Intensity of Myocardial Expression of Inducible Nitric Oxide Synthase Influences the Clinical Course of Human Immunodeficiency Virus-Associated Cardiomyopathy

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Background—Increased levels of tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) have been reported in patients with dilated cardiomyopathy. We investigated the myocardial expression of TNF-α and iNOS in patients with HIV-associated cardiomyopathy (HIV-DCM) compared with patients with idiopathic dilated cardiomyopathy (IDCM).

Methods and Results—Endomyocardial biopsy specimens from 82 HIV-DCM and 80 IDCM patients were processed for determination of the immunostaining intensity of TNF-α and iNOS and for virological examination. Negative controls were derived from autopsy myocardium specimens from 32 HIV-negative patients without known heart disease. The mortality rate for congestive heart failure between groups according to the intensity of iNOS staining was also evaluated. The mean intensity of both TNF-α and iNOS staining was greater in patients with HIV-DCM (0.81 and 1.007, respectively) than in patients with IDCM (0.44 and 0.49, respectively) and controls (0.025 and 0.027, respectively). The staining intensity of both TNF-α and iNOS was inversely correlated with CD4 count. The staining intensity of iNOS was greater in HIV-DCM patients with HIV/coxsackievirus B3 (CVB3) or with HIV/cytomegalovirus coinfection than in IDCM patients showing infection with CVB3 and adenovirus alone. The staining intensity of iNOS correlated to mortality rate, because it was higher in HIV-DCM patients and, in particular, in those with an optical density unit >1.

Conclusions—Cytokine activation seems to play a significant pathogenetic role in both HIV-DCM and IDCM. In HIV-DCM patients, the state of immunodeficiency may favor the selection of viral variants of increased pathogenicity, influencing the clinical course of cardiomyopathy by enhancement of the inflammatory process. (Circulation. 1999;100:933-939.)

Key Words: AIDS ▪ viruses ▪ cardiomyopathy ▪ hormones ▪ nitric oxide synthase

The acquired immunodeficiency syndrome (AIDS) represents a unique opportunity to review the vulnerability of the heart to infections and the relation between myocarditis and dilated cardiomyopathy (DCM).1 The human immunodeficiency virus (HIV)-1 genome has been demonstrated within myocytes in cardiac autopsy and biopsy tissue from patients with congestive cardiomyopathy.1–4 Nevertheless, the pathogenesis of the heart muscle disease in AIDS is still unclear.4,5

Increased levels of tumor necrosis factor-α (TNF-α) and increased basal production of nitric oxide (NO) have been reported in experimental myocarditis6,7 and in patients with DCM.8

NO, synthesized from l-arginine by 3 NO synthase (NOS) enzymes, is a biological mediator with multiple actions.9 Two constitutively present enzymes are found in neuronal and endothelial cells, respectively; the third form is inducible in many cells by endotoxin and cytokines, such as interleukin-1, interferon-γ, and TNF-α.6,9

Experimental studies have shown that induced NO production has a negative inotropic effect on cardiac myocytes7 and that high levels of NO produced by inducible NOS (iNOS) are cytotoxic.6,10,11 We investigated the myocardial expression and the immunostaining intensity of TNF-α and iNOS in HIV-positive subjects with dilated cardiomyopathy (HIV-DCM) compared with patients with idiopathic dilated cardiomyopathy (IDCM) in relation to both immunohistological and virological findings.

Methods

Study Population

Eighty-two HIV-positive and 80 HIV-negative patients with echocardiographic diagnosis of dilated cardiomyopathy were selected for the study. HIV-positive patients came from a cohort of 952 HIV-positive subjects recorded in the Gruppo Italiano per lo Studio Cardiologico dei pazienti affetti da AIDS (GISCA) files who were selected according to criteria previously described.9 In these patients,
the diagnosis of AIDS was based on the Centers for Disease Control and Prevention (CDC) criteria.12 IDCM patients came from a cohort of 323 HIV-negative outpatients recorded at GISCA centers. In the group of patients with IDCM, the exclusion criteria were age <18 years; use of drugs with a definite cardiotoxic action; a history of chronic alcoholism; or a history of coronary heart disease, valvular heart disease, arterial hypertension, systemic diseases (malnutrition, diabetes, or rheumatic, neurological, and neoplastic diseases), congenital heart diseases, and cor pulmonale.

Negative controls were derived from autopsy myocardium specimens from 32 HIV-negative patients without known heart disease. The study protocol was approved by the Institutional Review Board of the Coordinating Center of the study (Department of Infectious Diseases, University of Pavia, Italy).

Echocardiographic Definition of DCM
In both study groups, the diagnosis of DCM was made by echocardiography using a Hewlett Packard Sonos 500 model 77020A with either a 3.5- or 5.0-MHz transducer. All echocardiographic images were stored on videotape and analyzed blindly by 2 independent investigators. Echocardiographic diagnosis of DCM was based on the presence of diffuse left ventricular hypokinesia (ejection fraction <45%) and left ventricular dilatation (left ventricular end-diastolic volume index >80 mL/m²), as previously described.4,13

Endomyocardial Biopsy
All patients selected for the study underwent right ventricular endomyocardial biopsy. This procedure was performed within 1 month (range, 13 to 42 days) after echocardiographic demonstration of cardiomyopathy via the right internal jugular vein under fluoroscopic control with a modified Caves-Schultz biopsytope (Millar Instruments Inc) in accordance with the Stanford technique.14 Five to 8 samples of 1.5 to 3 mm³ were obtained from the middle-distal portion of the right ventricular septum.9

Histology
The endomyocardial biopsy samples were fixed in 10% buffered formalin and embedded in paraffin. Six serial sections 4 to 5 µm thick were cut on a microtome and routinely stained with hematoxylin-eosin. Specific stains (eg, Gram, Ziehl-Neelsen, and periodic acid–Schiff) could be used to identify bacteria, fungi, and mycobacteria.1 Histological diagnosis of active or borderline myocarditis was defined in accordance with the Dallas criteria.15

Immunohistochemistry
Immunoperoxidase staining for identification of lymphocyte and antigen markers and major histocompatibility complex (MHC) classes I and II was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4 The avidin-biotinylated-peroxidase complex method with primary antiserum to a synthetic peptide from human iNOS and TNF-α was performed in accordance with Beschorner et al16 as previously described.1,4

Virology
The technique of in situ hybridization using 35S-labeled RNA or cDNA virus-specific probes was used for detection of cardiotropic viruses (coxackievirus B3 [CVB3], cytomegalovirus, adenovirus, herpes virus, and Epstein-Barr virus).17 A mixture of 35S-labeled RNA probes encompassing the entire HIV genome was used to detect the presence of HIV in the myocardial tissue of the patients with HIV-DCM, patients with IDCM, and in controls according to the method described by Grody et al.2 Positive controls were derived from HIV-infected lymphocyte cultures.

Clinical Follow-Up
The patients selected for the study underwent clinical examination every 3 months and echocardiographic examination every 6 months. Patients’ functional classes were defined in accordance with the New York Heart Association criteria.

End Points and Data Collection
The assessment of the myocardial immunostaining intensity of TNF-α and iNOS and the evaluation of the mortality rate for congestive heart failure (CHF) between groups of patients with DCM according to the intensity of iNOS staining were the end points of the study. Data regarding both clinical and echocardiographic parameters were reported on the charts of the subjects selected for the study. Each chart was provided with a computer-generated code of identification. All data coming from the GISCA Centers were filed by a centralized computerized system, and the filed data were then analyzed blindly by an independent investigator using a computerized database.

Statistical Analysis
One-way ANOVA and Student’s t test for independent samples (with 95% CI for the differences) were used for analysis of continuous data. The χ² test with Yates’s correction and Fisher’s exact test were used for analysis of categorical data.18 A linear regression test was used for calculation of correlation coefficients between variables when appropriate. The Kaplan-Meyer test was used for analysis of survival between groups of patients with DCM according to the follow-up times. In this analysis, the patients who died of CHF were considered as event, whereas the patients lost to follow-up and those who died of noncardiac causes were considered as censored. Survival curves were compared by log-rank test.18 A value of 2-sided P<0.05 was considered statistically significant.

Informed Consent
The research was carried out in accordance with the Helsinki Declaration. The study protocol was explained to all the patients selected for it. All the patients selected for the study gave their informed consent.

Results

Characteristics of the Patients and Duration of Follow-Up
Patient selection started in December 1996. The Executive Committee decided to stop the study in December 1998. The mean duration of follow-up was 24±3.2 months. No patient was lost to follow-up. The principal characteristics and the echocardiographic measurements of the patients selected for the study at enrollment are reported in Table 1.

Among the HIV-DCM patients, 49 were homosexuals, 31 were heterosexuals, and 2 were blood transfusion recipients. Nine patients fulfilled the CDC criteria for AIDS. All patients, after demonstration of cardiomyopathy, received therapy with digoxin, diuretics (furosemide), and ACE inhibitors (enalapril) without significant differences between groups with regard to either the type of treatment or the posology of drugs. HIV-DCM patients also received antiretroviral treatment. Specifically, 33 patients received zidovudine (500 mg/d PO), 24 patients received didanosine (4 mg/kg body wt q12h PO), and 25 received zalcitabine (0.75 mg q8h PO).

Histology and Immunohistochemistry
Histological diagnosis of myocarditis was made in 67 patients with HIV-DCM (38 with active and 29 with borderline
myocarditis) and in 18 patients with IDCM (8 with active and 10 with borderline myocarditis) (P<0.001). In the other 15 HIV-DCM patients and in the other 62 patients with IDCM, microscopic study of endomyocardial biopsy specimens revealed a mixture of myocardial cells and areas of interstitial and perivascular fibrosis without inflammatory cell infiltrate.

The immunopathological findings documented in the patients of the 2 study groups with a histological diagnosis of myocarditis are reported in Table 2. Compared with IDCM patients, in HIV-DCM patients the inflammatory-cell infiltrates were composed predominantly of CD3 and CD8 lymphocytes, possibly reflecting the number of circulating lymphocytes in these patients in relation to the state of immunodeficiency.

### TABLE 2. Immunopathological Findings Documented in Patients of the 2 Study Groups With Histological Diagnosis of Myocarditis

<table>
<thead>
<tr>
<th>Lymphocytes or Membrane</th>
<th>HIV-DCM (n=67), n (%)</th>
<th>IDCM (n=18), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2 (OKT11)</td>
<td>22 (33)</td>
<td>7 (39)</td>
</tr>
<tr>
<td>CD3 (OKT3)</td>
<td>38 (57)</td>
<td>7 (39)</td>
</tr>
<tr>
<td>CD4 (OKT4)</td>
<td>5 (8)</td>
<td>15 (83)*</td>
</tr>
<tr>
<td>CD8 (OKT8)</td>
<td>57 (85)</td>
<td>6 (33)*</td>
</tr>
<tr>
<td>CD25 (IL2-R)</td>
<td>2 (3)</td>
<td>9 (50)*</td>
</tr>
<tr>
<td>B lymphocytes (MB1)</td>
<td>2 (3)</td>
<td>12 (67)*</td>
</tr>
<tr>
<td>NK cells, CD16 (Leu1B)</td>
<td>2 (3)</td>
<td>8 (44)*</td>
</tr>
<tr>
<td>Monocytes, CD11B (OKM1)</td>
<td>2 (3)</td>
<td>7 (39)*</td>
</tr>
<tr>
<td>CD57 (Leu7)</td>
<td>25 (37)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Increased MHC class I staining</td>
<td>47 (70)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Increased MHC class II staining</td>
<td>20 (30)</td>
<td>6 (33)</td>
</tr>
</tbody>
</table>

IL2-R indicates interleukin-2 receptor; NK, natural killer.

*P<0.001 for the comparison with patients with HIV-DCM.

### Virology

The in situ hybridization test was positive for HIV in 62 patients with HIV-DCM, whereas it was negative in biopsy specimens from patients with IDCM and from controls.

Other cardiotropic viruses were documented by in situ hybridization test from endomyocardial biopsy samples of 27 patients with HIV-DCM and 6 patients with IDCM (P<0.001). Specifically, in the group of patients with HIV-DCM, the in situ hybridization test was positive for CVB3 in 15 patients, for cytomegalovirus in 5 patients, for Epstein-Barr virus in 4 patients, and for adenovirus in 3 patients. All these patients had a positive hybridization test for HIV. In the group of patients with IDCM, the in situ hybridization test was positive for CVB3 in 7 patients and for adenovirus in 3 patients. All these patients showed active myocarditis at histological examination of biopsy specimens.

In 41 of 62 HIV-DCM patients with a positive hybridization test for HIV, an active myocarditis was documented at histological examination of endomyocardial biopsy specimens. Among them, the in situ hybridization test was also positive for CVB3 in 8 patients, for cytomegalovirus in 3 patients, and for Epstein-Barr virus in 2 patients.

### TNF-α and iNOS

Cardiac myocytes of patients with HIV-DCM showed a strong immunoreactivity for both TNF-α and iNOS. In these patients, a significant optical density of staining for both TNF-α and iNOS was also found in the endothelium of intramyocardial blood vessels (Figure 1).

The mean optical density of staining for TNF-α and iNOS in cardiac tissue was greater in patients with HIV-DCM than in patients with IDCM and controls (Figure 2). Specifically, the mean intensity of TNF-α staining (in optical density units) was 0.81 (range, 0.45 to 1.45) in patients with HIV-DCM and 0.44 (range, 0.10 to 1.10) in patients with IDCM (P<0.001). The mean intensity of iNOS staining was 1.007 (range, 0.33 to 1.51) in patients with HIV-DCM and 0.49 (range, 0.11 to 1.12) in patients with IDCM (P<0.001). In controls, the mean intensity of TNF-α and iNOS staining was 0.025 (range, 0.010 to 0.041) and 0.027 (range, 0.017 to 0.041), respectively.

Among patients with HIV-DCM, the mean intensity of TNF-α staining was greater in patients showing HIV/CVB3 coinfection (0.98; range, 0.54 to 1.42) than in patients showing HIV/cytomegalovirus coinfection (0.65; range, 0.48 to 0.82) or those showing HIV/Epstein-Barr virus or HIV/adenovirus coinfection (0.50; range, 0.45 to 0.56). Similarly, the mean intensity of iNOS staining was greater in patients showing HIV/CVB3 coinfection (1.00; range, 0.60 to 1.51), compared with patients showing HIV/cytomegalovirus (0.67; range, 0.51 to 0.83), HIV/Epstein-Barr virus (0.62; range, 0.48 to 0.76), or HIV/adenovirus coinfection (0.48; range, 0.33 to 0.64). In patients showing only HIV infection by in situ hybridization test, the mean intensity of TNF-α and iNOS staining was 0.67 (range, 0.47 to 0.88) and 0.69 (range, 0.49 to 0.90), respectively.

Among patients with IDCM, the mean intensity of TNF-α staining was 0.61 (range, 0.12 to 1.10) in patients showing myocardial CVB3 infection and 0.42 (range, 0.10 to 0.60) in
patients showing adenovirus infection. In these patients, the mean intensity of iNOS staining was 0.62 (range, 0.13 to 1.12) and 0.43 (range, 0.11 to 0.72), respectively.

In the 2 study groups, the intensity of both TNF-α and iNOS staining was inversely correlated to CD4 count (Figure 3) and was not influenced in HIV-DCM patients by antiretroviral treatment, because it was similar among patients with an equal value of CD4 count receiving a different antiretroviral treatment. Furthermore, in both groups of patients with DCM, the intensity of both TNF-α and iNOS staining was inversely correlated to ejection fraction ($r=0.875$, $P<0.001$ and $r=0.821$, $P<0.001$, respectively) and to left ventricular

Figure 1. Endomyocardial biopsy specimen from a patient with HIV-associated DCM showing strong immunostaining (optical density unit, 1.36) for iNOS both in cardiac myocytes (white arrow) and in endothelium of a small blood vessel (black arrow). Avidin-biotin complex method. Magnification ×100.

Figure 2. Mean optical density of staining of TNF-α and iNOS in patients with HIV-DCM, IDCM, and controls. Horizontal bars represent mean values.

Figure 3. Correlation between optical density of staining for TNF-α and iNOS and CD4 count.
end-diastolic volume index \((r=0.712, P<0.001\) and \(r=0.742, P<0.001\), respectively). Other variables, such as age and duration of documented cardiomyopathy, did not correlate significantly with the intensity of TNF-\(\alpha\) and iNOS staining or with echocardiographic functional parameters.

**Survival**

During the follow-up period, 25 patients with HIV-DCM and 11 patients with IDCM died of CHF \((P=0.017)\). The mean survival time, after enrollment, was 10.8±4.03 months for HIV-DCM patients and 15.6±5.3 months for IDCM patients \((95\% \text{ CI}, -8.07\text{ to } -1.53; P=0.005)\).

Other 11 HIV-DCM patients died of Pneumocystis carinii pneumonia during the follow-up period and were considered as censored for the survival analysis. The Kaplan-Meier curve comparing the survival rate between patients with HIV-DCM and those with IDCM is shown in Figure 4.

In the group of patients with HIV-DCM, according to the value of intensity of iNOS staining, the survival rate was significantly lower in patients with an optical density unit \(>1\) (Figure 5). Below this value, no significant difference was observed in the survival rate compared with the group of patients with IDCM.

**Autopsy Findings**

Autopsy was performed in all patients of both groups who died of CHF. In these patients, the heart showed dilatation of both ventricular cavities, with a weight ranging from 450 to 735 g (mean, 590.5 g), with no significant difference between HIV-DCM and IDCM patients. Intraventricular thrombi were observed in 3 HIV-DCM patients. Atherosclerotic plaques with a reduction of <50% in luminal diameter were documented in 14 patients (8 HIV-DCM and 6 IDCM), involving the left anterior descending artery in 10 (6 HIV-DCM and 4 IDCM) and the right coronary artery in 4 (2 HIV-DCM and 2 IDCM). Five other patients (3 HIV-DCM and 2 IDCM) had mild stenosis in ≥2 main coronary vessels, with a reduction of <70% in luminal diameter. All these subjects did not present clinical and pathological evidence of ischemic heart disease.

**Discussion**

The role of viral myocarditis in the development of DCM has not been fully characterized.\(^1,3,19\)

In our study, myocarditis was documented in 82\% of HIV-DCM patients. A positive in situ hybridization test for HIV was demonstrated in 62\% of patients with myocarditis. Among these latter, a coinfection with CVB3, with cytomegalovirus, and with Epstein-Barr virus was documented in 20\%, 7\%, and 5\% of the cases, respectively. In 38\% of HIV-DCM patients with myocarditis, however, the in situ hybridization test was negative for HIV and for other cardiotropic viruses. Furthermore, in 18\% of HIV-infected patients, myocarditis was not documented at histological examination of endomyocardial biopsy specimens. In the group of patients with IDCM, myocarditis was documented in 23\% of the cases. Among them, CVB3 and adenovirus myocardial infection was documented in 39\% and 17\% of the cases, respectively. All IDCM patients with a positive hybridization test for cardiotropic viruses showed myocarditis at histological examination of endomyocardial biopsy specimens, but 44\% of patients with myocarditis had a negative hybridization test.

Immunological factors and cytokines may play an important role in development and progression of both HIV-DCM and IDCM.\(^8,20\) The myocardial expression of MHC class I in patients with both HIV-DCM and IDCM strongly suggests the presence of an active immune process within the myocardium.\(^1,3,4\) and the finding of a strong immunoreactivity for both TNF-\(\alpha\) and iNOS in cardiac tissue of patients with DCM may be relevant to the pathogenesis of this disease.\(^8\)

In HIV disease, dendritic cells have the capacity to initiate primary immunological response and to present the antigen to T lymphocytes. The interaction between dendritic cells and T lymphocytes, particularly with CD8 cells, could promote a local increase of multifunctional cytokines, such as TNF-\(\alpha\), which can also be produced and secreted by infected macrophages.\(^21\)

TNF-\(\alpha\) has a negative inotropic effect by altering intracellular calcium homeostasis, possibly by inducing NO synthesis, which also reduces myocyte contractility.\(^10,22\) A relationship between TNF-\(\alpha\) and iNOS is suggested by the correlation between the plasma concentration of acid-labile nitroso compounds, representing the end products of NO metabolism, and TNF-\(\alpha.\)\(^23\) The local availability of TNF-\(\alpha\) could be relevant to the induction of iNOS and consequent high production of NO.\(^8,24–26\)

Lowenstein et al demonstrated that iNOS is induced in mice infected with CVB3\(^8\) and that NO inhibits CVB3 replication by inactivation of CVB protease 3C.\(^27\) Therefore, in murine myocarditis, iNOS is crucial for the host response to CVB3,\(^28\) although the chronic activation of iNOS by cytokines in ventricular myocytes alters the contractile func-

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**Figure 4.** Kaplan-Meier curve comparing survival rate between patients with HIV-DCM and those with IDCM in relation to follow-up times.

**Figure 5.** Kaplan-Meier curve comparing survival rate among patients with HIV-DCM according to value of optical density unit (ODU) of staining for iNOS in relation to follow-up times.
tion and decreases the responsiveness to β-adrenergic agonists. The increased levels of iNOS observed in both HIV-DCM and IDCM patients coincide with an abundant local source of TNF-α that is not present in controls. The source is predominantly vascular, although the cytokine was also found in cardiac myocytes. Thus, it is possible that TNF-α diffuses from vessels to cause paracrine stimulation of the myocytes. TNF-α may also be having effects on the vessels themselves. Vascular production of NO could lead to vasodilation, but conversely, TNF-α could also be downregulating the isof orm of iNOS in the endothelium, because it reduces the stability of endothelial NOS RNA. It can also induce tissue factor expression via 55-kDa TNF-α receptor with a potential procoagulant action.

In our study, a greater intensity of both TNF-α and iNOS immunostaining was observed in patients with HIV-DCM than in patients with IDCM. The intensity of myocardial expression of iNOS was greater in patients showing a myocardial viral infection. Interestingly, in patients with HIV-DCM, the intensity of both TNF-α and iNOS staining was greater in patients showing infection with viruses other than HIV (particularly CVB3 and cytomegalovirus) compared with patients showing infection with HIV only. Moreover, patients showing coinfection with HIV and CVB3 had a greater intensity of iNOS staining than did IDCM patients showing myocardial infection with CVB3 alone.

Considering that the intensity of both TNF-α and iNOS staining was inversely correlated with the CD4 count, it is possible that the state of immunodeficiency may either favor the selection of viral variants of increased pathogenicity or enhance the cardiovirulence of specific viral strains, influencing the clinical course of cardiomyopathy. This could explain the fact that all HIV-DCM patients with an optical density unit >1 showed a myocardial infection with CVB3 or with cytomegalovirus in addition to HIV, whereas IDCM patients with a positive in situ hybridization test for CVB3 or adenovirus alone showed an optical density unit <1. According to the value of intensity of iNOS staining, we found that in HIV-DCM patients, a value of 1 optical density unit could act as a cutoff value. Above this value, in fact, the survival rate was significantly lower than in patients showing an optical density unit <1.

The cardiac myocyte seems to be the principal site of the enzyme. The negative inotropic effect of NO, exerted over long periods, may lead to a permanent depression of myocardial cells, negatively influencing the clinical course of cardiomyopathy.

Our study highlighted the role of the immunological factors and cytokines in inducing structural and functional changes of the myocardial tissue as response to an inflammatory process, which is frequent, but not exclusively, induced by a viral agent. Considering the low prevalence of coronary artery lesions documented at autopsy of patients who died of CHF, the finding of myocardial fibrosis observed at histological examination of biopsy samples obtained from both HIV-DCM and IDCM patients may represent the final expression of an intramyocardial inflammatory process in the absence of coronary artery disease.

In HIV-DCM, in which a viral infection is more frequently documented, the HIV myocardial infection, the interaction between HIV and other cardiotropic viruses, and the state of immunodeficiency may enhance the inflammatory response and increase both the expression and the cytotoxic activity of specific cytokines, such as TNF-α, and iNOS. The role of immunological factors in the pathogenesis of HIV-DCM is further supported by the improvement of both ventricular structure and function with monthly intravenous infusions of immunoglobulins in HIV-1–infected patients, as described by Lipshultz et al.

However, further efforts should be made to elucidate the pathogenesis of HIV-related heart disease. From studies of HIV-positive patients and heart disease, we may learn more about myocardial virology and immunology, with significant implications for other non-HIV cardiovascular diseases. In fact, because the role of infection and inflammation in so many other cardiovascular diseases is only now becoming recognized, discovery of the molecular mechanisms of HIV-related heart disease may have broader implications and provide the basis for rational therapeutic strategies and improved care.

Appendix

In addition to the study authors, the members of the GISCA included Franco De Rosa, MD (Department of Infectious and Tropical Diseases, University La Sapienza, Rome); Fabrizio D’Andrea, MD (Division of Cardiology, Aurelia Hospital, Rome); Willi Calderon, MD, Francesca Cadario, MD, Gaetano Filice, MD (Department of Infectious and Tropical Diseases, Policlinico S. Matteo, Pavia); Maurizio Viani, MD, Francesco Caccamo, MD, Gianissto Garavelli, MD (AIDS Center, Pavia); Alfonso Barbieri, MD, Rossano Vitali, MD (AIDS Center, Gorgonzola); Fiorenzo Edo, MD, Fausto Barbieri, MD (AIDS Center, Vigevano); Silvano Lopez, MD (AIDS Center, Abbiategrasso); Giovanni Rizzo, MD (Division of Infectious Diseases, General Hospital, Novara); Bruno Caccianotti, MD, Giovanni Salandra, MD (AIDS Center, Foggia); Alfonso Catanzaro, MD (AIDS Center, Lucera).

References


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