HIV and Mitochondria: More Than Just Drug Toxicity

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(See the article by Morse et al, on pages 1778–87.)

Potent antiretroviral therapy (ART) is more widely available and safer than ever and has made human immunodeficiency virus (HIV) infection a treatable chronic infection in many settings. Cohort studies and clinical trials have demonstrated the need for early ART to optimize individual [1] and public health [2] outcomes. Nonetheless, complications of ART still hold an inauspicious and prominent place in the history of HIV. There was early recognition of generalized and tissue-specific mitochondrial toxicities associated with older nucleoside reverse transcriptase inhibitors (NRTIs), including myopathy, peripheral neuropathy, lactic acidosis, and lipoatrophy (recently reviewed elsewhere [3]). In many parts of the world, persons have been or are currently treated with these older NRTIs and remain at risk for overt mitochondrial toxicities. More recently, metabolic syndrome and increased cardiovascular risk have been noted in patients treated with potent ART including protease inhibitors (PIs) [4]. Other contemporary complications of chronic HIV infection and ART include renal [5], bone [6], and neurocognitive [7] abnormalities and "premature" aging in otherwise healthy, chronically ART-treated patients [8]. Mitochondrial dysfunction likely plays a role in all of these complications [9–13].

Mitochondria are organelles that exist in virtually all mammalian cells (except red blood cells) and are the site of arguably the most fundamentally important process of the cell: oxidative phosphorylation (OXPHOS) and generation of adenosine triphosphate (ATP). They harbor multiple copies of their own DNA molecules (mtDNA) that encode 13 of the 90 proteins that must be coordinated to produce ATP. Mitochondria have numerous other critical functions, including homeostasis of cellular reactive oxygen species and regulation of the intrinsic apoptotic pathway via cytochrome *c* release. Accumulation of somatic mtDNA mutations and/or progressive mitochondrial dysfunction are involved in human aging processes and diseases [14]. It is also plausible that chronic infection and inflammation and/or drugs with adverse effects on mitochondrial function would contribute to long-term complications in HIV-infected persons. HIV and HIV polypeptides—in the absence of ART—have been shown to contribute to mitochondrial dysfunction and apoptosis [15, 16], including in $CD4^+$ and $CD8^+$ T cells [17]. A recent study has also demonstrated mitochondrial damage—decreased mtDNA quantity and function—in adipose tissue from ART-naive individuals compared with ART-treated or HIV-seronegative controls [18].

Despite the recognition of a role for mitochondrial toxicity in HIV and ART complications for >2 decades, the pathophysiology of these complications is still poorly understood. The study by Morse et al [19] in this issue of the *Journal of Infectious Diseases* provides some insights. In this small cross-sectional study of HIV-infected, ART-untreated and -treated patients on NRTIs (without PIs), and HIV-seronegative controls, mitochondrial genetic and histological analysis was performed in peripheral blood mononuclear cells (PBMCs), adipose, and muscle tissue. Their population was characterized by long-term HIV infection (median time since diagnosis, almost 12 years) and relatively long-term ART exposure among the treated subjects (median, >8 years). Of the patients on ART, 34% had clinically defined lipoatrophy. By comparing untreated subjects to those on NRTI-containing ART and HIV-uninfected subjects, Morse et al [19] observed that HIV infection was associated with increased adipose mtDNA levels in the absence of ART. These results may have differed from other recent data [18] due to differences in populations; their untreated HIV-infected population was substantially older (median age, 44 vs 37 years).

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In addition, levels of activated ($CD38^+$/$HLA\text{-}DR^+$) and central memory ($CD27^+$/$CD45RO^+$) $CD8^+$ (and to a lesser extent, $CD4^+$) lymphocytes in blood were positively correlated with mtDNA in adipose tissue from either the bicep or quadricep region. This novel observation suggests that activated peripheral T cells are related to mitochondrial biogenesis in adipose tissue. Earlier studies identified negative correlations between T-cell activation and mtDNA in blood [20, 21], but to our knowledge, the present study represents the first to report a correlation with adipose mtDNA. Morse et al [19] did not observe a correlation between T-cell activation and PBMC mtDNA. Given the cross-sectional design, one cannot determine causality or the direction of the relationship (did T-cell activation lead to increased mtDNA quantity in adipose, or vice versa?). Immune activation in adipose tissue was not assessed, so whether peripheral T-cell activation was a marker for tissue-level immune responses in these subjects is not known. The authors also do not present data on peripheral or tissue inflammation, so relationships between immune activation, adipose mtDNA, and inflammation are not clear. Nonetheless, these data provide further evidence that HIV infection can affect mitochondria at the tissue level in the absence of ART and indicate that there are possible interactions between immune activation and tissue mitochondria that should be studied further.

A strength of this study is the use of the huMITOchip microarray analysis in PBMCs and in adipose and muscle tissues. PBMCs of untreated, HIV-infected subjects had increased expression of genes related to cell activation, cell death, and inflammatory cytokines and chemokines compared with the HIV-infected subjects on NRTIs and HIV-negative controls. In contrast, mitochondrial genes were downregulated in PBMC and adipose tissue from HIV-infected, untreated persons relative to the other groups. These data again suggest that HIV infection has direct effects on mitochondria and that immune cell activation and inflammation are involved in this process. How might mitochondria, inflammation, and immune activation be interrelated? One possibility was highlighted in an important recent study demonstrating that free plasma mtDNA released from damaged or dead cells can bind Toll-like receptor-9 (TLR9), an intracellular receptor that responds to bacterial or viral DNA molecules [22]. The activation of TLR9 in monocytes or neutrophils results in a potent proinflammatory reaction and cytokine production. A subsequent study has demonstrated increased plasma mtDNA in acute HIV seroconverters and ART-naive subjects compared with HIV-seronegative controls and long-term nonprogressors, and a positive correlation between plasma HIV RNA and plasma mtDNA [23].

Interpretation of studies assessing mitochondrial complications have been limited by lack of consensus on the optimal in vivo and ex vivo measure(s) by which to quantify mitochondrial dysfunction. There are 2 generally accepted methods to diagnosis mitochondrial dysfunction in symptomatic persons: elevated lactate levels in the blood, or histologic changes in mitochondria and/or decreased OXPHOS enzymes in skeletal muscle tissue—neither of which are feasible for screening asymptomatic persons. Morse et al [19] evaluated mitochondria histopathologically by staining muscle tissue and found no differences between their groups, in contrast to other studies [9, 24, 25]. The authors suggest that differences between their results and earlier studies may have been in part due to preferential inclusion of persons more tolerant of ART. Mitochondrial DNA quantity in peripheral blood cells is a potentially more feasible screening tool. However, both increases and decreases in mtDNA have been reported in pathogenic conditions, there is no standard for defining what constitutes an abnormal mtDNA quantity, and data from heterogeneous HIV-infected populations have been inconsistent [26–29]. Only very recently has there been consensus on methods for quantifying mtDNA copy number [30]. Morse et al [19] observed a lack of correlation between PBMC and tissue mtDNA and differences in adipose tissue mtDNA between HIV-infected, ART-treated, and seronegative subjects, both of which have been shown elsewhere [31]. Ex vivo measures of OXPHOS protein levels and/or enzyme activities were not performed in the present study but have been used to quantify mitochondrial enzyme quantity and/or function in PBMCs and adipose tissue from HIV-infected persons [29]. This study showed that OXPHOS proteins (NADH dehydrogenase and cytochrome *c* oxidase) in PBMCs and adipose were correlated; thus, this method could represent a promising approach for studying effects of HIV and/or ART in peripheral blood cells that are associated with metabolic alterations in adipose tissue.

The study by Morse et al [19], together with other recent studies, should stimulate continued research in HIV and mitochondria. Our understanding of the role of mitochondria in HIV disease pathogenesis and ART complications remains partial. To improve this understanding, the limited view of mitochondrial toxicity as an outdated complication of older NRTIs must be revised and expanded, while still acknowledging this scenario remains a present reality in many resource-limited settings around the world. Carefully performed, tissue-based studies like that by Morse et al [19] (ideally longitudinal in design) are still needed to determine the effects of age (both at infection and ART initiation) and aging, contemporary ART, baseline inflammation and/or immune activation status, and other as-yet-undiscovered viral and host factors on mitochondrial function. Substudies of ongoing or planned clinical trials and cohort studies could be feasible settings in which to perform such assessments. There is much yet to be learned, and it

may well be that a clearer understanding of inflammation and immune activation, "premature" aging, and non-AIDS comorbidities in HIV-infected persons will be found by revisiting the site of the earliest ART toxicities that many have forgotten.

Notes

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