Cardiomyopathy in a murine model of AIDS: evidence of reactive nitrogen species and corroboration in human HIV/AIDS cardiac tissues

Alysia A. Chaves a,b, Michael J. Mihm a,b, Brandon L. Schanbacher a,b, Anupam Basuray a, Cynthia Liu a, Leona W. Ayers c, John Anthony Bauer a,b,d,*

a Center for Developmental Pharmacology and Toxicology, Columbus Children’s Research Institute, Columbus, OH 43205, USA
b Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA
c Department of Pathology, College of Medicine, The Ohio State University, Columbus, OH 43210, USA
d Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, OH 43210, USA

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Abstract

Objective: Cardiomyopathy and other vascular complications are now recognized as significant components of HIV/AIDS pathogenesis. Although the mechanisms involved in cardiomyopathy are poorly defined, a role for direct retroviral action and/or focal infiltration of activated immune cells have been postulated. Here we investigated mechanisms in retrovirus associated cardiomyopathy using a well-defined mouse model of acquired immunodeficiency. Methods: Mice were dosed with LPBM5 retrovirus; cardiac performance was assessed by echocardiography followed by tissue collection at 5 and 10 weeks post-infection. Results: Contractile deficits were observed at 5 and 10 weeks post-retrovirus infection and preceded the development of overt immunodeficiency. Selective and widespread cardiac infiltration of CD68+ cells, but not neutrophils, mast cells, or eosinophils was also observed at both 5 and 10 weeks. LPBM5 retrovirus was readily detectable in cardiac samples by RT-PCR. Time dependent increases in cardiac protein nitration (biomarker of reactive nitrogen species) were observed and were correlated to the extent of cardiac dysfunction whereas no changes in NOSII occurred at 5 and 10 weeks. We corroborated the mouse findings using cardiac tissues and clinical findings from human HIV/AIDS autopsies. Conclusions: These studies demonstrated that cardiac myocyte protein nitration in AIDS related cardiomyopathies, rather than focal immune cell lesions characterize retrovirus associated cardiomyopathies and differentiate them from non-retroviral cardiomyopathies.

Keywords: Nitric oxide; Reactive nitrogen species; HIV; AIDS; Cardiomyopathy; Retrovirus

1. Introduction

Following the advent of highly active antiretroviral therapy (HAART) regimens, AIDS-free living and overall survival have significantly improved for patients infected with human immunodeficiency virus (HIV) [1]. However, a variety of HIV-related complications have become increasingly evident in this population. For example, HIV-related pulmonary, neurological, and cardiovascular complications represent important contributors to the overall morbidity and mortality of HIV/AIDS patients [1,2]. HIV-related cardiovascular disease was first recognized in the early 1980s, primarily via autopsy cases in which myocarditis and cardiac inflammatory lesions were observed in hearts from AIDS patients with no documented evidence of cardiovascular disease [3,4]. Later studies demonstrated that HIV/AIDS patients develop an array of cardiovascular pathologies, ranging from left ventricular deficits in pre-symptomatic seropositive individuals to pulmonary hypertension, dilated cardiomyopathy and congestive heart failure [5,6]. The prevalence of cardiac complications in

*Corresponding author. Tel.: +1-614-722-2835; fax: +1-614-722-2774.
E-mail address: bauerj@pediatrics.ohio-state.edu (J.A. Bauer).

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HIV patients has been estimated to be as high as 80%, with an estimated 5–20% progressing to dilated cardiomyopathy and failure [1.7]. As the worldwide incidence of AIDS approaches an estimated 5,000,000 new cases annually, HIV-related cardiovascular disease may become a significant etiology of cardiac failure [8,9]. The mechanisms by which this unique form of cardiovascular disease develops and its relation to more traditional settings (non-retroviral) of cardiomyopathy remains incompletely defined. As a result, no specialized therapeutic approaches for this patient population currently exist.

Unfortunately, the study of relationships between human HIV infection and cardiac alterations is often complicated by the use of various drug therapies, illicit drugs known to be cardiotoxic, limited tissue availability and variable disease progression [10]. Demonstration of relevant animal models for the investigation of retrovirus-related cardiac dysfunction and pathologies could provide opportunities for further mechanistic insight and therapeutic intervention. In this study, we employed a well-established murine model of retroviral infection (LPBM5 virus) and defined the time-dependencies of retroviral progression and cardiac dysfunction, in an attempt to extend its relevance as a disease model. The LPBM5 model of retroviral infection is commonly called the ‘murine AIDS’ model, due to its high similarity to many of the immune-related complications seen during human HIV infection, including aberrant cytokine release, changes in T-cell populations, and increased susceptibility to opportunistic infections (for a detailed review see Watson 1989 [19]).

An additional goal was to define the contributions of nitric oxide (NO)-related biochemistry during murine retrovirus related cardiac dysfunction. Under normal physiological conditions, NO is known to modulate myocardic contractility and blood flow distribution [11]. In contrast, high levels of NO production (via inducible nitric oxide synthase, NOSII) are associated with several forms of cardiac disease, and have recently been implicated in human HIV-related cardiomyopathy [12]. Once formed, NO rapidly reacts with available superoxide anion to form peroxynitrite, a reactive nitrogen species known to conduct several oxidative reactions in a cellular environment [13]. Of these, nitration of protein tyrosine residues, producing 3-nitrotyrosine, is an unique feature of reactive nitrogen oxide synthase, NOSII are associated with several forms of cardiac disease, and have recently been implicated in human HIV-related cardiomyopathy [12]. Once formed, NO rapidly reacts with available superoxide anion to form peroxynitrite, a reactive nitrogen species known to conduct several oxidative reactions in a cellular environment [13]. Of these, nitration of protein tyrosine residues, producing 3-nitrotyrosine, is an unique feature of reactive nitrogen species when compared to actions of reactive oxygen species (e.g., superoxide anion, hydroxyl radical, etc.) [14].

We and others have demonstrated that reactive nitrogen species formation may be an important participant in the initiation and/or progression of a wide array of cardiac pathologies and that nitration of protein tyrosine residues can inhibit cardiac enzyme function, and promote cardiac myocyte death via necrosis and/or apoptosis [15–17]. In addition, peroxynitrite mediated protein nitration has previously been demonstrated in the setting of acute viral myocarditis, but its contribution to cardiac dysfunction during retroviral pathogenesis has not been established [18]. Therefore, we tested the hypotheses that cardiac NOS II expression and/or cardiac protein nitration are associated with time dependent cardiac dysfunction during murine AIDS progression.

To corroborate our experimental findings in the murine AIDS model, we explored the relevance of our experimental results in a sample of cardiac autopsy tissues from HIV/AIDS patients with dilated cardiomyopathy and relevant controls.

2. Methods

2.1. Murine AIDS model and study design

All aspects of our animal use were in accordance with the guidelines of the National Institutional of Health and approved by the Institutional Animal Care and Use Committee. Active LP-BM5 virus was prepared according to the methods of Watson et al. [19]. Retrovirus-containing cell-free supernatant was collected from infected SCI/ MuLV cells (AIDS Research and Reference Reagent Program, Bethesda, MD, USA), and concentrated by centrifugation (Advanced Biotechnologies, Columbia, MA, USA). Titers of esotropic MuLV were determined by the standard S’L plaque assay [20] and by units of reverse transcriptase activity using a commercially available kit (Boehringer Mannheim, Germany).

Pathogen-free female C57BL/6 mice (Harlan Laboratories, Indianapolis, IN, USA) were housed in a sterile cage rack system with HEPA filtered air circulation (approximately 50 air changes/h, Allentown Caging, Allentown, PA, USA). Following 1–2 weeks of acclimatization LP-BM5 retrovirus was dosed via a single intraperitoneal injection (100 μl dose containing 200 reverse transcriptase units). Control animals received an identical injection of vehicle. At the time of injection all mice were 6 weeks old and weighed 16–18 g.

Cardiac performance was assessed by echocardiography, as previously described [21], at selected times throughout 10 weeks of retroviral infection (8–12 per group). To account for time-variations, animal age and body weight, two groups of control animals were studied at 0 and 10 weeks post injection (n = 6 in each group). Immediately following echocardiography, animals were sacrificed by pentobarbital overdose. Spleen weight was measured as an index of retroviral progression, as documented by others [19,22].

2.2. Reverse transcriptase-polymerase chain reaction (RT-PCR) for LPBM5

Total RNA was isolated from frozen cardiac and splenic tissue (Trizol, Gibco BRL). RNA quantity was monitored at 260 nm. RNA integrity was verified by fractionating on agarose gels. Two μg of total RNA was reverse transcribed.
to cDNA (cDNA cycle kit, Invitrogen) in 20 µl reverse transcription reaction mix at 42 °C (60 min). The reaction was stopped by heat inactivation at 95 °C (2 min) and chilled on ice. Subsequently 5 µl of the resulting cDNA was amplified using primers specific for the p12 region in the gag gene in the LP-BM5 genome [23] and β-actin [24]. The RTV primers: sense primer, 5’T-CTTATGCACTTCCCCCT-3’; antisense primer: 5’T-CCGCCCTCTTCTAAGTGC-3’, similarly β-actin primers: sense primer: 5’T-ATGGATGACGATATCGCT-3’; antisense primer: 5’T-ATGAGGTAGTCTGCTAGGT-3’ were used in PCR reactions under the same conditions which included an initial denaturation at 95 °C (5 min), followed by a cycle of denaturation (95 °C/1 min), annealing (55 °C/1 min), and extension (72 °C/1 min). Each sample was subjected to 30 cycles followed by a final extension (72 °C/10 min). PCR products were separated and visualized on a 2% agarose ethidium bromide stained gel. Band intensity was assessed using imaging software (UVP-Labworks analysis), normalized to β-actin expression in each tissue. Cardiac RTV expression was expressed as a percentage of splenic RTV expression.

2.3. Blood sampling and analysis

Whole blood was collected at sacrifice. Liver, pancreatic and muscle enzyme activity (AST, ALT, lipase and amylase), as well as total cholesterol and triglyceride levels were measured. Complete blood chemistries using whole blood smears were used to measure eosinophils, neutrophils, monocytes and lymphocytes (Antech diagnostics).

2.4. Histology and immunohistochemistry

Following functional analyses, hearts were rapidly isolated, equatorially sectioned (mitral valve), and processed for immunohistochemical studies using standard protocols [17]. Cardiac and splenic cross-sections were stained using hematoxylin/eosin and Masson’s trichrome for routine morphologic and histologic assessments. Cardiac 3-nitrotyrosine (anti-3NT antibody, Upstate Biotechnology, Lake Placid, NY, USA, 1:400 dilution) and nitric oxide synthase 2 (anti-NOS2, Transduction Labs, Lexington, KY, USA, 1:400 dilution) were assessed in cardiac cross-sections [17]. Staining controls included antibodies preadsorbed with purified 3NT or murine NOS II; addition of antigen eliminated positive staining in each case, demonstrating antibody specificity. Histological stains for mast cells (Astra Blue stain, Sigma, St. Louis, MO, USA) and eosinophils (Vital Red stain, Sigma, St. Louis, MO, USA), as well immunohistochemical probes for neutrophil (anti-myeloperoxidase (MPO) antibody, Neomarkers, 1:2000 dilution) and monocyte/macrophage presence (anti-CD68+, Neomarkers, 1:400) were employed. In additional studies, NOSII stained tissues were assessed for NOSII positive cell bodies as well. Diaminobenzidine (0.06% w/v) was used to provide visualization of immunoreactivity, with methyl green counterstaining.

2.5. Digital image analysis

Digital images were acquired using a Polaroid DMC camera and Olympus microscope (model BX40) and transferred to Image Pro Plus software (Media Cybernetics, Silver Spring, MD, USA) for both area and intensity analyses. Total cross-sectional images of spleen and heart were captured at 4× magnification and circumferential traces were used to calculate tissue areas. Images from cardiac samples assessed for specific immune infiltrates (MPO+, CD68+, mast cells, NOSII+) were captured at 400× magnification, positive cells were segmented and gated based on size, then counted and expressed per tissue area. In NOSII and 3NT studies, left ventricular images were captured at 400× and relative intensity was determined using image threshold analysis, as we have previously described [17].

2.6. HIV/AIDS cardiac specimens

Left ventricular sections (LV anterior wall) were obtained as paraffin-embedded autopsy specimens from AIDS patients and non-HIV infected controls from the National Cancer Institute AIDS Cancer and Specimen Resource. All autopsy samples studied were collected within 4 h of death. Autopsy samples were collected between 1983 and 1998. Patient histories were reviewed to subclassify samples into two groups: HIV infected patients with documented evidence of dilated cardiomyopathy (HIV-DCM, n = 4) and non-HIV infected patients with no evidence of cardiac disease (CTRL, n = 3). HIV/AIDS patients with confounding risk factors for cardiovascular disease (smoking, diabetes, protease inhibitor therapy, etc.) were omitted from analysis. Age at autopsy was not different between patient groups (CTRL: 40±2.3 yrs, HIV-DCM: 36.5±3.2yrs, P = NS), and was not a significant factor for cardiovascular disease. Average ejection fraction in HIV+ patients with dilated cardiomyopathy was 27.5±7.5%, mean CD4+ counts were 123.5±46.5 cells/mm³. These cardiac specimens were then assessed for evidence of NOS II and protein-3NT, using immunohistochemical methods identical to those described above.

2.7. Statistical analysis

All data presented represent 6–12 observations per group. Statistical comparisons were made by one-way ANOVA’s with Student–Newman–Keuls post hoc tests. Significant correlations were assessed using Spearman’s non-parametric correlation analysis. A total of 30–35 data points were used for each regression analysis (CTRL, 5, 10 weeks RTV), providing statistical power >0.95 at r² = 0.5 and α = 0.05. P < 0.05 defined statistical significance.
3. Results

3.1. Murine AIDS studies

Two groups of control animals were studied at 0 and 10 weeks post-vehicle injection to account for time-variations, animal ages and body weights. In all parameters presented below, no statistically significant differences were observed in these two control groups. Therefore, they were pooled for further comparisons to retrovirus-infected mice.

3.2. Splenomegaly and cardiac expression of LP-BM5 during murine retroviral pathogenesis

Splenomegaly is consistently observed in murine AIDS and used as a marker of retroviral progression and immune dysfunction [19]. Representative mid-line splenic cross sections from control and LPBM5-treated mice at 10 weeks post-injection are shown in Fig. 1A (Upper panel), illustrating increases in organ size and disordered organ architecture (hematoxylin and eosin staining shown). Total spleen weight in control vs. retrovirus infected animals at sacrifice are shown in Fig. 1A (lower panel). Spleen weight was significantly increased, indicating retroviral progression and immune dysfunction. Additional staining with Masson’s trichrome did not reveal evidence of splenic fibrosis (data not shown).

LPBM5 infectivity is facilitated by the gag, pol, and env genes [25]. The p12 region in the gag gene is unique to the LPBM5 virus and important to infectivity [26]. Shown in Fig. 1B (Upper panel) are representative RTV expression bands in cardiac and splenic tissue from RTV infected mice at 5 and 10 weeks. The 229bp RT-PCR product, specific for the p12 gag region of LPBM5, was confirmed by restriction enzyme analysis with Sma I (data not shown). Viral load in cardiac tissue (expressed as percentage of splenic viral load) is depicted in Fig. 1B (Lower panel). Virus was present in both cardiac and splenic tissue (homogenates) in RTV-treated animals at 5 and 10 weeks post-treatment; cardiac load was approximately 50% of splenic load (normalized to β-actin) at each time point.

3.3. Blood leukocytes during retroviral pathogenesis

LPBM5 infection results in an immunosuppressive profile that closely resembles human AIDS. Shown in Fig. 2 are average total circulating blood monocytes, neutrophils, lymphocytes and eosinophil counts from control and retrovirus infected animals. Total levels of monocytes in whole blood were reduced at 5 and 10 weeks post infection with RTV (Fig. 2, Upper left panel). In contrast, circulating levels of neutrophils, lymphocytes and eosinophils remained unchanged.

3.4. Cardiac morphology and histology

Shown in Fig. 3 are representative photomicrographs of equatorial cardiac cross-sections from control and RTV-treated mice at 10 weeks. Hearts from RTV-treated mice developed a hypertrophic response by 5 weeks, which persisted throughout the study (Fig. 3, middle panel). LPBM5-treated animals did not develop significant cachexia until after 10 weeks; the increase in normalized heart weight was not artifactualy caused by body weight loss.

Fig. 1. Splenomegaly and increased viral load in cardiac and splenic tissue during the progression of murine AIDS. Panel A: Upper panel–representative Hematoxylin and Eosin mid-line splenic cross sections of a control mouse and RTV-infected mouse at 10 wks. Lower panel–average data for total spleen weight in control vs. retrovirus infected animals at sacrifice. Data are expressed as mg per g of body weight (n = 6–12). *, P < 0.05 as compared to control. Panel B: Upper panel–representative images of retroviral RT-PCR products from cardiac (C) and splenic (S) tissue in mice infected with LPBM5. Lower panel–total cardiac viral load at 5 and 10 weeks post RTV infection (expressed as a percentage of splenic RTV expression). Data is represented as mean±S.E.M. (n = 3–6); *, P < 0.05 as compared to control.
significant increase in LV cross-sectional area (at the level of the mitral valve) was observed at 5+ weeks post infection. This measure of LV mass (roughly a 25% increase from control) paralleled changes in total heart weight observed at 5 and 10 weeks.

3.5. Time-dependent reduction in cardiac performance during murine AIDS

Cardiac performance was assessed at 5 and 10 weeks using echocardiography (Fig. 4). No difference in heart rate was detected at any time point. Murine AIDS was associated with progressive decrements in left ventricular (LV) fractional shortening, cardiac output, and stroke volumes. At 10 weeks retroviral infection, reductions in these parameters were approximately 15, 30, and 35% respectively, relative to control (Fig. 4, Lower panel). Reductions in fractional shortening were predominantly related to increases in LV end systolic dimension (1.72±0.04 vs. 1.60±0.02 mm at 10 weeks, RTV vs. control).

3.6. Cardiac immune cell prevalence during retroviral pathogenesis

Leukocyte infiltration into cardiac tissue plays an important role in promoting and sustaining infectious cardiac diseases [27]. Shown in Fig. 5 are averaged data of cardiac infiltrating immune cells as measured by histochemical techniques. Nominal presence of neutrophils and eosinophils were detected at 10 weeks post-infection, whereas cardiac infiltration of CD68+ cells (monocytes/macrophages) was significantly increased at 10 weeks post-infection, compared to controls (Fig. 5, Upper middle panel). Cardiac presence of neutrophils, eosinophils, and mast cells were not significantly elevated with retrovirus infection (P = NS). The levels of CD68+ and neutrophils (MPO+ cells) were not measured at 5 weeks post-infection, due to technical problems. Although changes in immune cell prevalence were detected by our imaging approach, we observed limited evidence of classical focal lesion sites in any region of the cross-sectional areas studied.

3.7. NOSII immunohistochemistry and cardiac protein nitration

Representative photomicrographs of cardiac NOS II and protein-3NT immunoprevalence during murine AIDS are shown in Fig. 6, where darker staining indicates positive immunoprevalence. NOSII content was not significantly
altered in cardiac myocytes at 5 and 10 weeks post-infection, whereas sustained and extensive cardiac myocyte protein nitration was observed at 5 and 10 weeks during retroviral infection ($P < 0.05$). Additional observation of the cross-sections revealed the presence of NOS II positive mononuclear cells, which was consistent with the general distribution of CD68+ infiltrates. Using automated cell counts by methods identical to those in Fig. 5, we found that NOS II positive cell bodies are significantly elevated in hearts from MAIDS mice at 10 weeks of retroviral infection, relative to control (NOS II positive cells: $14.8 \pm 2.1 \text{ vs. } 21.0 \pm 3.0 \text{ cells/mm}^2$, CTRL vs. 10 week RTV, $P < 0.05$). The relative increases in these NOS II positive cells matches that observed for CD68+ cells over the same time course (CD68+ cells: $14.8 \pm 2.2 \text{ vs. } 21.1 \pm 1.4 \text{ cells/mm}^2$), thus confirming that these NOS II positive cells are apparently monocyte/macrophage lineage. In contrast to this focal distribution of NOS II, immunoprevalence of 3NT was widely distributed throughout myocardium and was therefore dissociated from the distribution of NOS II.

### 3.8. Relationships of cardiac performance and immunohistochemical measures

Non-parametric correlation analyses were used to test for statistical relationships among in vivo cardiac functional parameters and immunohistochemical results. A total of 60 data points were used for each regression analysis, providing a statistical power of 0.986 at $r^2 = 0.5$ and $\alpha = 0.05$. Cardiac immunoprevalence of 3NT was statistically correlated to LV fractional shortening % ($r^2 = -0.600$, $P < 0.01$), maximal aortic flow velocity ($r^2 = -0.559$, $P < 0.01$), and LV cross-sectional area ($r^2 = +0.515$, $P < 0.02$). In contrast, no statistically significant
3.9. Human tissue investigations

3.9.1. Cardiac NOSII versus protein nitration

Shown in Fig. 7 are representative photomicrographs in human cardiac autopsy tissues from AIDS patients with...
non-ischemic dilated cardiomyopathy (HIV-DCM) and seronegative controls with no documented evidence of cardiac disease. Identical to our mouse studies, we observed striking evidence of cardiac myocyte protein-3NT formation, with no corresponding increases in NOSII presence. Similar to the murine studies described above, NOS II positive mononuclear cells were detected among sial; a few recent studies have documented its presence in non-ischemic dilated cardiomyopathy (HIV-DCM) and was confirmed by RT-PCR. Interestingly, LPBM5 was found to be present in cardiac tissue as well. The retroviral load in cardiac tissue was substantial (approximately 50% of the splenic retroviral load), suggesting that retrovirus is likely infecting cardiac myocytes. The presence of HIV in cardiac myocytes themselves has been highly controversial; a few recent studies have documented its presence in human myocytes in vivo [31] and its potential to weakly infect rat cardiomyocytes, but not replicate, in vitro [32]. However, studies in simian models of retroviral infection have shown that SIV does not infect myocytes [33]. Our studies in cardiac homogenates did not establish the cell types infected, and cardiac load did not increase from 5 to 10 weeks, despite significantly worsening cardiac performance in RTV-treated mice. Further studies defining the direct role of cardiac myocyte infection in RTV-related dysfunction are ongoing in our laboratory.

LV systolic performance was significantly impaired within 5 weeks of infection, seen measured by LV fractional shortening and stroke volume. These early performance deficits occurred at a time known to precede overt immune deficiency. Cardiac dysfunction in this model, a phenomenon similar to observations in asymptomatic HIV patients [5]. Further declines in stroke volume and cardiac output were observed at 10 weeks—a 35–45% reduction in cardiac output is consistent with moderate to severe heart failure in mice. This biphasic decay of cardiac performance is similar to the presentation of cardiac dysfunction in HIV/AIDS patients and suggests that more than one mechanism may be involved [34–36]. The relationships between initial cardiac contractility deficits and later, more severe cardiac impairments are not well understood in this model or in humans. The identification of early markers of dysfunction, and a more complete understanding of their relevance to the development of progressive cardiac failure, may have predictive and/or preventative value in this patient population. Recent studies have also shown that the effects of relevant cardiotoxins (alcohol, cocaine, coxsackievirus B3) are aggravated by concurrent retroviral infection [37,38]. Thus, important interactions may exist in the development of RTV-induced cardiac injury in both mice and humans.

A pathogenic role for the immune system in acute cardiac injury as well as progressive cardiac and vascular disease is becoming increasingly apparent [27,39]. The interactions between immune cells and cardiac myocytes may have particular relevance in the setting of RTV- and AIDS-related cardiac disease. While LPBM5 infection was associated with a striking reduction in circulating monocytes, we observed selective infiltration of CD68+ cells into cardiac tissue, with no corresponding increases in the cardiac residence of any other leukocyte studied (neutrophils, mast cells, eosinophils). Monocytes/macrophages are carriers for both HIV and LPBM5, and recent studies in humans have demonstrated that trafficking of HIV-infected CD68+ cells to the heart occurs in patients with AIDS-
related cardiomyopathy [40,41]. Macrophages are high capacity production sites for reactive oxygen and reactive nitrogen species, and may contribute to cardiac oxidative damage in this setting [42]. The factors involved in the recruitment and/or activation of these immune cells to the heart remain unknown; further studies defining the molecular interactions and consequences involved are ongoing in our laboratory.

We observed a progressive increase in the cardiac presence of protein-3NT during the development of RTV-related cardiac dysfunction. In contrast, NOSII levels were not increased at any time point studied. In addition, significant associations between several measures of cardiac dysfunction and extent of cardiac 3NT immunoprevalence were detected, whereas no such relationships were observed for NOS II. These findings suggest that cardiac RNS formation and attendant protein nitration may participate in RTV-related cardiac pathology, rather than NOS II induction. This is consistent with the bimolecular reaction kinetics of RNS formation, and supports the under-appreciated concept that NOS II induction is not obligatory for promotion of RNS formation and the sustained presence of protein-3NT in vivo [15,17]. Staining patterns indicate that cardiac myocytes themselves carried a majority of the heart’s protein nitration burden—staining was widespread throughout the myocardium and was not confined to focal immune infiltrates. Further studies defining the putative sources for RNS and intracellular targets for protein nitration in this setting are ongoing in our laboratory.

An important consideration in these studies was our capacity to detect NOSII in situ—demonstrating that the lack of NOSII positive staining was not a ‘false negative’ result. While we observed only nominal evidence of NOSII prevalence in cardiac myocytes from murine RTV hearts, we did observe intense positive staining for NOSII in infiltrative cells. We therefore quantified these cell bodies in controls and at 10 weeks of RTV infection, using methods identical to our cardiac infiltrate counts. We found that the residence of NOSII positive infiltrates was increased at 10 weeks relative to controls, and that the relative prevalence in these cells paralleled that of CD68+ cells (NOS II positive cells: 14.8±2.1 vs. 21.0±3.0 cells/mm²; CD68+ cells: 14.8±2.2 vs. 21.1±1.4 cells/mm², CTRL vs. 10 week RTV). These studies illustrate our capacity for the in situ detection of NOSII in cardiac tissue, and further validate the unexpected spatial dissociation between cardiac myocyte protein nitration and NOSII prevalence. In contrast to our finding of statistical association between cardiac protein nitration and LV function, we detected no such relationship between NOS II intensities or positive cell prevalence and any of the functional parameters measured, thus suggesting that reactive nitrogen species may be more directly related to dysfunction than NO production capacity per se). Further studies defining the contributions of these infiltrates to the protein oxidative events and functional impairments in this setting are warranted, and are ongoing in our laboratory.

To validate our murine findings, we conducted further histopathological studies in human cardiac autopsy tissues from HIV seropositive patients with documented evidence of AIDS-related cardiomyopathy, that were in decompensated heart failure (average ejection fraction 27%). We observed striking increases in protein-3NT presence in cardiac tissue from patients with AIDS-related cardiomyopathy compared to HIV seronegative controls, while cardiac NOSII protein levels remained unchanged. These results are in opposition to recent results published by Barbaro et al., which showed that increased cardiac NOSII staining may be a consistent phenomenon in AIDS patients with cardiac disease [12,30]. As has been shown in experimental studies, important context- and time-dependencies may exist between the induction of NOSII (and other extramitochondrial oxidases) and the formation of protein-3NT in cardiac tissue. Consistent with our experimental studies, staining for protein-3NT predominated in cardiac myocytes themselves; immune cell infiltration may incompletely explain the oxidative events (and perhaps the cardiac myocyte dysfunction) that were observed in this setting.

In summary, LPBM5 infection, a well-established experimental model for the immunologic complications of HIV-infection, caused early cardiac contractility deficits and later, more severe cardiovascular impairment in mice, with a time course that preceded the development of overt immunodeficiency; the ‘murine AIDS’ model appears appropriate for the mechanistic study of RTV-induced cardiac complications. Although the infectious agent was not HIV itself, the murine AIDS model recapitulates many of the important features of AIDS-related cardiac disease in humans, suggesting that the systemic and/or cardiovascular response to RTV infection may mediate many of these changes, rather than direct effects of the virus itself. Although we observed dramatic changes in immune cell levels and trafficking patterns, a number of structural and biochemical alterations occurred in the cardiac myocytes themselves in RTV-treated animals. Immune cell interactions incompletely explain the global cardiac muscle changes that occur in this setting, and the cardiac myocyte itself may represent an important site of RTV-related pathogenesis. Here, we describe first-time evidence that cardiac RNS formation and oxidative injury may play a significant role in the development of cardiac complications in both the murine AIDS model and in a relevant population of AIDS-related cardiomyopathy patients. Further studies defining the sources and putative intracellular targets of these oxidants will provide important mechanistic insights, and may reveal new therapeutic opportunities for this unique and important cardiovascular disease setting.
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