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Infection with HIV-1 Induces a Decrease in mtDNA

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Cross-sectional studies have suggested that infection with human immunodeficiency virus (HIV) type 1 could reduce the mitochondrial DNA (mtDNA) content of blood cells. We investigated mtDNA content in peripheral blood mononuclear cells (PBMCs) obtained from 36 antiretroviral therapy-naive documented HIV-1 seroconverters, before and after seroconversion. mtDNA content statistically significantly decreased 1 year after seroconversion and showed a nonsignificant decrease during the subsequent 4 years. These findings confirm that infection with HIV-1 may, itself, reduce mtDNA content, at least within PBMCs. This could have implications for the subsequent development of mitochondrial toxicities associated with the use of nucleoside analogue reverse-transcriptase inhibitors.

Immunodeficiency is the hallmark of HIV-1 infection. The availability of highly active antiretroviral therapy (HAART) for HIV-1 infection has led to a dramatic decrease in mortality and disease progression, causing HIV-1 infection to assume the characteristics of a chronic disease. Nucleoside analogue reverse-transcriptase inhibitors (NRTIs) are major components of HAART and are known to inhibit in vitro DNA polymerase-γ, the enzyme responsible for mtDNA replication, to various extents [1]. In patients, NRTIs, by way of their effects on mitochondria, have been suggested to contribute to a range of treatment-associated adverse effects, which include peripheral neuropathy, myopathy (including cardiomyopathy), hepatic steatosis, lactic acidosis, and peripheral lipodystrophy [1]. HIV-1 infection itself may cause various complications, including peripheral neuropathy, cardiomyopathy, wasting, and nephropathy [2]. Interestingly, some of these complications resemble the aforementioned adverse effects of NRTIs. One could therefore speculate that HIV-1 infection itself has adverse effects on mitochondria. In fact, the results of recent cross-sectional studies, which included HIV-1 infected patients receiving HAART as well as those who were therapy naive and uninfected control subjects, have indeed suggested that HIV-1 infection may be responsible for a decrease in mtDNA in peripheral blood mononuclear cells (PBMCs) [3–5]. To address more directly the question of whether HIV-1 infection may result in mtDNA depletion, we performed a longitudinal study in which we assessed the mtDNA content in PBMCs obtained from a group of individuals with documented HIV-1 seroconversion.

Patients and methods. The Amsterdam Cohort Study (ACS) was set up between October 1984 and March 1986—961 asymptomatic men living in and around Amsterdam who had multiple homosexual contacts were enrolled in a prospective study of HIV infection and AIDS. Clinical and epidemiological data were collected, and blood samples were drawn every ∼3 months for serological and immunological assessment, as well as for storage of both serum and PBMCs for future studies. Written, informed consent was obtained from all participants. This resulted in a cohort consisting of men who were found to already be HIV-1 seropositive at entry, as well as men who were seronegative and remained so or who seroconverted during follow-up.

The samples in our study consisted of serial collections of cryopreserved PBMCs from 36 men with documented HIV-1 seroconversion who had been enrolled in the ACS. Preseroconversion samples had been obtained at a median of 3 years (interquartile range [IQR], 1–6 years) before seroconversion. Postseroconversion samples were selected that had been obtained at ∼1 and ∼5 years after seroconversion. Participant selection from the overall group of documented HIV-1 seroconverters in the ACS was determined by the availability of samples at the repository laboratory and whether antiretroviral therapy (ART) had been administered within the period of observation after seroconversion. The moment of seroconversion was considered to be time point 0.

Patient PBMCs were obtained from the repository laboratory, where they had been freshly isolated from heparinized blood by means of the Ficoll-Hypaque method and then cryopreserved in liquid nitrogen by use of an automated freezing program. Before lysis with L6, a lysis buffer that contains chaotropic guanidinium thiocyanate [6], all PBMCs were thawed.

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Reprints or correspondence: Miriam Casula, Meibergdreef 9, PO Box 22700, 1100 DE Amsterdam, The Netherlands (m.casula@amc.uva.nl, m.casula@iatec.com). The Journal of Infectious Diseases 2005;191:1468–71 © 2005 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2005/19109-0013$15.00.
and washed, thereby limiting contamination with platelets to a level that did not alter the result of mtDNA quantification [7, 8]. Total DNA was extracted from an equivalent amount of $3 \times 10^5$ patient PBMCs by use of the silica-based method of Boom et al. [6]. An amount of total nucleic acid equivalent to $3 \times 10^5$ cells was used as input in the amplification reaction, and each sample was tested in duplicate. As a calibrator, known amounts of plasmid mtDNA and nuclear DNA (nDNA) were mixed and included in each assay.

mtDNA and nDNA of each isolate were simultaneously amplified by means of real-time duplex nucleic acid sequence-based amplification (Retina Mitox). Results for each isolate were calculated as the mean value of the mtDNA:nDNA ratios of duplicate measurements. In addition, patient data such as age and laboratory measurements—including HIV-1 load and CD4+ and CD8+ cell counts in peripheral blood—were also retrieved from the ACS database. Although the objective of the study was to determine the relationship between the relative time since seroconversion and changes in mtDNA content per cell, the effect of other parameters (i.e., age) was also taken into consideration.

Changes in the number of mtDNA copies per cell among the 3 available time points were assessed by use of the paired Student’s $t$ test. mtDNA data were logarithmically transformed, to obtain a normal distribution. The change in mtDNA content per cell was also expressed as a percentage relative to the preseroconversion value. Spearman’s correlation analysis was used to determine any association between mtDNA content and both immunological parameters and HIV-1 load after seroconversion. At 1 and 5 years after seroconversion, the association between the change in mtDNA content and HIV-1 load was also examined. Group size in the analyses was determined by the availability of samples at the 3 time points and of CD4+, CD8+, and HIV-1 load data from the ACS database. Furthermore, a correction by exclusion was made for the use of ART. Analyses were conducted by use of SPSS (version 11.0 for Windows; SPSS). The level of significance was set at $P < .05$ throughout the analysis.

**Results** Although all 36 patients were therapy naive at 1 year after seroconversion, only 18 remained therapy naive and had samples available at 5 years after seroconversion. The remaining 18 patients were excluded from the analysis at 5 years after seroconversion, because of unavailability of samples ($n = 10$) or the commencement of ART ($n = 8$).

mtDNA content decreased significantly, from a median of 264 copies/cell (IQR, 202–404 copies/cell) before seroconversion to a median of 234 copies/cell (IQR, 178–306 copies/cell) 1 year after seroconversion ($P = .004$) (figure 1). In terms of the percentage relative to preseroconversion values, the mtDNA content 1 year after seroconversion decreased significantly, by 11% (95% confidence interval, 2%–21%).

Although mtDNA content showed an additional decrease between 1 (median, 240 copies/cell [IQR, 196–279 copies/cell]) and 5 (median, 213 copies/cell [IQR, 177–293 copies/cell]) years after seroconversion, this did not reach statistical significance ($P = .894$) (figure 1). At the time points assessed, characteristic trends in CD4+ and CD8+ cell counts were observed. There was a significant decrease ($P < .001$) in CD4+ cell count (figure 2) from preseroconversion values (median, 761 cells/mm$^3$ [IQR, 578–960 cells/mm$^3$]) through 5 years after seroconversion (median, 480 cells/mm$^3$ [IQR, 345–600 cells/mm$^3$]), whereas the CD8+ cell count increased significantly between 1 (median, 715 cells/mm$^3$ [IQR, 515–998 cells/mm$^3$]) and 5 (me-
dian, 875 cells/mm³ [IQR, 782–1303 cells/mm³]) years after seroconversion \((P = .001)\). Analysis of the mtDNA content within fractionated CD4+ and CD8+ cells from a healthy donor showed identical mtDNA content (270 copies/cell) for each of these 2 cell types (data not shown), which suggests that changes in the CD4+ :CD8+ ratio cannot explain the altered mtDNA content of PBMCs that we found.

Cross-sectional and longitudinal analysis of the association between mtDNA and CD4+ cell count, CD8+ cell count, or HIV-1 load did not yield any statistically significant results (data not shown). Furthermore, the mtDNA content in PBMCs at the preseroconversion time point was not influenced by participants’ age \((r^2 = 0.004; P = .714)\), which indicates that older age does not explain the decrease in mtDNA after infection with HIV-1. Although the individuals in the ACS might have been exposed to other factors, such as hepatitis coinfection, because of a lack of data, we could not determine the effects of these additional factors on mtDNA.

Discussion. Our longitudinal study confirms the finding from earlier cross-sectional studies that HIV-1 infection, in the absence of any ART, is associated with a decrease in the mtDNA content of PBMCs. The mechanism underlying this mtDNA decrease remains to be established. However, considering that mtDNA, under normal circumstances, may already be exposed to reactive oxygen species (ROS) produced within mitochondria \([9]\), increased generation of ROS in response to viral infection might further affect mtDNA integrity by inducing mutations and deletions, which possibly leads to a measurable decrease \([10, 11]\). Furthermore, certain HIV-1–encoded proteins, such as Vpr, have been shown to adversely affect mitochondrial integrity \([12]\), which may occur both in HIV-1–infected cells and in surrounding uninfected bystander cells \([13]\). This possibly renders the normal ROS scavenging mechanism ineffective, resulting in accelerated damage to mtDNA in such cells and in apoptosis and thereby contributing to certain HIV-1–associated disease manifestations. One may expect that, under such circumstances, tissues with a high energy demand—such as nervous tissue, cardiac muscle, and renal tissue—would be the ones most affected. Furthermore, the analysis performed on samples from 18 ART-naive patients showed that mtDNA in PBMCs continued to decrease throughout the 5 years after seroconversion, although the decrease did not reach statistical significance. The statistical power of our analysis was limited, however, by the availability of samples.

In agreement with our data, both functional mitochondrial damage and a decrease in mtDNA in PBMCs in HIV-infected subjects, compared with healthy control subjects, has been reported by a number of other investigators \([3–5, 14]\), whereas others have reported no significant difference in mtDNA content \([15]\). The discrepancy between the results of these cross-sectional studies has yet to be explained.

In conclusion, our results are consistent with the concept that HIV-1 infection itself may be responsible for a decrease in mtDNA within PBMCs. Although subsequent treatment with ART, including NRTIs, may further aggravate the decrease in mtDNA by inhibiting DNA polymerase-γ and, thereby, possibly rendering patients more prone to developing certain NRTI toxicities, the suppression of HIV-1 infection may be expected to exert a restorative effect on mtDNA. The net effect of these 2 counteracting factors may ultimately represent the balance between the intrinsic mitochondrial toxicity of the specific NRTIs in the regimen and the overall antiviral potency of the ART regimen.

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