AKT Network of Genes and Impaired Myocardial Contractility During Murine Acute Chagasic Myocarditis

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Abstract. Chagasic disease is associated with high morbidity in Latin America. Acute Chagasic myocarditis is consistently found in acute infections, but little is known about its contribution to chronic cardiomyopathy. The aim of the study was to phenotypically characterize two strains of mice with differential Chagas infection susceptibility and correlate strain myocarditis phenotypes with heart tissue gene expression. C57BL/6J and Balb/c mice were injected intraperitoneally with 0 or 150–200 tissue-derived trypomastigotes (Tulahuen strain). Echocardiograms, brain natriuretic peptide, and troponin were measured. Heart tissue was harvested for histopathological analysis and gene expression profiling on microarrays. Genes differently expressed between infected Balb/c and C57BL/6J mice were identified. Echocardiograms showed differences in Balb/c versus C57BL/6J infected mice in heart rate (413 versus 476 beats per minute; $P = 0.0001$), stroke volume (31.9 ± 9.3 versus 39.2 ± 5.5 μL ; $P = 0.03$), and cardiac output (13.1 ± 3.5 versus 18.7 ± 3.2 $\mu\text{L}/\text{min}$; $P = 0.002$). Gene expression at 4 weeks analysis showed 32 statistically significant (q value < 0.05) differentially expressed genes between infected Balb/c and C57BL/6J mice that were enriched for genes related to the protein kinase B (AKT) pathway. These specific phenotypic features of cardiac response during acute Chagasic myocarditis may, in part, be related to host AKT network regulation.

INTRODUCTION

Chagasic disease, perhaps one of the most neglected tropical diseases, has a high morbidity not only in Latin America but also, among immigrants to the United States. It is estimated that 8–10 million people are infected worldwide,¹ and approximately 300,000 individuals are infected with *Trypanosoma cruzi* in the United States.² The primary mechanisms of mortality are sudden death and end-stage cardiomyopathy. Acute Chagasic myocarditis is consistently found in acute infections, but little is known about its contribution to chronic forms of cardiomyopathy. Parasitic myocardial invasion seems to be universal during the initial infection.³ Furthermore, there is a significant proportion of patients with electrocardiographic abnormalities and abnormal echocardiographic findings during this period.^{4–6} However, even with these abnormal electric and echographic cardiac changes, acute Chagasic myocarditis is vastly asymptomatic and clinically silent. Chronic Chagas cardiomyopathy (CCC), however, is markedly symptomatic and a source of significant morbidity, but it only develops in approximately 30% of the chronically infected patients after an average of 10 years.⁷ The link between acute and chronic myocarditis and cardiomyopathy is not clear. However, similar to other forms of chronic cardiomyopathy, the initial insult and injury may carry a significant prognostic factor for the development of the chronic forms of the disease. Additionally, several environmental, host, and parasitic factors may play a role in this process; these include polymorphisms in host genes associated with chemotaxis and other ancillary immune factors that are associated with the development of CCC.⁸ C57BL/6J and Balb/c strains of mice have been identified as differentially susceptible to infection with *T. cruzi*;^{9,10} with C57BL/6J mice considered to be resistant and Balb/c mice considered to be susceptible. However, susceptibility has significant variability depending on the *T. cruzi* strain used. The aim of this study was to phenotypically

characterize the two strains of mice with differential susceptibility to acute Chagasic infection for myocarditis and correlate strain phenotypes with heart tissue gene expression.

METHODS

Ethics statement. This study, including the procedures for the treatment of the animals, was conducted under an approved protocol by The Institutional Animal Care and Use Committee (IACUC) at the University of Colorado Denver [IACUC B-95911(06)1E]. This protocol and the animal facility adhere to national and international regulations: Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accreditation file number 00235, Policy on Humane Care and Use of Laboratory Animals (PHS) animal assurance of compliance A 3269-01, and United States Department of Agriculture (USDA) license 84-R-0059. Mice were maintained under pathogen-free conditions with unlimited access to water and food. They were euthanized by CO₂ asphyxiation, and all methods available were used to minimize their suffering.

Mice and infection. Seven- to eight-week-old Balb/c and C57BL/6J male mice were obtained from Jackson Laboratories (Bar Harbor, ME) and hosted in a controlled environment. Eight mice were infected, and four mice were used as controls for each strain. Experiments were done in duplicates. The Tulahuen strain of *T. cruzi* was obtained from American Type Culture Collection Company (ATCC) (Manassas, VA) (ATCC 30208). The parasites were then transferred to an NIH/3T3 fibroblast cell culture (ATCC CRL-1658) under the following sterile conditions: 37°C, 5% CO₂, and Dulbecco's Modified Eagle Medium (DMEM) culture media with 10% fetal bovine serum (FBS), 10 mM 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (HEPES), 0.2 mM sodium pyruvate, and 50 $\mu\text{g}/\text{mL}$ gentamicin. On days 5–7 of culture, tissue culture-derived trypomastigotes (TCTs) were harvested from the supernatant to obtain the parasite. TCTs were then resuspended in sterile filtered 1 $\times$ phosphate buffered saline (PBS) plus 1% glucose at concentrations of 750–1,000 TCT/mL. Mice were inoculated with TCTs after at least 1 week of adaptation

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through intraperitoneal (i.p.) injections in a 200 μ L volume. PBS/1% glucose was used as vehicle control.

Monitoring of acute infection. Weight and parasitemia were monitored weekly. Blood samples were obtained through retro-orbital bleeding under isoflurane anesthesia at weeks 1, 2 and 3; 2 μ L whole blood was added to 8 μ L red blood cells (RBC) lysis buffer (10 μ L total) to count the parasites on a hemocytometer. At 4 weeks, plasma was obtained from whole blood collected by cardiac puncture post-mortem. Plasma cardiac troponin-I and brain natriuretic peptide (BNP) enzyme-linked immunosorbent assay (ELISA) were measured by following the manufacturer's instructions (K-ASSAY; KT-470, 8578; Kamiya Biomedical Company, Seattle, WA). All samples were analyzed in duplicate. Absorbance was measured at 450 nm using the Synergy HT Plate Reader (Biotek, Winooski, VT), and concentrations were calculated using the standard curve.

Echocardiograms. Vevo 770 Visual Sonics was used with a 707b 30-MHz high-resolution mechanical transducer to perform echocardiograms under isoflurane anesthesia at 4 weeks post-infection. Euthermia was conserved during the whole procedure. The following measures were obtained: left ventricular anterior wall in diastole (LVAWd; millimeters), left ventricle internal diameter in diastole (millimeters), left ventricle posterior wall in diastole (millimeters), left ventricle anterior wall in systole (millimeters), left ventricle internal diameter in systole (millimeters), left ventricle posterior wall in systole (millimeters), early diastolic velocity (millimeters per second), atrial velocity (millimeters per second), deceleration time of early diastolic velocity (milliseconds), early diastolic velocity from tissue Doppler (E'), and atrial velocity from tissue Doppler (A'). Based on these measurements, the following calculations were done: left ventricle volume in diastole (microliters), left ventricle volume in systole (microliters), ejection fraction (EF; percentage), fractional shortening (FS; percentage), stroke volume (SV; microliters), cardiac output (CO; milliliters per minute), heart rate (HR; beats per minute [bpm]), mitral valve (MV) E/A , and E'/A' .

RNA extraction. Mice were euthanized after the echocardiograms were performed. Tibia length in millimeters was obtained. Hearts were harvested and weighted, and the apex was cut. Tissue was flash-frozen in liquid nitrogen, then disrupted, and homogenized using a TissueRuptor (Qiagen, Frederick, MD) followed by total RNA extraction using the RNeasy Plus Universal Mini Kit (Qiagen). RNA quality was determined using the Agilent 2100 Bioanalyzer with the RNA 6000 Nano Kit Assay (Santa Clara, CA).

Heart histology. Remaining heart tissue was fixed in 10% buffered formalin and embedded in paraffin. Paraffin blocks were prepared, and two serial sections were stained: one with hematoxylin and eosin stain (H&E) and one used for immunohistochemistry using the immunoperoxidase method for detection of *T. cruzi* antigens. Briefly, sections were deparaffinized and incubated with anti-*T. cruzi* lipophosphoglycan antibody (Tcr7D3; Abcam, Cambridge, MA). Anti-mouse immunoglobulin M (IgM) biotinylated was used as the secondary antibody. Myocardial and pericardial degrees of inflammation and tissue parasitism were determined by examination of the mononuclear and polymorphonuclear cellular infiltrations and the number of amastigotes by an independent blinded pathologist. A score was assigned between zero (no involvement) and four (100% involvement). Additionally, the number of myocardial-

infiltrating cells was measured using a computer morphometric analysis (NIH ImageJ) of images obtained using an Aperio Scan Scope T3 System (Vista, CA).

Statistical analysis. Bivariate analysis was conducted using a χ^2 test for dichotomous variables and a *t* test or linear regression for continuous variables or multivariable analysis. Data from the duplicate experiments were analyzed in conjunction. We considered a two-sided *P* value < 0.05 to be statistically significant. All analyses were performed using STATA software, version 12.0 (College Station, TX).

Gene expression microarrays. Gene expression profiling of heart tissue was performed on the Agilent G3 SurePrint 8 $\times$ 60 Mouse Gene Expression One-Color Microarray Platform. We included six mice per experimental group per strain for a total of 24 samples. Agilent's labeling kits for one-color assays were used followed by hybridization and wash procedures according to the manufacturer's protocols. Arrays were scanned on the Nimblegen MS200 High-Resolution Microarray Scanner on the 2- μ m setting. Images were processed in the Agilent Feature Extraction software, and intensities were exported from the Feature Extraction software. Feature Extraction quality control (QC) reports were used to monitor data quality.

Gene expression data analysis. Array data were quantile-normalized and log₂-transformed before statistical analysis was performed with Partek software (St. Louis, MO). Principal components analysis was then used to perform additional QC and detect and exclude potential technical outlier samples. A two-factor analysis of variance (ANOVA) was used to identify differentially expressed genes. The first factor was the within-strain comparison between infected and uninfected mice, and the second factor compared Balb/c mice with C57BL/6J mice after accounting for the within-strain infected/uninfected comparison. Statistical significance was determined using the Benjamini-Hochberg false discovery rate method,¹¹ and genes with *q* values < 0.05 were considered significant. Pathway analysis was performed using Ingenuity Pathway Analysis (Redwood City, CA).¹²

RESULTS

Acute infection. We first examined the results associated with signs of acute infection. Four-week mortality was 56.3% and 12.5% for Balb/c and C57BL/6J mice, respectively (*N* = 16; *P* = 0.009) (Figure 1A), in accordance with the established susceptibility differences between the two strains. Among the infected mice who survived, the weight loss between Balb/c and C57BL/6J mice was not statistically different (*P* = 0.32) (Supplemental Figure 1). Parasitemia peaked at 2 weeks (Figure 1B) and was higher in the susceptible Balb/c mice, but it was not significantly different from that in C57BL/6J mice because of the high variability in parasite counts: 500,781 $\pm$ 866,464 (Balb/c) versus 140,625 $\pm$ 280,606 (C57BL/6J) parasites/mL (*P* = 0.12). Parasitemia levels at 4 weeks were also not significantly different. BNP and troponin levels were not significantly different between strains, but BNP was elevated in infected compared with uninfected Balb/c mice: 61.88 $\pm$ 20.9 versus 32.26 $\pm$ 17.1 ng/L (*P* = 0.01). This difference was not observed in C57BL/6J mice (Figure 2). Heart weight and heart weight/tibia length ratios did not differ among infected Balb/c and C57BL/6J mice (Supplemental Figure 2). These data generally support published findings of the difference in susceptibility of the two strains of mice to *T. cruzi* acute infection, although Tulahuen is considered a high-virulence strain.

FIGURE 1. (A) Mortality at 4 weeks was 56.3% and 12.5% for Balb/c and C57BL/6J mice ($P = 0.009$), respectively. Significance was assessed using a χ^2 test. (B) Parasitemia peak levels developed at 2 weeks (2w). Parasitemia at 4 weeks (4w) is also displayed. Significance was assessed using a t test ($P = 0.12$). Bars represent SE.

Echocardiograms. We next examined differences in acute myocarditis phenotypes from echocardiogram data collected at 4 weeks among survivors post-injection.

Differences among non-infected Balb/c and C57BL/6J mice. Echocardiograms showed no significant differences in non-infected Balb/c versus non-infected C57BL/6J mice in HR (406 versus 438 bpm; $P = 0.4$), SV (40.6 ± 6.4 versus 39.7 ± 6.1 μL ; $P = 0.77$), or CO (16.6 ± 4.8 versus 17.4 ± 3.7 $\mu\text{L}/\text{min}$; $P = 0.71$). There was a significant difference on baseline EF (56% versus 65%; $P = 0.04$) but not on LVAWd ($P = 0.64$).

FIGURE 2. BNP levels in nanograms per liter at 4 weeks post-infection. BNP was elevated in infected compared with uninfected Balb/c mice: 61.88 ± 20.9 versus 32.26 ± 17.1 ng/L. Although not statistically significant, it was also elevated in infected versus non-infected C57BL/6J mice: 52.4 ± 19.7 versus 36 ± 15 ng/L. Bars represent SE. * P value = 0.01; ** P value = 0.05.

FIGURE 3. Differences in CO at 4 weeks post-infection. Infected Balb/c versus infected C57BL/6J mice: CO: 13.1 ± 3.5 versus 18.7 ± 3.2 $\mu\text{L}/\text{min}$. Significance was assessed using a t test. Bars represent SE. * $P = 0.0016$.

Differences among infected Balb/c and C57BL/6J mice. Echocardiograms showed significant differences in infected Balb/c versus infected C57BL/6J mice in HR (413 versus 476 bpm; $P = 0.0001$), SV (31.9 ± 9.3 versus 39.2 ± 5.5 μL ; $P = 0.03$), and therefore, CO (13.1 ± 3.5 versus 18.7 ± 3.2 $\mu\text{L}/\text{min}$; $P = 0.0016$) (Figure 3). Similarly, CO differences between infected strains remained significantly associated after adjustment for weight loss at 28 days ($P = 0.005$). Infected Balb/c mice had a higher EF percentage compared with both non-infected Balb/c and infected C57BL/6J mice, although this last difference was not statistically significant (73.22 ± 10.8 versus 68.98 ± 9.11 ; $P = 0.36$) (Supplemental Figure 3). LVAWd was not different between infected Balb/c and C57BL/6J mice; however, it differed in infected compared with non-infected for Balb/c mice (0.78 ± 0.09 versus 0.65 ± 0.04 mm; $P = 0.039$) and C57BL/6J mice (0.75 ± 0.06 versus 0.68 ± 0.04 ; $P = 0.023$). Taken together, these data show that the most striking hemodynamic differences between the two infected strains were reflected in CO and contractility.

Heart histology. Finally, to characterize phenotypic differences in susceptibility between the two strains of mice, we examined heart tissue histology at 4 weeks post-infection. Automated calculations of cellular myocardial infiltrates did not show differences between infected Balb/c and C57BL/6J strains ($2,648.8 \pm 336.7$ versus $2,330.6 \pm 554.7$ nucleated cells/ mm^2 ; $P = 0.16$) (Figure 4). However, infected Balb/c mice had more infiltrates compared with uninfected controls ($2,648.8 \pm 336.7$ versus $1,938 \pm 262.1$ nucleated cells/ mm^2 ; $P = 0.0003$). There was no difference in infected compared with uninfected C57BL/6J mice or between non-infected groups. Manual pathological evaluation by a blinded board-certified pathologist showed significant differences between infected and non-infected groups for both Balb/c and C57BL/6J strains in terms of degree of acute myocarditis ($P = 0.0001$ and $P = 0.005$, respectively), pericarditis ($P = 0.0001$ and $P = 0.001$, respectively), and tissue parasitism ($P = 0.008$ and $P = 0.028$, respectively). Figure 4 displays representative changes in the Balb/c myocardium. Pathology data indicated that infected Balb/c mice developed more pronounced myocardial infiltrates during the first 4 weeks.

Gene expression analysis. **Differences among non-infected Balb/c and C57BL/6J mice.** Differences at baseline among the two strains encompassed mostly genes involved in networks with cellular assembly and organization, heme biosynthesis, and calcium signaling.

Differences among infected Balb/c and C57BL/6J mice. Given the differences in response to *T. cruzi* infection in the two strains of mice, we profiled RNA from heart tissue on

FIGURE 4. Representative Balb/c myocardial histopathology changes during infection. (A) Foci of inflammation with associated necrotic myocardial fibers (arrowheads). *T. cruzi* amastigotes in myocardial fiber (arrow). Magnification: $\times 200$ (H&E stain). (B) Necrotic myocardial fibers (arrowheads). *T. cruzi* amastigotes in myocardial fiber (arrow). Magnification: $\times 400$ (H&E stain). (C) Foci of pericarditis (white arrowheads) overlying foci of inflammation in the myocardium (black arrowheads). Magnification: $\times 200$ (H&E stain). (D) *T. cruzi* amastigotes (arrows). Magnification: $\times 400$ (immunoperoxidase stain).

microarrays with the goal of determining molecular processes underlying these differences in susceptibility. Gene expression analysis showed 144 statistically differentially expressed genes in infected Balb/c compared with infected C57BL/6J mice (q value < 0.05). Similarly, there were 163 genes differentially expressed in the two strains at baseline (uninfected), with 66 genes shared among the two analyses. We subtracted the genes in common and were left with 32 genes that are unique to the comparison of the infected strains of mice; these genes are listed in Table 1. Ingenuity Pathway Analysis of 32 genes identified three significant transcriptional networks, including networks centered on protein kinase B (AKT) (network 2) (Figure 5A), ubiquitin C, and human leukocyte antigen - class II histocompatibility antigen, DR alpha chain (HLA-DRA) (networks 1 and 3) (Supplemental Figure 4A and B). Examination of expression patterns across the groups shows that these genes of interest, such as *Ncam1*, are (1) more abundant in BALB/c mice at baseline and (2) induced in response to *T. cruzi* in BALB/c mice but not in C57BL/6J mice (Figure 5B). This suggests that expression patterns of the genes that we have identified in our analysis are, at least in part, responsible for differences in susceptibility of the two strains.

DISCUSSION

Although clinically evident myocarditis manifested by heart failure and/or sudden death caused by arrhythmias is uncommon, acute myocarditis can be a source of significant morbidity and mortality in humans when present. Furthermore, the contributions of the myocardial parasitic burden, the degree of inflammation, and the host immune-mediated response during the acute infection to the development of CCC are unknown.

To better understand the host susceptibility to acute myocarditis, our study used an animal model to identify novel host susceptibility factors.

As anticipated based on previous reports, Balb/c mice were susceptible and C57BL/6J mice were resistant to infection with *T. cruzi*.⁹ The increased mortality in Balb/c mice was not characterized by a more pronounced weight loss. However, weight loss is not always an accurate measure of morbidity in acutely infected mice, because loss of solid tissue mass can be masked by an increase in weight caused by fluid retention related to cardiac/renal functional impairment. Although plasma troponin and BNP levels and myocardial infiltration were not different between infected Balb/c and C57BL/6J mice, they were significantly higher in infected compared with non-infected Balb/c mice (but not C57BL/6J mice). The difference in parasitemia between strains may be biologically very significant, because parasite numbers were several times higher in Balb/c mice. This difference could also, in part, explain the worse myocardial response. The absence of statistical significance likely reflects the small number of mice studied. Greater mortality in Balb/c mice may indicate a more severe septic inflammatory state and extracardiac organ injury (e.g., renal failure) in this strain. However, the CO was not increased; this finding goes against a hyperdynamic state characteristic of severe sepsis. As an alternative, intraventricular conduction abnormalities have been described in mice infected with *T. cruzi*,¹³ eliciting arrhythmias and sudden death as possible causes of mortality.

According to most measurements, myocarditis affected both types of strains in a similar fashion. However, we noted some relevant hemodynamic differences between these strains of mice during acute Chagasic infection; there were limited decreases in HR, SV, and therefore, CO ($\text{CO} = \text{HR} \times \text{SV}$) in Balb/c mice.

TABLE 1
List of significantly differently expressed genes between the two infected strains of mice

Entrez gene name	Symbol	Location	Type(s)	FDR	Fold Δ
Major histocompatibility complex, class II, DR α	HLA-DRA	Plasma membrane	Transmembrane receptor	4.68E-04	77.01
Guanylate binding protein 2, interferon-inducible	GBP2	Nucleus	Other	3.69E-02	27.371
BAI1-associated protein 2-like 1	BAIAP2L1	Cytoplasm	Other	4.64E-02	9.989
Histidine ammonia-lyase	HAL	Cytoplasm	Enzyme	3.08E-02	7.805
RIKEN cDNA D630045M09 gene	D630045M09Rik	Unknown	Other	3.69E-02	7
Family with sequence similarity 227, member B	FAM227B	Unknown	Other	4.95E-02	6.536
Neuronal cell adhesion molecule	NRCAM	Plasma membrane	Other	4.93E-02	5.576
NOP56 ribonucleoprotein	NOP56	Nucleus	Other	4.01E-02	4.945
Synapsin III	SYN3	Plasma membrane	Other	3.74E-02	4.896
Exostosin-like glycosyltransferase 1	EXTL1	Cytoplasm	Enzyme	2.21E-02	4.67
CUE domain containing 1	CUEDC1	Unknown	Other	3.69E-02	4.429
Histone cluster 2, H4	Hist2h4	Nucleus	Other	5.41E-03	3.975
Neural cell adhesion molecule 1	NCAM1	Plasma membrane	Other	1.76E-02	2.569
Acyl-CoA oxidase 2, branched chain	ACOX2	Cytoplasm	Enzyme	4.07E-02	2.462
Nucleolar protein 12	NOL12	Nucleus	Other	5.46E-03	2.441
Mitochondrial fission regulator 1	MTFR1	Cytoplasm	Other	4.15E-02	2.418
Matrix Gla protein	MGP	Extracellular space	Other	1.47E-02	1.992
Dynein, light chain, Tctex-type 1	DYNLT1	Cytoplasm	Other	5.17E-03	1.981
Protein phosphatase 1, regulatory (inhibitor) subunit 14B	PPP1R14B	Cytoplasm	Phosphatase	1.76E-02	1.92
Procollagen C-endopeptidase enhancer	PCOLCE	Extracellular space	Other	1.59E-03	1.835
Kelch-like family member 40	KLHL40	Unknown	Other	4.76E-02	1.783
RIKEN cDNA 4930511J24 gene	4930511J24Rik	Unknown	Other	4.44E-02	1.743
Vacuolar protein sorting 52 homolog (<i>S. cerevisiae</i>)	VPS52	Cytoplasm	Other	4.15E-02	1.696
Activating transcription factor 5	ATF5	Nucleus	Transcription regulator	4.93E-02	1.68
Decorin	DCN	Extracellular space	Other	2.83E-02	1.587
Solute carrier organic anion transporter family, member 3A1	SLCO3A1	Plasma membrane	Transporter	4.71E-02	-1.673
Coiled-coil-helix-coiled-coil-helix domain containing 2	CHCHD2	Cytoplasm	Other	1.76E-02	-1.708
CD59a antigen	Cd59a	Plasma membrane	Other	1.52E-02	-1.977
Clusterin	Clu	Cytoplasm	Other	3.86E-02	-2.009
Diacylglycerol O-acyltransferase 2	DGAT2	Cytoplasm	Enzyme	2.97E-02	-2.055
$\beta\gamma$ -crystallin domain containing 3	Crybg3	Unknown	Other	4.03E-03	-2.129
CDC42 effector protein (pGTPase binding) 1	CDC42EP1	Extracellular space	Other	4.07E-02	-4.373

FDR = false discovery rate q values; fold Δ = fold change in gene expression difference of Balb/c versus C57BL/6J mice.

SV is determined by contractility, pre-load, and afterload. Therefore, the possible mechanisms for the observed decreased CO in Balb/c mice are a reduction in HR, a decrease in contractility, a decrease in pre-load (intravascular volume), or a combination thereof. However, preload is least likely given the finding that the differences in CO remained after adjustment for weight (a surrogate for hydration and intravascular volume), thus leaving the reduction in HR and decreased contractility as the more likely mechanisms. No differences were seen in cardiac hypertrophy (CH) between the two strains; this could be because of the insensitive detection of CH by echo or because more time was needed for its development. Pathology findings suggest a trend toward increase myocardial cellular infiltration in Balb/c mice, which may partly explain the observed decrease in myocardial contractility.

The most important finding of our study is the involvement of the AKT gene network in host response to acute Chagasic infection in the myocardium. AKT is a serine-threonine protein kinase that regulates key cellular signaling cascades, such as cell growth and survival and glucose and cardiovascular homeostasis. *T. cruzi* targets AKT in host cells as an intracellular antiapoptotic strategy.¹⁴ Similarly, *Akt* overexpression produces CH and a remarkable increase in cardiac contractility.^{15,16} Finally, AKT also mediates the activation of endothelial nitric oxide (NO) synthase, leading to increased NO production,¹⁷ and its inhibition produces hypotension and bradycardia.¹⁸ Additionally, within the AKT network, neural cell adhesion molecule 1 (NCAM1) has been identified as overexpressed in Chagas

myocarditis and may act as receptor for tissue targeting and cellular invasion by *T. cruzi*.¹⁹ Several other genes from this network have also been implicated in cardiac function and inflammation (Table 2).

Based on the already described cardiovascular effects of the AKT gene, it seems that its up-regulation may be beneficial during acute Chagasic myocarditis for the host and perhaps, the parasite as well.¹⁴ The phenotypic differences in CO and contractility between the two strains may be driven by the regulation of this network. Although its up-regulation may be beneficial during acute infection, it may have completely different implications for chronic Chagasic cardiomyopathy. Additional studies based on AKT modulation (genetic or pharmacologic) on acute or chronic models of infection may prove useful to determine its potential role in cardiac phenotype alteration during Chagasic myocarditis. Additionally, nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) in the center of the network may also have important implications for responses to stress or parasite antigens. Polymorphisms of the NFKB1 gene have been implicated in susceptibility to left ventricular dysfunction and heart failure.^{20,21} Other differentially regulated networks, centered around HLA-DR and ubiquitin C may suggest important differences in antigen recognition and intracellular parasitic protein amastigote assembly and degradation and may increase immune recognition of cardiac oxidized antigens.^{22,23} However, differences in HLA-DR might be a reflection of its great genetic variability and therefore, difficult to interpret.

FIGURE 5. Gene expression analysis of BALB/c and C57BL/6J heart tissues in response to *T. cruzi* infection. (A) Akt gene network. Genes are displayed with symbols. Red indicates up-regulated genes and green indicates down-regulated genes in the BALB/c to C57BL/6J mice. A continuous line indicates a direct interaction; dashed lines are indirect interactions. (B) Representative dot plot of expression levels of *Ncam1* in heart tissue.

Although previous studies have been done for heart gene expression during *T. cruzi* infection, most of them analyzed chronic myocardial changes and compared them with non-infected groups. Changes observed during acute infection involve up-regulation of the immune system (interferon-induced), HLA class II, fibroblast growth factors (FGF 15 and 12), cell cycle (Cyclin E), cytoskeleton genes, and deficiency of mitochondrial oxidative phosphorylation.^{24,25} In contrast, chronic infection is associated with up-regulation of immune inflammatory responses (chemokines, adhesion molecules, cathepsins, and major histocompatibility complex molecules) and fibrosis (extracellular matrix components, lysyl oxidase, and tissue inhibitor of metalloproteinase 1).²⁶

There are several limitations to our study. The echocardiogram and heart histology findings at 4 weeks may reflect a survival effect, particularly among Balb/c mice. Thus, sepsis

could still be an earlier cause of mortality. Diagnosing and grading the severity of myocarditis are still a challenge because of the lack of uniformity and consensus. As pointed out above, CO and contractility have several determinants that are difficult to take into account based on the available data. Microarray data can produce erroneous conclusions, and these findings need to be followed up with some functional or protein-based approaches. Finally, human studies must be performed to correlate the importance of the *AKT* gene network in the outcome of acute Chagasic infection and the role of the host-mediated immunity in the development of CCC.

CONCLUSIONS

Differences in mortality, myocardial contractility, and HR were seen among different strains in a murine model of acute

TABLE 2
Selected genes implied in cardiac function and inflammation

Symbol	Documented associations
EXTL1	Function in the chain polymerization of heparan sulfate and heparin
NCAM1	Overexpression of NCAM in Chagas myocarditis; NCAM may act as a receptor for tissue targeting and cellular invasion by <i>T. cruzi</i> ; marker for cardiomyocytes apoptosis, remodeling
MTFR1	Mouse homolog protects cells from oxidative stress
MGP	Matrix Gla protein system dysregulation could be involved in left ventricular dysfunction in patients with chronic HF; progression of subclinical coronary atherosclerosis
KLHL40	Expression is increased by Adcyap1 (which might play a role in ischemic heart disorders)
ATF5	Stress remediation
DCN	Matrix assembly; angiogenesis, cardiac remodeling, inhibit TGF-B1, indirectly inhibited by CD82
CLU	Cell survival, cardiomyocytes, ischemic-induced death protection
CDC42EP1	Mediates actin cytoskeleton reorganization at the plasma membrane

Chagasic infection. These specific phenotypic features of cardiac response during acute Chagasic myocarditis may be, in part, related to host AKT network regulation. Therefore, our study confirmed previously described differences in mouse strain susceptibility to *T. cruzi* infection but also, identified additional differences in measures of acute myocarditis. Importantly, several of the genes in the AKT network might prove to be useful biomarkers for estimation of acute myocarditis severity and/or increased risk of CCC development.

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