Dilated Cardiomyopathy Associated With Simian AIDS in Nonhuman Primates

Richard P. Shannon, MD; Meredith A. Simon, DVM; Michael A. Mathier, MD; Yong-Jian Geng, PhD; Sunil Mankad, MD; Andrew A. Lackner, DVM, PhD

Background—Cardiomyopathy is being recognized with increasing frequency in patients with AIDS, yet the relationship between HIV infection and cardiac contractile dysfunction remains obscure. The purpose of the present study was to determine if infection with simian immunodeficiency virus (SIV) in nonhuman primates is associated with cardiac dysfunction and myocardial injury.

Methods and Results—Left ventricular size and function were determined by 2D echocardiography in 16 rhesus macaques before and at weekly intervals following infection with cloned pathogenic SIVmac 239 or the highly attenuated SIVmac 239 nef deletion mutant. A second group of 15 rhesus macaques chronically infected with pathogenic (n=6) or nonpathogenic (n=9) virus were studied at >2 years following infection. Cardiac tissues from 24 rhesus macaques chronically infected with pathogenic SIV were reviewed for evidence of cardiac pathology. Acute infection (≤6 weeks) with either pathogenic or nonpathogenic SIV caused neither contractile dysfunction nor cardiac pathology. However, LV ejection fraction was significantly (P<0.05) depressed (43±6%) in rhesus macaques chronically infected with pathogenic SIV compared with rhesus macaques chronically infected with nonpathogenic SIV (61±3%). Furthermore, two thirds of rhesus macaques that succumbed to simian AIDS had myocardial pathology including lymphocytic myocarditis (n=9) and coronary arteriopathy (n=6), with complete vessel occlusion (n=4) and associated myocardial infarction and necrosis.

Conclusions—This unique model is valuable in understanding the pathogenesis of cardiac injury associated with retroviral infection in a relevant nonhuman primate model of AIDS. (Circulation. 2000;101:185-193.)

Key Words: AIDS ▪ myocarditis ▪ cardiomyopathy

There has been considerable progress in the treatment of patients with AIDS with resultant reductions in morbidity and prolonged survival. One consequence of the increased survival is the emergence of new manifestations of HIV infection, including cardiac dysfunction.1–7 Dilated cardiomyopathy and associated symptoms of congestive heart failure are being recognized with increasing frequency, with estimates that 10% to 18% of HIV seropositive individuals will manifest evidence of left ventricular dysfunction giving rise to 21 000 to 40 000 new cases of symptomatic heart failure each year by the year 2000. Despite this clinical recognition, the pathogenesis of HIV cardiomyopathy remains unclear, limiting application of both specific treatments and preventive strategies. Limitations in the understanding of the relationship between HIV infection and development of dilated cardiomyopathy are confounded by the use of illicit drugs and antiretroviral agents which, in and of themselves, may be cardiotoxic.8,9

In addition, there is a paucity of experimental animal models of HIV infection which are relevant to the human condition. However, simian immunodeficiency virus (SIV) infection in Asian macaques is associated with the development of a chronic clinical syndrome reminiscent of human AIDS.10–13 Simian AIDS is characterized by a prolonged clinical latency, weak neutralizing antibody responses, persistent viremia, and a particular tropism of the virus for CD4+ lymphocyte and monocytes/macrophages10 similar to the syndrome in humans. Accordingly, the purpose of the present study was to examine the cardiovascular, functional, and pathological consequences of both acute and chronic SIV infection in nonhuman primates in an attempt to discern whether SIV infection is associated with the development of dilated cardiomyopathy in the absence of the confounding influences observed in humans.

Methods

The Simian Model of AIDS

The model of simian AIDS has been studied extensively at the New England Regional Primate Research Center (NERPRC), and the methods for developing the model have been described previous-
ly. Naive, juvenile macaques were inoculated intravenously with cell-free supernatants of various strains of SIV. All animals demonstrated persistent infection with SIV, determined by virus isolation from peripheral blood mononuclear cells and a rise in serum antibody response against SIV proteins, as described previously.15,16

**Experimental Protocol**

The Cardiovascular Effects of Acute SIV Infection

Sixteen rhesus macaques (aged 2 to 4 years) were infected with pathogenic SIVmac 239 (50 ng/mL, p27 antigen), or SIV 239 Δ nef, a cloned virus lacking most of the nef gene and thus infectious but highly attenuated.16,17 Animals underwent M-mode and 2D echocardiography before infection and at 2, 3, 4, and 5 weeks following infection while sedated with ketamine (10 to 20 mg/kg). Serial CBCs, total lymphocyte counts, and lymphocyte subsets (CD4+, CD8+, CD2+, and CD20+) were monitored during the course of the infection. Cardiac tissues were made available for pathological study at the time of euthanasia.

**Cardiovascular Effects of Chronic SIV Infection**

Echocardiographic studies were performed in a cohort of 15 rhesus macaques chronically infected with either pathogenic or nonpathogenic SIV as part of the NERPRC program in SIV pathogenesis and vaccine development. Pathogenic strains included uncloned SIVmac 251 (n = 3), cloned pathogenic SIVmac 239 (n = 1), and cloned pathogenic SIVmac 239 with a mutation in a negative regulatory element (n = 2) which did not alter pathogenicity of the virus. Animals were infected intravenously (n = 5) or mucosally (n = 1). Nonpathogenic strains included those with deletions of the nef gene (Δ nef) alone or in combination with other mutations of regulatory genes of SIV (vif, vpr, vpx). Pathogenicity was confirmed by the presence of persistent viremia and decreases in CD4+ T cells (the hallmark of the immunodeficiency syndrome). M-mode and 2D echocardiograms were performed in macaques sedated with 10 to 20 mg/kg of ketamine. Importantly, the echocardiographer was blinded to the specific virus with which the animals were infected.

**Data Analysis**

M-mode and 2D echocardiograms were performed using a HP 500 (Hewlett Packard) and a 2.5- or 3.5-MHz transducer. Standard parasternal long- and short axes views were obtained, and data were stored on a VHS tape and analyzed using an ImageView DCR (Nova Microsonics). End-systolic and end-diastolic volumes were calculated on the basis of maximal and minimal cavity diameters and an area-length method obtained from the standard apical 4-chamber view. Heart rate was recorded using lead II of standard ECG.

**Pathological Studies**

Acute SIV Infection

Three to 4 transmural sections of the left ventricle of rhesus macaques infected with SIVmac 239 or SIVmac 239 Δ nef were selected...
TABLE 3. Clinical Characteristics of Rhesus That Died of Simian AIDS and Manifested Cardiac Pathology

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age, y</th>
<th>Duration, mos</th>
<th>Weight, kg</th>
<th>Myocarditis</th>
<th>Arteriopathy</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>95–346</td>
<td>4</td>
<td>8</td>
<td>5.3</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94–433</td>
<td>4</td>
<td>24</td>
<td>6.5</td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>93–448</td>
<td>4</td>
<td>26</td>
<td>6.08</td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>92–655</td>
<td>4.75</td>
<td>15</td>
<td>6.2</td>
<td>*</td>
<td></td>
<td>Pneumocystis</td>
</tr>
<tr>
<td>92–502</td>
<td>7</td>
<td>8</td>
<td>3.1</td>
<td>*</td>
<td></td>
<td>Cryptosporidiosis</td>
</tr>
<tr>
<td>92–95</td>
<td>6.5</td>
<td>20</td>
<td>7</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94–421</td>
<td>3.75</td>
<td>24</td>
<td></td>
<td>*</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>94–158</td>
<td>5.5</td>
<td>38</td>
<td>4</td>
<td>*</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>94–89</td>
<td>19.5</td>
<td>45</td>
<td>5.9</td>
<td>†</td>
<td>†</td>
<td>MAI</td>
</tr>
<tr>
<td>94–412</td>
<td>5.5</td>
<td>17</td>
<td>8.3</td>
<td>†</td>
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<tr>
<td>92–797</td>
<td>6.8</td>
<td>38</td>
<td>4.9</td>
<td>†</td>
<td>Pneumocystis</td>
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</tr>
<tr>
<td>92–765</td>
<td>3.5</td>
<td>22</td>
<td>3.65</td>
<td>*</td>
<td>†</td>
<td>MAI</td>
</tr>
<tr>
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<td>12</td>
<td>7.18</td>
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</tr>
<tr>
<td>92–3</td>
<td>5.75</td>
<td>30</td>
<td></td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>91–614</td>
<td>4</td>
<td>24</td>
<td>3.76</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.9±1.5</td>
<td>23±5</td>
<td>5.45±0.5</td>
<td>5 (33%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Age indicates age at time of death; duration, time since infection with SIV; weight, weight at time of death; MAI, mycobacterium avium intracellulare.
*Indicates mild, focal myocarditis and cardiomyopathy; †, moderate; ††, severe, diffuse.

Figure 1. Sections of myocardium from the left ventricle of a rhesus macaque that died of simian AIDS. a, Area of normal myocardium. b, Myocyte hypertrophy. c, Area of focal myocarditis with a mononuclear cell infiltrate but without necrosis. d, Area of myocyte hypertrophy and reparative fibrosis associated with myocyte drop out.
randomly from apex to base and stained with hematoxylin and eosin and examined by a staff pathologist. Sections were graded for the presence of lymphocytic infiltrate and associated necrosis.

We reviewed the gross and microscopic cardiac pathology from 24 SIV-infected rhesus macaques that had succumbed to AIDS and compared these findings to a group of chronically infected rhesus macaques (n=5) which had received nonpathogenic deletion mutants of SIV. Tissue preparation was performed as described above. Myocardial fibrosis was determined using paraffin sections stained with picrosirius red for collagen from 3 control animals and 9 animals found to have histopathologic evidence of myocardial involvement. The collagen content was determined morphometrically using a Metamorph image analysis system.

Immunohistochemistry

Immunohistochemical stains for T cells, B cells, and macrophages in the myocardium were performed on formalin-fixed, paraffin-embedded sections as described previously. The CD3+ epitope on T cells was identified using a rabbit polyclonal antibody. The CD68+ epitope on macrophages was identified using a monoclonal antibody KP1. The CD20+ epitope on B cells was identified using a monoclonal antibody L-26. All antibodies were obtained from Dako (Carpinteria, Calif). A negative control for every section consisted of irrelevant antibody at the same concentration as the primary antibody. A biotinylated secondary antibody against immunoglobulin was applied, and a avidin-biotin complex method (ABC Standard, Vector Labs, Burlingame, Calif) was used to detect antibody labeling, diaminobenzidine served as the chromogen.

Opportunistic Infections

The presence of opportunistic infections was determined by routine bacteriologic staining for pneumocystis carinii, mycobacterium avium intracellulare, and cryptosporidiosis in blood, lung, and cardiac tissues as described previously. In situ hybridization for macaque cytomegalovirus (CMV) was performed as previously described on formalin-fixed, paraffin-embedded sections mounted on Superfrost/Plus slides (Fisher Scientific). The DNA probe, a 9.2-kb restriction fragment containing the macaque CMV immediate-early gene and 3′ flanking region (provided by Dr. Peter A. Barry, UC Davis) in the plasmid pSP72 (Amp) was random-prime labeled with digoxigenin-dUTI following the manufacturer’s recommended protocol (Boehringer Mannheim, Indianapolis, Ind). Hybridization procedures were performed under denaturing conditions to localize DNA as well as RNA. As a negative control, sections were hybridized with the plasmid PUC19 labeled with digoxigenin at the same time as the CMV probe. Sections were then immunostained using the ABC technique using a sheep monoclonal anti-digoxigenin antibody (Boehringer Mannheim, Indianapolis, Ind) as the primary antibody and diaminobenzidine as the chromogen.

Statistical Analysis

The echocardiographic and pathological data from macaques infected with pathogenic or nonpathogenic SIV were analyzed using an unpaired Student’s t test, run using Excel, Version 4.0 for Macintosh (Microsoft Corp).
Results

Cardiovascular Effects of Acute SIV Infection

Table 1 depicts the resting left ventricular volumes, heart rates, and cardiac indices in the groups of animals infected acutely (3 weeks) with SIV\textsubscript{mac} 239 or the nonpathogenic virus, SIV\textsubscript{mac} 239 \(\Delta\) nef. Within 2 weeks, viral antigens were detectable in the plasma of animals infected with SIV\textsubscript{mac} 239. There were associated decreases \((P<0.05)\) in CD2\textsuperscript{+} cells (T lymphocytes), CD4\textsuperscript{+} cells (T helper cells), and CD20\textsuperscript{+} cells (B cells), but no change in CD8\textsuperscript{+} cells, consistent with acute SIV infection. No such changes were observed in the animals infected with the SIV\textsubscript{mac} 239 \(\Delta\) nef. There was neither echocardiographic evidence of cardiac dysfunction nor histopathological evidence of acute myocardial inflammation. There were no other associated structural changes within the myocardium over the initial 5 week period of infection from any of the 16 acutely infected rhesus macaques.

Cardiovascular Effects Associated With Chronic SIV Infection

Table 2 reveals the effects of chronic SIV infection on resting left ventricular ejection fraction and cardiac volumes. There was a marked reduction in left ventricular ejection fraction in the animals infected with pathogenic virus \((43\pm7\%)\) compared with the group infected with nonpathogenic virus \((61\pm3\%)\). Blood pressure was not significantly different between the 2 groups. In addition, there was evidence of left ventricular dilatation \((21\pm3\) versus \(28\pm3\) mL/m\(^2\)) and contractile dysfunction associated with significant increases in LV end-systolic volume indices \((9\pm1\) versus \(16\pm3\) mL/m\(^2\), \(P<0.05\)). However, the stroke volume index \((13\pm2\) versus \(12\pm2\) mL/m\(^2\)) was not significantly different between the 2 groups, suggesting that ventricular dilatation had compensated for the impairment in left ventricular systolic performance. At this stage of the myopathic process, cardiac index was not significantly depressed. Although the time from initial viral infection was significantly greater in the animals infected with nonpathogenic virus, only those rhesus chronically infected with pathogenic strains of SIV manifest evidence of immunosuppression in both absolute and the percent of CD4\textsuperscript{+} cells (Table 2).

Retrospective review of 24 rhesus macaques who had been infected chronically with pathogenic SIV\textsubscript{mac} 251 for a comparable duration and had succumbed to simian AIDS revealed a high incidence of cardiac involvement (Table 3). Both the

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Animal \& Duration, & Weight, & Infection \\
\hline 95–212 & 2.5 & 28 & 8 & \\
93–526 & 5.3 & 27 & 6.7 & Pneumocystis \\
93–457 & 4.75 & 31 & & \\
93–240 & 14 & 42 & 8.79 & MAI \\
93–141 & 4.7 & 32 & 6.8 & Cryptosporidiosis \\
92–539 & 2.8 & 16 & 6.14 & Pneumocystis \\
92–530 & 1.75 & 8 & 5.65 & Pneumocystis \\
91–605 & 14 & 16 & 7.1 & Pneumocystis \\
92–004 & 2 & 5 & 5.66 & Pneumocystis \\
\hline
Total & 5.8\(\pm\)1.6 & 23\(\pm\)5 & 6.86\(\pm\)0.4 & 7 (78\%)
\hline
\end{tabular}
\caption{Clinical Characteristics of Rhesus That Died of Simian AIDS and Manifested No Cardiac Pathology}
\end{table}

MAI indicates mycobacterium avium intracellulare.
and the duration of SIV infection was comparable in the 2 groups. However, the macaques with simian AIDS and cardiac pathology (Table 3) were emaciated to a greater extent compared with macaques with simian AIDS but no cardiac involvement (Table 4). In contrast, the macaques with simian AIDS but no cardiac involvement (Table 4) had a greater frequency of opportunistic infections (7 of 9 animals) compared with those where cardiac involvement was identified (5 of 15 animals, P = 0.05). None of the myocardial samples demonstrated evidence of cytomegalic inclusion bodies, indicative of this herpes virus infection which is known to be cardiotropic and none demonstrated CMV nucleic acid sequences in the cardiac tissues examined.

Nine animals had evidence of myocarditis, whereas 9 had evidence of coronary arteriopathy either alone (n = 6) or in combination with myocarditis (n = 3). In 4 of the animals with coronary arteriopathy, there was evidence of vessel occlusion and recanalization, with associated areas of myocardial infarction. Two of these 4 animals were observed to die in acute cardiogenic pulmonary edema. Two animals had marantic endocarditis and 1 had demonstrated mural thrombus within the left ventricle. Nine animals had no evidence of cardiac involvement. Figure 1 reveals a spectrum of histopathological changes observed in the myocardium of animals that succumbed to simian AIDS. There were areas of normal myocardium (Figure 1a), areas of myocyte hypertrophy (Figure 1b), areas of focal myocarditis with a mononuclear cell infiltrate (Figure 1c), and areas of the myocardium characterized by myocyte hypertrophy, myocyte drop out, and reparative fibrosis (Figure 1d). Quantitative assessment of the myocardial fibrosis (Figure 2) revealed as much as a 4-fold increase in collagen content (range, 1.7 to 5.1 volume percent of myocardium) compared with hearts from animals infected with nonpathogenic strains (range, 0.3 to 1.3 volume percent of myocardium). Figure 3 illustrates an example of severe acute myocarditis (Figure 3a) with associated giant cells and acute myocyte necrosis (Figure 3b) in an animal that died of simian AIDS. Giant cells were found in only 2 of the 9 animals observed to have myocarditis and were associated with a severe and diffuse inflammatory infiltrate. Immunohistochemical staining (Figure 4) identified the cellular constituents of the inflammatory infiltrates as predominately CD3+ T lymphocytes (e), whereas the giant cells were composed of macrophages (f).

Discussion

In the present study, we report direct evidence of LV contractile dysfunction and ventricular dilatation in nonhuman primates with simian AIDS associated with chronic, but not acute SIV infection. Specifically, there was a 28% reduction in LV ejection fraction, a 33% increase in LV end-diastolic volume index, and a near doubling of the LV end-systolic volume index in 6 rhesus macaques chronically infected with pathogenic virus, compared with rhesus macaques infected chronically with nonpathogenic virus. We
believe that the animals chronically infected with the non-pathogenic strains of SIV serve as an important control because the SIVmac239 Δ nef virus is infectious but attenuated in its ability to replicate and thereby cause immune suppression. Taken together, these data prove that retroviral infection alone without associated immune suppression is insufficient to explain cardiac involvement in simian AIDS.

Several recent reports have suggested a direct role for retroviral infection in the pathogenesis of myocarditis in patients with AIDS. These studies, which used highly sensitive in situ hybridization or PCR techniques, lack the requisite specificity to identify whether virus is present in myocytes or inflammatory cells for which these viruses are known to be tropic. In support of the notion that the virus is not resident within the cardiac myocytes is the observations of Robedollo et al., who showed that HIV is incapable of infecting human neonatal cardiocytes. We did not perform in situ studies to detect SIV nucleic acid sequences in the present study because of the lack of specificity of the technique to localize the virus to cardiac myocytes or cells which express the CD4+ receptor.

Figure 5. Sections of myocardium from left ventricle of macaque with simian AIDS, demonstrating an extensive perivascular infiltrate involving an epicardial coronary artery (a). In the area of perivascular infiltrate (b), there is an intense intimal and smooth muscle hyperplastic response.

The inverse relationship between the incidence of opportunistic infection and the presence of cardiac pathology in the 24 rhesus macaques which succumbed to simian AIDS (Table 3) is curious and as yet unexplained. A greater burden of opportunistic infections may simply be a marker for more severe immune suppression, suggesting that these animals may be incapable of mounting the inflammatory responses which appear to characterize both the myocarditis (Figure 3) and coronary arteriopathy (Figure 5) in those animals with cardiac involvement. Indeed, the cellular constituents of the myocardial inflammatory response (CD3+ T lymphocytes and CD68+ macrophages, Figure 4) suggest a cell-mediated immune response and is consistent with findings in humans.1 We did not identify cytomegalic inclusion bodies nor early immediate gene products of CMV in the myocardium from any animal with myocardial involvement, despite the endemic nature of this herpes virus in the macaques colony as manifest by >85% seropositivity for CMV. CMV accounted for only 9% of opportunistic infections in rhesus macaques with simian AIDS19 in a previous report. Thus, opportunistic infection with CMV appears neither necessary nor sufficient to explain the nature and extent of inflammatory myocardial lesions in simian AIDS.

Although myocarditis is a well recognized pathology in both human and simian AIDS, the finding of extensive coronary arteriopathy observed here is less appreciated in humans. The arteriopathy is characterized by both an acute perivascular inflammation (Figure 5) and intimal and smooth muscle hyperplasia (Figure 6), with occasional thrombotic occlusion of these arterioles leading to regional myocardial necrosis and fibrosis. Whether these findings represent a continuum or distinct lesions remains to be determined. Similar coronary vascular findings have been reported in children with AIDS, suggesting that the juvenile nature of the rhesus macaques may be germane to the pathogenesis. Pulmonary arteriopathy has been recognized more commonly in both human and simian AIDS.25,26 We found pulmonary arteriopathy in 9 of the 24 rhesus examined in the present study. Five of the 6 animals with coronary arteriopathy had pulmonary vascular involvement as well. Chalifoux et al reported a 20% incidence of pulmonary arteriopathy in macaques with chronic simian AIDS with histopathological features similar to those observed here in coronary arteries. Inflammatory infiltrates were characterized as predominantly CD68+ macrophages. Thus, the pathogenesis of these vascular lesions has not been defined. Importantly, antiretroviral agents and associated dyslipidemias, which have been implicated in coronary syndromes in patients with AIDS, could not be implicated in the observed findings, suggesting that they are not likely causal.

These observations constitute the first description of extensive cardiac involvement in a relevant animal model of AIDS. The observation that 2 animals died in acute pulmonary edema and were found to have extensive SIV coronary arteriopathy and associated myocardial necrosis provides strong evidence in favor of a structural-functional relationships in some chronically infected animals. These issues will require further investigation in this model, which affords an unprecedented opportunity to examine the natural history and

Figure 5. Sections of myocardium from left ventricle of macaque with simian AIDS, demonstrating an extensive perivascular infiltrate involving an epicardial coronary artery (a). In the area of perivascular infiltrate (b), there is an intense intimal and smooth muscle hyperplastic response.
pathogenesis of this clinically relevant condition, devoid of the confounds of illicit drugs and antiretroviral agents.

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References


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