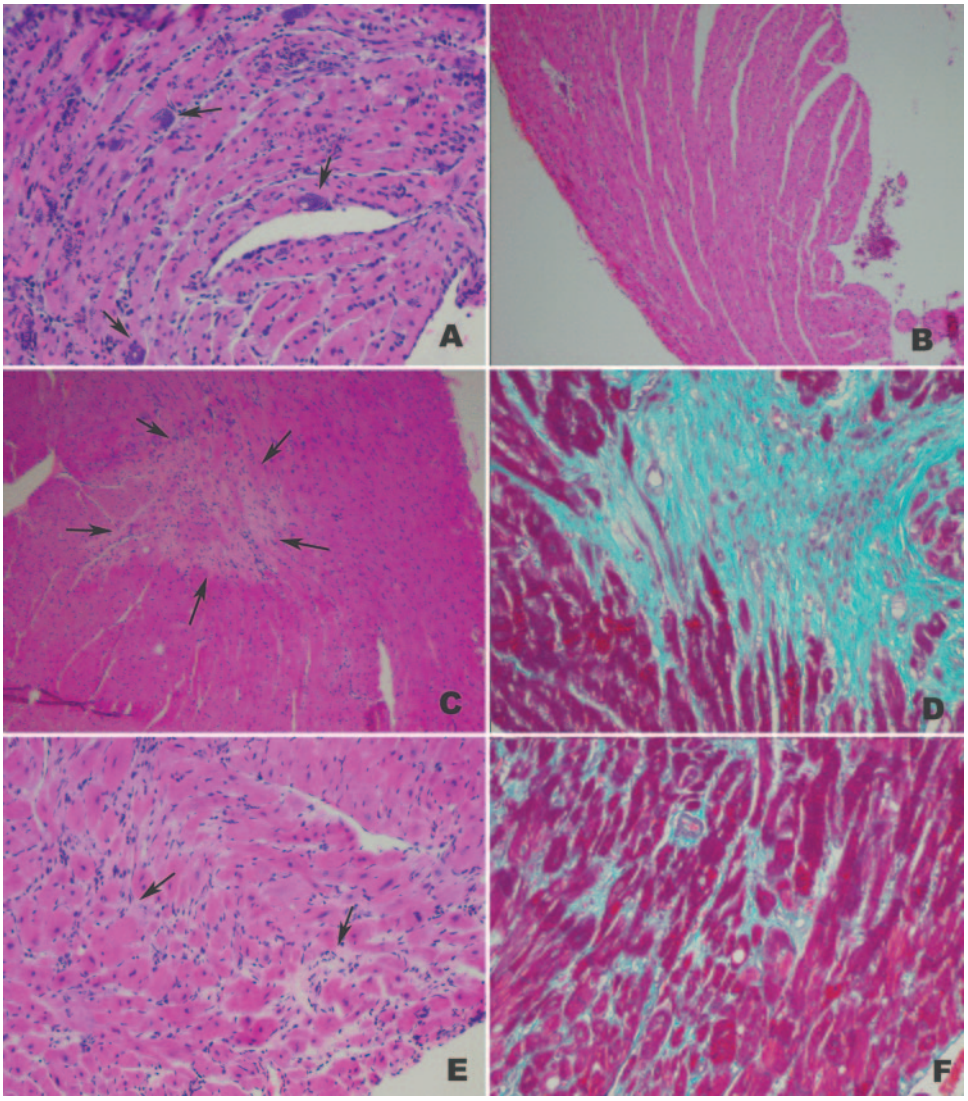

FIG. 2. Representative sections of myocardium of mice infected with *T. cruzi.* (A) Day 15 p.i. in cardiac myocyte-specific ET-1 KO mice infected with the Tulahuen strain. There is myonecrosis, inflammation, vasculitis, and parasite pseudocysts. Mice infected with the Brazil strain showed similar pathology. (B) Mice infected with the Brazil strain at 130 days p.i. There is dilation of the left ventricle in infected FLOX mice. (C and D) Cardiac myocyte hypertrophy and areas of replacement (arrows) and interstitial fibrosis in Brazil strain infected FLOX mice day 130 p.i., which is more evident by Trichrome staining, as seen in panel D. (E and F) Day 130 p.i. Brazil strain-infected ET-$1^{flox/flox}$;α-MHC-Cre(+) mice. Note the interstitial fibrosis, which is more evident by Trichrome staining in panel F. The genotype of the cardiac myocyte-specific ET-1 KO mice is ET-$1^{flox/flox}$;α-MHC-Cre(+) and of the FLOX mice is ET-$1^{flox/flox}$;Cre(−).

of replacement fibrosis, which correlates best with cardiac remodeling, was significantly less (Fig. 2E). In infected mice in which the ET-1 gene was deleted from endothelial cells, as well as in other genetic control mice that were infected with the Brazil strain, there were large areas of replacement fibrosis (Fig. 2C and D and data not shown). Uninfected mice over the age of 120 days had occasional focal areas of age-associated interstitial fibrosis. Replacement fibrosis was never observed in uninfected mice (data not shown).

Cardiac MRI. Previous observations from this laboratory indicate that this method in very useful in examining right ventricular dilation and hypertrophy. Thus, infected (Brazil strain) mice underwent cardiac MRI at 130 to 150 days p.i. We compared the right ventricular internal diameter (RVID) in four groups of infected and uninfected mice (Fig. 3). These mouse strains included FLOX mice, wild-type mice, and mice in which the ET-1 gene was deleted from the endothelial cells (Tie2) and cardiac myocytes [ET-$1^{flox/flox}$;α-MHC-Cre(+)]. There were no significant differences in the RVID in uninfected mice. There were significant increases in the RVID in all infected mice compared to their controls, except for the mice in which the ET-1 gene was deleted from the cardiac myocytes [ET-$1^{flox/flox}$;α-MHC-Cre(+)]. The RVID in the infected ET-$1^{flox/flox}$;α-MHC-Cre(+) mice was significantly smaller compared to other infected groups ($P < 0.05$). This was confirmed by echocardiography (data not shown).

Transthoracic echocardiography. In these studies there were five mouse groups studied by echocardiography, and for

Right Ventricular Internal Dimension

Genotype	Uninfected, mm mean +/- SEM	Infected, mm mean +/- SEM
WT	1.60 +/- 0.05 (n=6)	2.90 +/- 0.22* (n=7)
FLOX	2.04 +/- 0.20 (n=10)	2.76 +/- 0.28* (n=9)
Tie-2	1.90 +/- 0.15 (n=4)	2.57 +/- 0.08* (n=7)
ET-1 KO	1.83 +/- 0.11 (n=8)	2.14 +/- 0.14 (n=13)

*P < 0.05 vs. uninfected value

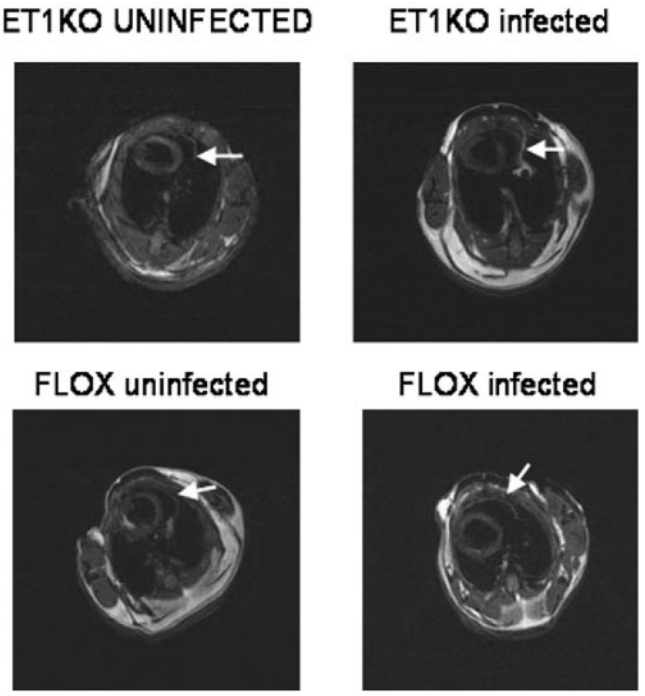

FIG. 3. Representative cardiac MRI analysis of mice infected with the Brazil strain of *T. cruzi*. The table shows that infection with the Brazil strain caused a significant increase in the RVID in all mice studied except for ET-$1^{flox/flox}$;α-MHC-Cre(+) mice. The second panel demonstrates transverse MRI images of the heart. These images demonstrate cross-sections of the mice positioned at the mid level of the left ventricle. The right ventricle is indicated by the white arrows. The upper left panel shows an image of an uninfected ET-1 KO mouse, and the upper right panel shows an image of an in infected ET-1 KO mouse. Note that infection did not significantly increase the RVID (see table). The lower left panel shows an image of an uninfected control FLOX mouse, and the lower right panel shows an infected control mouse. Note the enlarged RVID in the infected FLOX mouse. All images were acquired at 9.4 T by using a spin-echo sequence with a repetition time of ca. 200 ms and an echo time of 18 ms and are based on four averages. Abbreviations: ET1-KO, cardiac myocyte-specific ET-1 KO mice [ET-$1^{flox/flox}$;α-MHC-Cre(+)]; Tie-2, endothelial cell-specific ET-1 KO mice [ET-$1^{flox/flox}$;Tie 2 Cre(+)]; FLOX, control mice [ET-$1^{flox/flox}$;Cre(−)]; WT, ET-$1^{+/+}$;Cre(−) mice.

each group there were infected and uninfected mice: the two experimental groups were those mice in which the ET-1 gene was deleted from cardiac myocytes [ET-$1^{flox/flox}$;α-MHC-Cre(+)] and mice in which the ET-1 gene was deleted from the endothelial cells [ET-$1^{flox/flox}$;Tie 2 Cre(+)] [Tie2]. We used mice with the following genotypes as controls: ET-$1^{flox/flox}$; Cre(−) [FLOX]), ET-$1^{+/+}$;α-MHC Cre(+) [Cre(+)] and ET-$1^{+/+}$;Cre(−) [wild type, Cre(−), C57BL/6]. These controls were chosen to examine whether either Cre or Flox would affect the development of cardiomyopathy independent of the ET-1 deletions.

Echocardiography was performed 130 to 150 days p.i. The heart rate ranged from ca. 500 to 600 beats per minute in all groups, both control and infected. A slower heart rate was noted in some infected mice. The baseline values for the left ventricular end diastolic diameter (LVEDD) were <3 mm in all groups (Fig. 4). As a result of infection, there was a significant increase in the LVEDD in all groups. However, on average, there was a 30% increase in all infected groups compared to controls except for infected ET-$1^{flox/flox}$;α-MHC-Cre(+) mice, in which the increase was only 13%. There was a significant decrease in percent left ventricular fractional shortening in all infected groups compared to controls (Fig. 5). Interestingly, the degree of reduction in left ventricular fractional shortening was less in mice in which the ET-1 gene was deleted from the cardiac myocytes or the endothelial cells (Fig. 6). There was a significant decrease in relative wall thickness in diastole in all infected groups. However, the degree of reduction in relative wall thickness in diastole was lower in the infected ET-$1^{flox/flox}$;α-MHC-Cre(+) mice (Fig. 6).

DISCUSSION

Chagasic heart disease is a dilated cardiomyopathy, but it may also result in a vasculopathy. Recent evidence suggests that ET-1 contributes to the pathogenesis of Chagasic cardiomyopathy (2, 31, 32, 35, 36). We had previously demonstrated that treatment of *T. cruzi*-infected mice with phosphoramidon, a nonspecific ECE inhibitor, ameliorated chronic cardiomyopathy (15). That study did not distinguish between ET-1 derived from cardiac myocytes and endothelial cells. We sought to investigate this by utilizing mice in which the ET-1 gene was knocked out either in cardiac myocytes or endothelial cells. The highly virulent Tulahuen strain killed mice in every group, and this was accompanied by high blood and tissue parasitism. Mice infected with the cardiomyopathic Brazil strain did not kill the mice, and this strain faithfully recapitulates chronic Chagasic cardiomyopathy in humans. In the chronic stage surviving mice exhibited cardiac myocyte hypertrophy and interstitial myocardial fibrosis. There were large areas of replacement fibrosis in the myocardium of infected control mice, which was reduced in mice in which the ET-1 gene had been

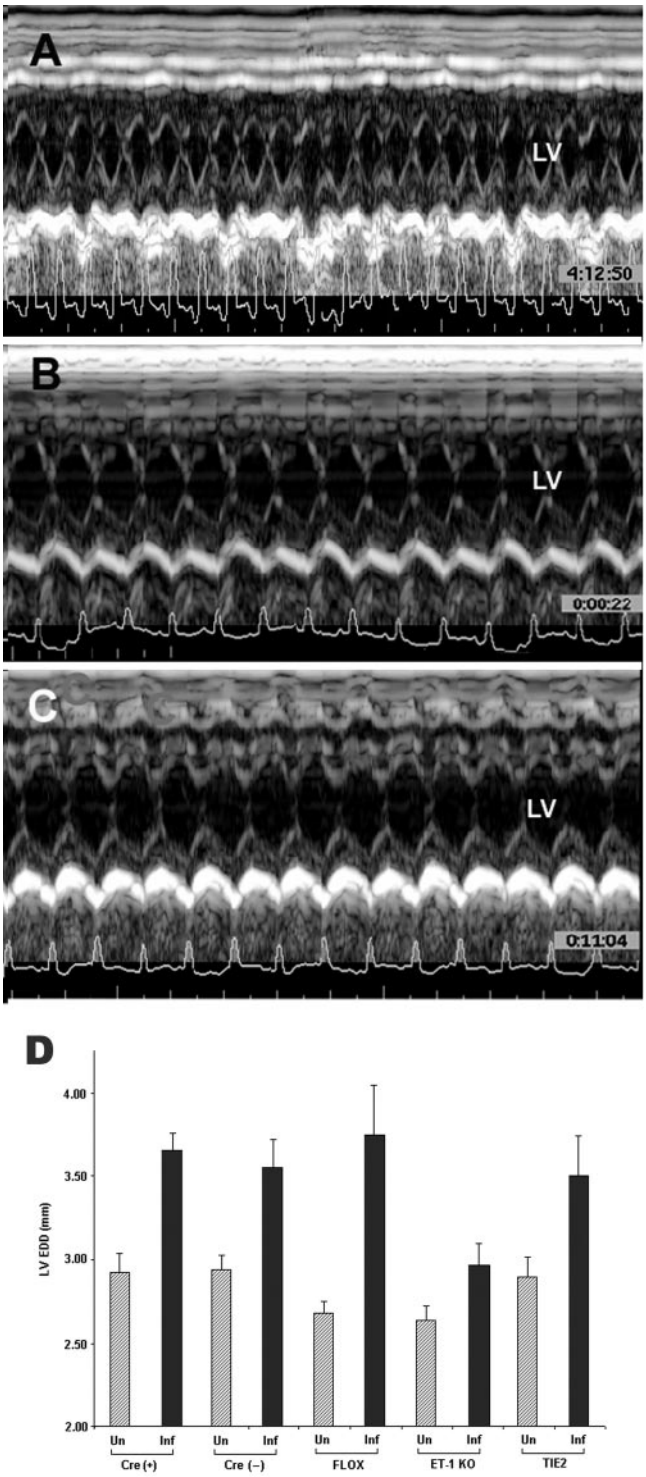

FIG. 4. Representative M-mode echocardiographic tracings of the left ventricle (LV) in an uninfected wild-type mouse (C57BL/6) (A), an infected ET-$1^{flox/flox}$;α-MHC-Cre(+) mouse (B), and an infected wild-type mouse (C). Whereas infection resulted in significant increase in left ventricular size as determined by the LVEDD and a significant decrease in left ventricular size fractional shortening in both groups of infected mice, the magnitude of these changes was greater in the wild-type control mice. (D) LVEDD of infected and uninfected mice at day 130 p.i. Note that there was a significant increase ($P < 0.05$) in LVEDD in all infected mice compared to their uninfected controls. However, the increase averaged 38% in all groups except for the ET-$1^{flox/flox}$;α-MHC-Cre(+) group, where the increase was only 13%. All mice were infected with the Brazil strain. LV, left ventricle. Results for cardiac-specific ET-1 KO [ET-$1^{flox/flox}$;α-MHC-Cre(+)] mice, endothelial cell-specific ET-1 KO [ET-$1^{flox/flox}$;Tie 2 Cre(+)] mice (TIE2), control [ET-$1^{flox/flox}$;Cre(−)] mice (FLOX), Cre(+) [ET-$1^{+/+}$; α-MHC-Cre(+)] mice [Cre(+)], and Cre(−) [ET-$1^{+/+}$; Cre(−)] mice [i.e., wild type, Cre(−), and Flox(−)] [Cre(−)] are shown. Un and Inf, uninfected and infected, respectively.

deleted from cardiac myocytes. Cardiac MRI analysis demonstrated that in mice in which the ET-1 gene was deleted from cardiac myocytes infection did not cause a significant increase in right ventricular inner diameter (RVID). In the other groups, such as mice in which the ET-1 gene had been deleted from endothelial cells (Tie2) and wild-type mice, infection resulted in a significant increase in RVID. These data demonstrate that the absence of the ET-1 gene in cardiac myocytes ameliorates right ventricular enlargement, as determined by MRI, and underscore the observations that the right ventricle is often more severely involved in this infection.

Echocardiography was then used to evaluate other aspects of myocardial structure and function in *T. cruzi*-infected mice at 130 days p.i. We observed an increase in LVEDD in infected mice in all groups, but the increase in ET-$1^{flox/flox}$;α-MHC-Cre(+) mice was significantly smaller (40 versus 13%). In addition, the percent LV fractional shortening, a measurement of myocardial systolic performance, was reduced in all mice, although the degree of change was less in mice in which the ET-1 gene was deleted from cardiac myocytes and endothelial cells. The relative wall thickness in diastole was significantly decreased in infected mice; that is, infection caused a thinning of the ventricular wall. However, the effect was less dramatic in infected mice in which the ET-1 gene was deleted from cardiac myocytes. Finally, echocardiography confirmed the MRI data that infection caused a dilation of the right ventricle but that the degree of enlargement was less in infected mice in which the ET-1 gene was deleted from cardiac myocytes. Taken together, these observations demonstrate that murine Chagasic cardiomyopathy is a dilated cardiomyopathy with enlargement of the left and right ventricles, reduced fractional shortening and thinning of the myocardium. However, in mice in which the ET-1 gene was deleted from cardiac myocytes the degree of change was markedly reduced. This suggests that ET-1 production from myocytes is involved in the pathogenesis of this cardiomyopathy.

Originally described as a vasoconstrictor (59), ET-1 is one of three endothelin isoforms (ET-1, ET-2, and ET-3) (19). The synthesis of ET-1 is mediated by ECE, and the actions of ET-1 are mediated via two G-protein-coupled receptors ET_A and ET_B. The contributions of the endothelin system to normal development and to disease states have been aided by the use of knockout (KO) mice. For example, ET-1 and ET_A KO mice die at birth from asphyxia as a result of malformations of neural crest derived facial and pharyngeal structures (6, 25). Mice in which the ET-1, ET_A, ET_B, or ECE-1 are lacking have cardiovascular and gastrointestinal abnormalities (10, 24, 58, 60).

The contributions of ET-1 to the cardiovascular system have

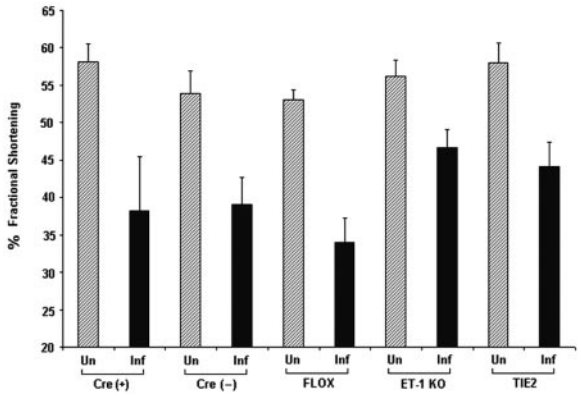

FIG. 5. Left ventricular percent fractional shortening of infected and uninfected mice at day 130 p.i. (Brazil strain). There was a significant ($P < 0.05$) decrease in the percent fractional shortening in all infected groups, but the magnitude of the decrease in the infected ET-$1^{flox/flox}$;α-MHC-Cre(+) mice and ET-$1^{flox/flox}$;Tie 2 Cre(+) mice was less. The results for cardiac-specific ET-1 KO [ET-$1^{flox/flox}$; α-MHC-Cre(+)] mice, endothelial cell-specific ET-1 KO [ET-$1^{flox/flox}$; Tie 2 Cre(+)] mice (TIE2), and control [ET-$1^{flox/flox}$;Cre(−)] (FLOX), Cre(+) [ET-$1^{+/+}$;α-MHC-Cre(+)], and Cre(−) [ET-$1^{+/+}$;Cre(−)] mice [i.e., wild type, Cre−), and Flox(−)] are as indicated. Un and Inf, uninfected and infected, respectively.

been well studied. In the heart, cardiac myocytes, cardiac fibroblasts, and endothelial cells synthesize ET-1 (8, 19, 47). Inflammatory cells found in the heart during inflammation may also synthesize ET-1 (45). Cardiac fibroblasts contribute to ET-1 production in other examples of myocardial injury (17). Interestingly, mice overexpressing ET-1 in cardiac myocytes exhibit a dilated cardiomyopathy accompanied by an increase in the inflammatory infiltrates (61). Therefore, the deletion of the ET-1 gene (62), as well as the overexpression of the ET-1 gene in cardiac myocytes (61), may both eventually result in progression to a dilated cardiomyopathy. This, however, is not the case in mice where the gene for the ET_A receptor has been deleted from cardiac myocytes (18).

Locally produced ET-1 acts in part by increasing smooth muscle contractility and inducing myocyte hypertrophy and injury (8, 14, 19). ET-1 may improve contractility in the failing heart, and upregulation of ET-1 may provide short-term inotropic support for the failing myocardium. In humans and in experimental models of congestive heart failure (CHF), ET-1 production is increased in the myocardium and levels in plasma often correlate with the severity of CHF (23, 27, 38, 55). In the setting of myocardial infarction, increased ET-1 levels in plasma correlate with infarct size (20). Therefore, in various disease states, increased ET-1 levels may reflect both the degree of endothelial and myocardial cell damage and a possible mechanism for myocardial damage. Treatment with an ET_A receptor antagonist improves the survival of animals with CHF and is accompanied by improvement in the degree of left ventricular dysfunction and remodeling (37, 40, 41, 43). These observations suggest that upregulation of ET-1 may be a potential target for therapeutic intervention in the treatment of CHF. In the past, studies on the role for ET-1 in the modulation of cardiovascular structure have focused on the role of ET-1 in the induction of smooth muscle cell proliferation, such as that after balloon angioplasty (7, 56).

A role for the vasculature in the pathogenesis of acute and chronic Chagasic cardiomyopathy has been proposed. *T. cruzi* infection of cultured endothelial cells activates the NF-κB pathway stimulating the synthesis of proinflammatory cytokines and the expression of vascular adhesion molecules (11). In addition, infection of endothelial cells causes increased synthesis of ET-1 (57) and the activation of ERK and AP-1 (32), which are important in the activation of ET-1. These and other infection-associated perturbations in endothelial-cell signal transduction mechanisms may contribute to focal pathology and vasospasm (28, 29, 30).

Petkova et al. (36) found elevated levels of ET-1 in plasma and increased expression of mRNAs for prepro-ET-1, ECE and ET-1 in the myocardium. The increase in ET-1 observed in the myocardium of infected mice is likely to have several sources. It may reflect damage to a variety of cell types found in the heart, such as endothelial cells, cardiac myocytes, and cardiac fibroblasts. In addition, an elevated ET-1 may explain, in part, the vascular spasm and ischemia observed as a consequence of this infection. Salomone et al. (42) reported elevated levels of ET-1 in plasma in patients with chronic Chagasic cardiomyopathy.

The extracellular signal-regulated kinase (ERK)/AP-1 pathway regulates ET-1 synthesis. *T. cruzi* infection of the myocardium and cultured endothelial and smooth muscle cells activates the ERK/AP-1/ET-1/cyclin D1 pathway (13, 32). In fact, ET-1 regulates ERK. In addition, cyclin D1, a downstream targets both ET-1 and ERK, mediates smooth muscle cell proliferation (32, 48) and possibly cardiac myocyte hypertrophy (1), as does ET-1. ET-1 also contributes to increased collagen deposition and subsequent myocardial fibrosis (9, 32, 37). ET-1 is significantly reduced in ET-$1^{flox/flox}$;α-MHC-

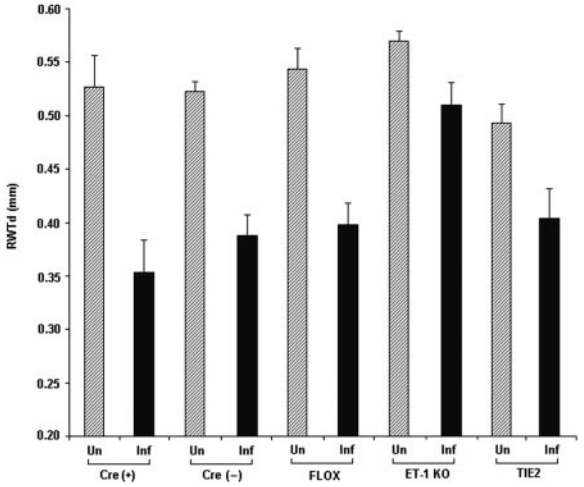

FIG. 6. Relative wall thickness in diastole of infected and uninfected mice at day 130 p.i. (Brazil strain). There was significant ($P < 0.05$) thinning of the wall in all infected groups compared to their uninfected counterparts. However, the magnitude of thinning was significantly less in the ET-1 KO group compared to other infected groups. Results for cardiac myocyte-specific ET-1 KO mice [ET-$1^{flox/flox}$; α-MHC-Cre(+)], endothelial cell-specific ET-1 KO mice [ET-$1^{flox/flox}$;Tie 2 Cre(+)] (TIE2), and control [ET-$1^{flox/flox}$;Cre(−)] (FLOX), Cre(+) [ET-$1^{+/+}$;α-MHC-Cre(+)], and Cre(−) [ET-$1^{+/+}$;Cre(−)] mice [e.g., wild type, Cre(−), and Flox(−)] are shown. Un and Inf, uninfected and infected, respectively.

Cre(+) mice. Therefore, the ET-1 stimulus for increasing fibrosis is reduced and perhaps this is the reason for less remodeling.

Petersen and Burleigh (34) have suggested that T. *cruzi*-induced cardiac myocyte hypertrophy in vitro is mediated by IL-1β. Previously, we have demonstrated that *T. cruzi* infection of endothelial cells results in a dramatic increase in interleukin- 1β (IL-1β) mRNA and protein (50). It is well known that ET-1 may be stimulated by cytokines and ET-1 in turn may stimulate cytokine production. Infection of endothelial cells and myocardium results in increased synthesis of ET-1 and cytokines, including IL-1β, tumor necrosis factor alpha, and gamma interferon (4, 5, 12, 35), all of which can stimulate the production of ET-1. As pointed out by Petersen and Burleigh (34), their experiments are in the setting of cultured cells, whereas the studies from our laboratory have primarily been in mice. We agree with the assessment of those authors that although IL-1β may be an important early factor in cardiac myocyte hypertrophy, ET-1 may be important at a later time point in the infection. It is possible that an important event is the ET-1-induced vasospasm which results in focal myocardial ischemia. Ischemia may result in myonecrosis, which in turn may cause further increases in ET-1. This then causes cardiac myocyte hypertrophy and enhanced fibrosis. As noted above, recent data clearly implicate ET-1 in the remodeling process (37).

Our observations, as well as those of others, underscore the complicated and redundant pathways involved in cardiovascular remodeling that attends *T. cruzi* infection. In addition, it is of interest that the most dramatic differences in myocardial function in infected mice were observed when the ET-1 gene was deleted in cardiac myocytes and to a lesser extent in mice in which the ET-1 gene was deleted in endothelial cells. This is consistent with *T. cruzi*-induced vasculopathy, since ET-1 synthesized by infected cardiac myocytes can cause vasospasm in adjacent microvasculature, but suggests that cardiac myocyte production of ET-1 may be a critical factor in the pathogenesis the vasculopathy seen in Chagasic cardiomyopathy. Finally, the present study also reiterates that noninvasive cardiac testing can provide complementary information when the pathogenesis of dilated cardiomyopathy in experimental models of chronic Chagas' disease is being studied.

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