AIDS-related vasculopathy: evidence for oxidative and inflammatory pathways in murine and human AIDS

Reshma S. Baliga,1,2 Alysia A. Chaves,2 Liang Jing,2 Leona W. Ayers,4 and John A. Bauer1,2,4

1Center of Cardiovascular Medicine, Columbus Children’s Research Institute, 2Division of Pharmacology, College of Pharmacy, College of Medicine and Public Health, and 4Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, Ohio

Submitted 29 March 2005; accepted in final form 25 May 2005

Vascular complications in HIV patients range from evidence of vascular lesions leading to advanced atherosclerosis (even in the absence of other risk factors) to early-onset acute coronary syndrome and evidence of endothelial dysfunction (even in immunocompetent HIV-positive patients) (1, 12, 15, 16, 20, 27, 31, 32, 42, 52). Furthermore, vascular complications during HIV infection may also contribute to the neurological and pulmonary complications already associated with HIV disease progression.

Despite substantial evidence of vascular abnormalities in HIV/acquired immune deficiency syndrome (AIDS) patients, the mechanisms involved and strategies for prevention are not well defined. The study of relations between human HIV infection and vascular alterations is often complicated by the use of various drug therapies, illicit drugs, limited tissue availability, and variable disease progression. Direct HIV infection of vascular cells (most notably vascular endothelium) remains controversial (9, 11, 24), and toxicities of HIV-associated proteins (gp120 and Tat protein), increased cytokine exposures, and, more recently, toxicities of HIV drug regimens have been suspected as participants (11, 28, 31, 43). Because most of these studies have been conducted in vitro, the in vivo setting of initial immune activation and subsequent deficiency during retroviral progression may not be recapitulated. For these reasons, we theorize that demonstration of relevant animal models for the investigation of retrovirus-related vascular dysfunction and pathologies would provide opportunities for further mechanistic insight.

In this study, we examined the potential value of a previously well-defined mouse model of retroviral infection (LP-BM5 virus), commonly called the “murine AIDS” model. This model exhibits many characteristics similar to human HIV infection, including splenomegaly, lymphadenopathy, hypergammaglobulinemia, and B cell hyperactivity at the early stages of retrovirus infection. Other similarities include aberrant and time-dependent cytokine release, changes in T cell populations, and progression to severe immune deficiency at ~20 wk after infection (29, 36, 46). Increased susceptibility to opportunistic infections and evidence of neurological abnormalities have also been documented, and we recently showed that this model recapitulates a dilated cardiomyopathy phenotype often observed in HIV patients (10, 21). In an attempt to extend its relevance as a disease model, we tested the hypothesis that time-dependent vascular dysfunction occurs in this well-defined murine model of retroviral pathogenesis.
It is now recognized that the vascular endothelium plays a major role in maintenance of cardiovascular health, and early impairment in this cell monolayer has been associated with, and, in some cases, may initiate many forms of disease (17, 30). We previously showed that endothelial cell impairment, in vivo or in vitro, may involve formation of reactive nitrogen species and altered nitric oxide (NO) bioavailability (35, 48, 49). Alterations in endothelial function have also been implicated in HIV-related vascular abnormalities. We therefore conducted specific mechanistic investigations to evaluate endothelial characteristics and inflammatory pathways as they relate to vascular function in the murine AIDS model. An additional component of the studies described here was our effort to relate changes we observed in this well-characterized murine model of AIDS to changes in vascular tissues, specifically coronary artery endothelium, in HIV/AIDS human autopsy samples.

METHODS

Murine AIDS model and study design. All aspects of our animal use were in accordance with the guidelines of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee. Active LP-BM5 virus was prepared according to the methods of Watson (45) and Zhang et al. (51). Retrovirus-containing cell-free supernatant was collected from infected SCI/MuLV cells (AIDS Research and Reference Reagent Program, Bethesda, MD) and concentrated by centrifugation (Advanced Biotechnologies, Columbia, MA). Titers of orthotropic MuLV were determined by the standard S’L plaque assay and by units of reverse transcriptase activity with the use of a commercially available kit (Boehringer Mannheim, Mannheim, Germany).

Pathogen-free female C57BL/6 mice (Harlan Laboratories, Indianapolis, IN) were housed in a sterile cage rack system with high-efficiency particulate-filtered air circulation (~50 air changes per hour; Allentown Caging, Allentown, PA). After 1–2 wk 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 wk old and weighed 16–18 g. Spleen weight was measured as an index of retrolental progression at the time of death, as documented by others (46).

Isolated vascular function studies. At 5 and 10 wk, animals were killed with a dose of pentobarbital sodium (100 mg/kg ip; Nembutal, Abbott Laboratories). Thoracic aortas were rapidly isolated for functional evaluations by methods similar to those previously described (49). Briefly, 2- to 3-mm segments of thoracic aorta were mounted on isometric force transducers (Grass Instruments, Quincy, MA) and incubated in 10-mL organ baths containing Krebs buffer bubbled with 95% O2 at 37°C. After 90 min of equilibration (resting tone = 1 g), maximal contractile force was evaluated for each segment with use of a high potassium concentration (modified Krebs buffer containing 90 mM KCl). Cumulative contractile responses were then evaluated for cumulative bath additions of phenylephrine (PE).

After precontraction with PE to 80% of maximum, relaxant responses to acetylcholine (ACh), an NO-mediated endothelium-dependent relaxant, and sodium nitroprusside (SNP), an NO-mediated endothelium-independent relaxant, were also determined. Concentrations and methods for cumulative dose-response curves were similar to those previously published by us (corresponding to 5.8e-11–7.7e-5 M for PE, 2.1e-11–2.8e-5 M for ACh, and 7.9e-12–1e-8 M for SNP) (33, 35, 49). To account for time variations, animal age, and body weight, two groups of control animals were studied before and 10 wk after injection (n = 6 per group). No statistically significant differences were observed in these two groups; hence, they were pooled for further comparisons with retrovirus-infected mice. Contractile and relaxant response data were fit to the four-parameter logistic equation using Graph Pad Prism software (Graph Pad, San Diego, CA).

After functional studies, the aortic segments were immediately weighed and then fixed in formalin, so that functional parameters could be related to the in situ staining results (see below).

Histology and immunohistochemistry. After functional analyses, aortas were rapidly processed for immunohistochemical studies using standard protocols. Aortic protein 3-nitrotyrosine (anti-3-NT antibody; Upstate Biotechnology, Lake Placid, NY; 1.5 μg/ml), intracellular adhesion molecule (anti-ICAM-1; Santa Cruz Biotechnology, Santa Cruz, CA; 1.0 μg/ml), and NO synthase (NOS) isoforms 2 and 3 (anti-NOS2 and anti-NOS3; Transduction Labs, Lexington, KY; 0.5 μg/ml each) were assessed in aortic cross sections. General morphology and evidence of fibrosis deposition were assessed using Masson’s trichrome stain (which stains cytoplasm red, collagen blue, and nuclei black) with a kit-based approach (Sigma Chemical). Staining controls included antibodies preadsorbed with purified 3-NT or murine NOS2; addition of antigen eliminated positive staining in each case, demonstrating antibody specificity. Diaminobenzidine (0.06% wt/vol) was used to provide visualization of immunoreactivity, with methyl green counterstaining.

Throughout the studies, care was taken to account for individual vascular tissue segments from each animal, allowing isolated segment functional responses to be directly related to immunohistochemical analyses from the same segment. This provided an opportunity to relate various vascular end points statistically by correlation analysis (see below).

Digital image analysis. Digital images were acquired using a Polaroid DMC camera and an Olympus microscope (model BX40) and transferred to Image Pro Plus software (Media Cybernetics, Silver Spring, MD) for area and intensity analyses. In protein 3-NT, NOS2, NOS3, and ICAM studies, aortic images were captured at ×800, and relative intensity was determined using image threshold analysis, as we previously described (49). Individual endothelial cells were analyzed for endothelial vs. surrounding smooth muscle immunostaining. Similarly, in human cardiac samples, images were captured around microvessels at ×800 to distinguish endothelial cells. Corroborative evidence was also obtained for murine coronary artery endothelial staining from images captured at ×800.

HIV/AIDS cardiac specimens. In an attempt to corroborate our animal studies in clinically relevant samples, we conducted in situ studies in human tissues. Left ventricular (LV) sections (LV anterior wall) were obtained as paraffin-embedded autopsy specimens from HIV/AIDS patients and non-HIV-infected controls (National Cancer Institute AIDS Cancer and Specimen Resource). All autopsy samples were collected between 1983 and 1998. Patient histories, including detailed autopsy reports and available clinical history information, were reviewed to subclassify samples into four groups: non-HIV-infected patients with no evidence of cardiovascular disease (HIV−/CVD−, n = 6), HIV-infected patients with no documented evidence of cardiovascular disease (HIV−/CVD−, n = 18), non-HIV-infected patients with documented evidence of cardiovascular disease (HIV+/CVD−, n = 7), and HIV-infected patients with documented evidence of cardiovascular disease (HIV+/CVD+, n = 7). The detailed autopsy report documented evidence of pericarditis (2 of 7), pericardial effusion (1 of 7), atherosclerosis (6 of 7), hypertrophy (2 of 7), and/or myocardial infarction (1 of 7) in the HIV−/CVD− group and myocarditis (2 of 27), pericarditis (5 of 27), pericardial effusion (9 of 27), atherosclerosis (4 of 27), hypertrophy (5 of 27), and myocardial infarction (1 of 27) in the HIV+/CVD+ group. If myocardial infarction was documented in the autopsy report, only regions of uninvolved myocardium were investigated. Age at autopsy was not different between patient groups [38 ± 6, 34 ± 6, 37 ± 8, and 38 ± 8 yr for HIV−/CVD−, HIV−/CVD+, HIV+/CVD−, and HIV+/CVD+, respectively, P = not
significant (NS) and was not a significant factor for cardiovascular disease. Average ejection fraction in HIV+/CVD+ patients was 27.5 ± 7.5% and mean CD4+ count was 123.5 ± 46.5 cells/mm³. Immunohistochemical methods identical to those described above were used to assess the microvasculature in the cardiac specimens for evidence of NOS2, protein 3-NT, and ICAM.

Statistical analysis. Values are means ± SE of 6–12 observations per group. Statistical comparisons were made by one-way ANOVA with Student-Newman-Keuls post hoc tests. Significant correlations were assessed using Spearman’s nonparametric correlation analysis. A total of 30–35 data points were used for each regression analysis (control and 5 and 10 wk of retrovirus), providing statistical power >0.95 at \( r^2 = 0.5 \) and \( \alpha = 0.05 \). \( P < 0.05 \) denoted statistical significance.

RESULTS

As described by other investigators and in our previous publication, time-dependent immunodeficiency was observed during LP-BM5 infection in mice, with substantial alterations at ≥10 wk (45–47). Progressive increases in spleen weight were indicative of disease progression (from 2.3 ± 0.1 mg/g body wt for control to 4.2 ± 0.6 mg/g body wt at 10 wk), and detectable levels of retrovirus were found in splenic and cardiac tissue homogenates at 5 and 10 wk, as we reported previously (10). Reduced circulating monocytes were also observed, similar to previous reports and as described in detail in our previous publication (8). In all aspects of this study of vascular tissues, control animals were studied at the first and last weeks of this protracted study; initial analyses of the control data showed no time-dependent differences in these two groups; therefore, these samples were combined for further statistical analyses.

Vascular contractile responses of isolated aortic ring segments from control mice and the murine AIDS model are shown in Fig. 1. The contractile response to tissue maximal depolarization (induced by 90 mM KCl) was significantly reduced at 1 and 5 wk in the murine AIDS model compared with the control response. The contractile response to cumulative concentrations of PE was also diminished in the murine AIDS tissues, with statistically significant reductions in maximal response (but not effective EC\(_{50}\) values) at 1, 5, and 10 wk during retroviral progression.

Isolated vasorelaxant responses in control and murine AIDS tissues are shown in Fig. 2. Maximal endothelium-dependent and NO-mediated relaxation responses induced by ACh were significantly reduced at 1 and 5 wk during murine AIDS progression, whereas increased EC\(_{50}\) was also observed at 10 wk. In contrast to the diminished endothelium-dependent response, no significant change in the concentration-dependent vasorelaxant response to SNP (an endothelium-independent NO-mediated vasorelaxant) was observed at any time throughout the study.

The distribution and prevalence of nitrated protein in control and murine AIDS vascular tissues are shown in Fig. 3. As previously reported, we observed modest basal protein 3-NT staining in control tissue in the endothelium and adjacent smooth muscle. Murine AIDS progression, however, resulted in extensive protein 3-NT staining, especially at 10 wk. Furthermore, this immunostaining appeared to be concentrated in the endothelial layer (Fig. 3C). Digital image analysis showed statistically significant increases in the endothelial layer at 5 and 10 wk during murine AIDS progression, with an apparent 10-fold increase from control levels. In contrast, increases in protein 3-NT were observed in the smooth muscle layer at 10 wk (Fig. 3C). Correlation analysis relating regional nitrination intensity to vascular function was also carried out (data not shown). Smooth muscle protein 3-NT prevalence was inversely correlated to the maximal PE response (\( r = -0.69, \ P < 0.01 \), as well as to the maximal KCl response (\( r = -0.2516, P < 0.05 \)). There was no significant correlation between the extent of endothelial 3-NT staining and the ACh maximal relaxation response (\( P = \text{NS} \)).

Immunohistochemical analyses of the distribution and prevalence of NOS3, NOS2, and ICAM are shown in Fig. 4. Using region-specific imaging techniques, we observed an increase of each analyte in the endothelial regions relative to adjacent smooth muscle in the same field of view as control tissues. As expected, NOS3 immunoreactivity was observed almost exclusively in the endothelium of control tissues and was increased at 1, 5, and 10 wk during murine AIDS. Correlation analysis also showed an inverse association between increased NOS3 immunostaining and ACh-induced maximal contraction (\( r = -0.2702, P < 0.05 \)).

Slight but detectable levels of NOS2 were apparent in control tissues, and this isoform was elevated in the endothelial and smooth muscle regions during retroviral infec-
tion. The increase in smooth muscle NOS2 prevalence was inversely correlated to the decrease in the contractile response to PE ($r = -0.72, P < 0.05$) and KCl ($r = -0.66, P < 0.05$), whereas increased endothelial NOS2 was not significantly correlated to the ACh maximal response. The endothelial activation marker ICAM was increased exclusively in the endothelial layer at 10 wk, and endothelial ICAM prevalence was also inversely correlated to the ACh maximal relaxation response (ACh $E_{\text{max}}$ vs. endothelial ICAM prevalence: $r = -0.3272, P < 0.05$).

In an attempt to corroborate our findings in the murine AIDS vascular tissues, cardiac specimens from HIV$^+/CVD^-$ (control), HIV$^+/CVD^-$, HIV$^+/CVD^+$, and HIV$^+/CVD^+$ tissues were investigated using immunohistochemistry and digital imaging techniques. Protein 3-NT, ICAM, and NOS2 were readily detectable in the tissues, and microvascular endothelial regions were specifically investigated using digital imaging techniques. Image analysis of the immunostaining showed increased endothelial 3-NT staining in the HIV$^+/CVD^+$ group, suggesting that the increased oxidative stress in the murine model is also seen in the human disease (Fig. 5). Increases in ICAM and NOS2 were also detected in endothelial regions in the HIV$^+/CVD^+$ group, as predicted by the murine model, but only ICAM staining was significantly different in the HIV$^-/CVD^+$ group.

**DISCUSSION**

Vascular complications were first identified in AIDS patients via observations of tissue samples at autopsy, but more recent studies suggest that initial changes are evident early in disease progression and in the absence of immunodeficiency (27, 42). Despite being recognized as an important complication in this patient group (1, 6, 9, 10), the mechanisms of vascular changes in HIV patients are not well understood and remain somewhat controversial. For example, although some studies have sug-

![Fig. 2](image1.png)

![Fig. 3](image2.png)
gested that HIV retrovirus can infect human cardiac and vascular cell types, it is unlikely that direct retroviral infection of these cells is obligatory for cardiovascular abnormalities (9, 11, 40). In various in vitro studies, investigators have shown that HIV-related proteins (gp120 and Tat protein) or components of highly active antiretroviral therapy [zidovudine (AZT) and HIV protease inhibitors] can be toxic to isolated cardiac or vascular cell types, although the concentrations required for toxicity have not always been physiologically relevant (6, 26). Furthermore, a key component of retroviral progression in humans and animals is an initial immune activation in response to pathogen (5), and this may play a central role in cardiovascular complications that occur in vivo. We recently found that the murine AIDS model recapitulates several key aspects of cardiac abnormalities documented in HIV patients, including decreased cardiac contractility, increased prevalence of infiltrative cell types, and evidence of oxidant-related pathways (10). Others have also shown that this model develops time-dependent immunodeficiency and several AIDS-related complications, including pulmonary infections, neurological changes, and increased tumor incidence (29, 36, 46). This model therefore has the following advantages: it has been used to investigate in vivo responses to retroviral infection, and it can recapitulate most features observed in HIV/AIDS patients. No previous studies have evaluated changes in the vasculature in this model.

Using isolated segments of vascular tissue, we investigated contractile and relaxant responses during LP-BM5 retroviral infection. Significantly reduced contraction responses to the nonspecific depolarizing agent KCl and the more specific /H9251-receptor agonist PE were observed at 1, 5, and 10 wk during infection, suggesting an early loss of vascular smooth muscle tone. Decreased vasorelaxant responses to ACh (an endothelium-dependent NO-mediated response) were also observed; in contrast, there were no changes in the relaxant response to the exogenous NO-mediated vasodilator SNP at any time point. Taken together, these data suggested decreased bioavailability of NO from the endothelium in these intact tissues. These changes in vascular function apparently preceded the time

Fig. 4. Changes in nitric oxide synthase (NOS) isoform distribution and intercellular adhesion molecule (ICAM) induction during murine AIDS. A: NOS isoform and ICAM distribution in control mouse aortic tissues. Regional optical densities were determined by automated digital imaging. NOS3, NOS2, and ICAM were more prevalent in the endothelial region than in adjacent smooth muscle in control tissues. B: relative change in NOS isoform and ICAM distributions during retroviral infection. Endothelium-specific increases in NOS3 were observed at 1, 5, and 10 wk, whereas NOS2 was increased in endothelial and smooth muscle regions during retroviral infection. ICAM-1 was only significantly induced later (10 wk). *P < 0.05 vs. control (0 wk).

Fig. 5. Evidence of oxidant and inflammatory activation in human microvascular endothelium during HIV/AIDS. A: representative images showing specific protein 3-NT immunostaining in human cardiac microvascular sections. B: histological examination of microvasculature from human autopsy samples: protein 3-NT (i), ICAM (ii), and NOS2 (iii) expression. CVD, cardiovascular disease. Statistically significant elevations were observed only in the HIV+/CVD+ patient group for 3-NT and NOS2 staining. ICAM immunostaining was elevated in HIV+/CVD+ and HIV-/CVD+ groups. *P < 0.05 vs. HIV-/CVD-.
period in which significant immunodeficiency occurs in this animal model, because many laboratories have shown significant immune dysfunction after 10 wk of retroviral infection (36, 46). Our observations of specific alterations of endothelium-dependent responses and vascular abnormalities that precede immunodeficiency are generally consistent with clinical evidence in HIV patients and further support the relevance of this animal model. Furthermore, because the LP-BM5 retrovirus is substantially different from HIV, it appears that mechanisms involving responses to viral pathogen, rather than the specific pathogen, may be key contributors to cardiovascular abnormalities in this setting.

Many studies have shown that NO bioavailability from the endothelium is a critical component of vascular homeostasis and that this process is indirectly modulated by the presence of reactive oxygen species (17, 30, 35, 39, 49). NO avidly reacts with superoxide anion to form peroxynitrite and other reactive nitrogen species; this reaction is diffusion rate limiting and can therefore reduce availability of endothelial NO for diffusion to smooth muscle and vasorelaxant effects (7). We and others have shown that nitration of protein tyrosine residues can be a sensitive and stable biomarker of reactive nitrogen species formation and dysregulation of NO in the endothelium (10, 23, 34, 44, 50). We therefore investigated the prevalence and distributions of protein 3-NT in vascular segments of control and murine AIDS tissues. In an attempt to delineate region-specific changes, we developed a digital image analysis approach to simultaneously investigate endothelial vs. adjacent smooth muscle regions. A statistically significant increase in protein 3-NT prevalence was observed during retroviral infection, with more prominent increases (~10-fold) in the endothelial layer than in smooth muscle. Inasmuch as we had also carefully cataloged tissues at the time of death, we were able to use nonparametric correlation analysis to investigate statistical associations between functional and immunohistochemical data in the same animal. A statistically significant correlation was observed between increased protein 3-NT and decreased contractile response to PE ($r = -0.69, P < 0.01$) and between increased smooth muscle protein 3-NT and decreased KCl response ($r = -0.2516, P < 0.05$). Endothelium-specific protein nitration was also statistically associated with diminished maximal response to ACh, suggesting that the in situ evidence of reactive nitrogen species was associated with functional evidence of diminished NO bioavailability.

Using the same region-specific immunohistochemistry and imaging approach, we tested the hypothesis that NOS isoforms are altered during retroviral progression and that these isoforms contribute to functional changes or reactive nitrogen species formation. The constitutive endothelial NOS isoform NOS3 was found almost exclusively in the endothelium and was upregulated in the endothelium at 5 and 10 wk. Correlation analysis also demonstrated an inverse correlation between NOS3 immunostaining and ACh maximal relaxation ($r = -0.2702, P < 0.05$), suggesting an uncoupling of NOS3 protein availability and ACh stimulation. An explanation for this contraintuitive finding could be an activated endothelium, leading to an increase in oxidant production in these aortas, which would result in more NO diverted to reactive nitrogen species pathways and less NO available for physiological functions. The finding of increased protein 3-NT in the aortas supports this hypothesis. Recent studies have shown that the regulatory aspects of NOS3 activity are complicated and that total NOS3 protein content is not necessarily an indicator of protein action. For example, NOS3 phosphorylation status, intracellular locations, and substrate availabilities play critical roles in actual endothelial NOS activity (18, 19, 41). Furthermore, reduced availability of arginine substrate, or cofactors, can convert NOS3 to an important source of oxidants and contribute to endothelial cell dysfunction/injury (25). Further studies investigating potential contributions of these mechanisms in this setting are warranted.

In contrast to the endothelium-specific prevalence of NOS3, the high capacity and calcium-independent isof orm NOS2 was increased in endothelial and smooth muscle regions during retroviral infection, and these increases were inversely associated with diminished contractile responses to KCl ($r = -0.66, P < 0.05$) and PE ($r = -0.72, P < 0.05$) but not maximal ACh vasorelaxant responses ($P = NS$). These findings suggest that the early phases of retroviral infection cause alterations in constitutive and inducible NOS isoforms in the vasculature, and this may contribute to endothelial oxidative injury and vasculopathy. Evidence of an activated endothelial state was provided by an endothelial activation marker, ICAM, which was increased at 10 wk during retroviral infection but demonstrated a statistically significant association with diminished endothelial function ($P < 0.05$).

Collectively, the data we observed in the murine AIDS model suggest that, during the early times preceding immuno-

![Fig. 6. Evidence of increased endothelial protein 3-NT in murine coronary vessels and murine aorta during murine AIDS. A: representative images showing specific protein 3-NT immunostaining in murine coronary vessels. B: histological examination of microvasculature from murine cardiac and aortic sections. Statistically significant elevation of protein 3-NT occurs in both vessels at later time points; $*P < 0.05$ vs. 0 wk.](http://ajpheart.physiology.org/Download/)
deficiency, a local vascular inflammatory/oxidative process occurs, predominantly in the endothelial region, and that these changes are apparently related to the observed vascular dysfunction. Although these specific mechanisms had not been previously investigated in HIV/AIDS tissues, each has been associated with vasculopathy and atherosclerosis in humans (26, 38). For example, atherogenesis and coronary artery plaque formation are now recognized as a localized inflammatory process, and several systemic markers of inflammation and cytokine activation (e.g., C-reactive protein and IL-6) have been shown to be associated with coronary heart disease risk and vascular disease progression (26, 38). Given these clinical observations and our findings in the murine AIDS model, we developed an approach to evaluate microvasculature in cardiac specimens from HIV/AIDS cases. We had unique access to a well-documented library of cardiac tissue from HIV patients, i.e., LV anterior wall obtained within 4 h of death. Patient histories were reviewed to subclassify samples into four groups: HIV+/CVD−, HIV+/CVD+, HIV−/CVD+, and HIV−/CVD+. Using analytic methods first developed for our murine tissues, we found that the human HIV−/microvascular endothelium demonstrated similar evidence of reactive nitrogen species (determined by protein 3-NT) and endothelial cell activation/inflammation (determined by ICAM and NOS2 prevalence). Interestingly, the observations from the murine model of chronic retroviral infection agreed well with measurements in human coronary microvessels (Figs. 5 and 6). Compared with tissues obtained from the HIV+/CVD− group, the HIV+/CVD− group had elevated intimal prevalence of NOS2 and protein nitration. ICAM was also shown to be elevated in the HIV+/CVD+ and HIV−/CVD+ groups. Overall, the findings from human tissues suggest that the alterations in coronary vasculature may be distinct in the HIV+/CVD+ group compared with other populations or more "traditional" forms of cardiac disease. Because our investigations were at sites that did not exhibit vascular lesions or evidence of plaque formation, we hypothesize that a widespread increase in endothelial perturbations may contribute to increased vascular risk in this population. In preliminary studies using corresponding cardiac specimens from the mouse model and a detailed intima-specific digital imaging method, we again found confluence between our mouse preparation and the data from human coronary arterioles and observed that the extent of intimal cell nitration in the thoracic aorta paralleled the changes in the coronary vasculature in these mice (Fig. 6). This consistent evidence of intimal protein nitration at multiple vascular sites suggests a general alteration of NO control during retroviral progression that may contribute to vascular dysfunction and/or other end-organ complications during AIDS. In addition to providing new and supportive mechanistic insight into human tissues, these findings also help corroborate our findings in the murine AIDS model. The degree of homology in these two species, despite substantial differences in retroviral pathogen, suggests that inflammatory and oxidant-related responses to pathogen are important contributors to AIDS-related vasculopathy and that the model may be useful for preclinical therapeutic trials. This concept is supported by a small preliminary clinical report demonstrating that high and frequent dosing of antioxidants prevented increases in markers of endothelial stress (13).

In summary, we have demonstrated evidence of vasculopathy in a well-defined murine model of AIDS. The observed functional deficits were similar to those described previously in HIV patients and include abnormal function of the vascular endothelium; they also preceded overt immunodeficiency and were apparently related to increased inflammatory pathways and reactive nitrogen species formation. Finally, these findings in the mouse model were recapitulated in relevant specimens from human HIV/AIDS patients. Further studies determining the sources and intracellular targets of oxidants in this setting may provide important mechanistic insights and identify new vasoprotective therapies for this increasingly important form of cardiovascular disease.

REFERENCES


