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Mitochondrial DNA, Neurologic and Systemic Inflammation, and Immune Dysregulation

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Background

- Cell-free mitochondrial DNA (mtDNA) is a highly immunogenic molecule[1] that can be detected in the cerebrospinal fluid (CSF) and is associated with neurologic disease[2, 3, 4].
- Chronic immune activation and persistent inflammation have been associated with the progression to AIDS.

Objectives

- **To characterize the relationship between free mtDNA in the CSF and inflammation during successful viral suppression in relation to AIDS status.**
- **To investigate the dynamics of mtDNA, HIV RNA, and inflammation following ART interruption.**

Methods

- We quantified levels of mtDNA in CSF by droplet digital PCR (ddPCR) in HIV-infected individuals:
  - ✓ (1) with completely suppressed RNA levels in CSF and blood (n=37)
  - ✓ (2) who underwent a structured ART interruption (n=5), with samples before, during, and after interruption (total n=41)
- Markers of immune activation (sCD14, sCD163, neopterin), and markers of inflammation and cellular trafficking (IP-10, MCP-1, IL-6, IL-2, IL-8, TNF-α, MIP-1α) were measured in CSF and blood plasma by ELISA and Meso Scale Discovery platform, respectively.
- Fixed-effects and mixed-effects regression analyses and all statistical testing were performed using R software.

References

1. Zhang et al. Nature. 2010.
2. Walko et al. Shock. 2014.
3. Kingwell. Nature Reviews. 2013
4. Wang et al. J. of Neurosurgery. 2013

Table 1. Patient Characteristics

	All participants (n=37)	AIDS (n=16)	No AIDS (n=21)	p-value*
Sex (M:F)	29:8	13:3	16:5	1
Race (Caucasian:No Caucasian)	26:11	12:4	14:7	0.72
CD4 Absolute	669 (476-904)	533 (452.5-748.75)	724 (559-904)	0.15
CD4 Percent	33.6 (24.4-39.7)	27.4 (19.25-32.75)	37.9 (33-44.8)	**<0.01**
CD8 Absolute	918 (689-1154)	1017 (732.25-1250.75)	785 (614-1067)	0.15
CD8 Percent	24.4 (33.5-50.6)	44.55 (33.2-60.48)	40.9 (35.1-46.4)	0.47
CD4/CD8	0.83 (0.51-1.02)	0.625 (0.2925-0.9675)	0.84 (0.71-1.29)	0.13

Table 1: Characteristics of our study participants. Median with IQR values are shown for the entire cohort and by groups defined by AIDS status. *Represent the p-value of a two-tailed Mann-Whitney or a Fisher test.

Results

Figure 1 and Table 2: mtDNA is associated with AIDS status, and systemic and CNS inflammation

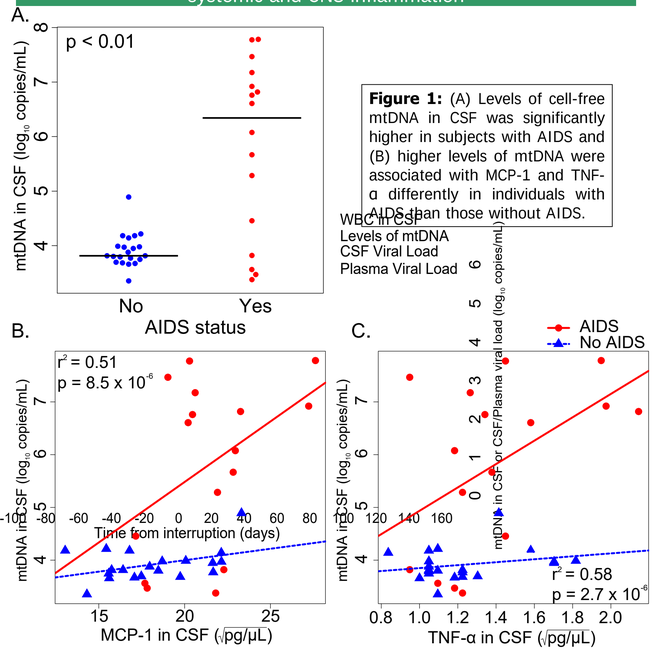


Figure 1: (A) Levels of cell-free mtDNA in CSF was significantly higher in subjects with AIDS and (B) higher levels of mtDNA were associated with MCP-1 and TNF-α differently in individuals with AIDS than those without AIDS.

Figure 2. mtDNA in CSF rise before pleocytosis and HIV RNA levels

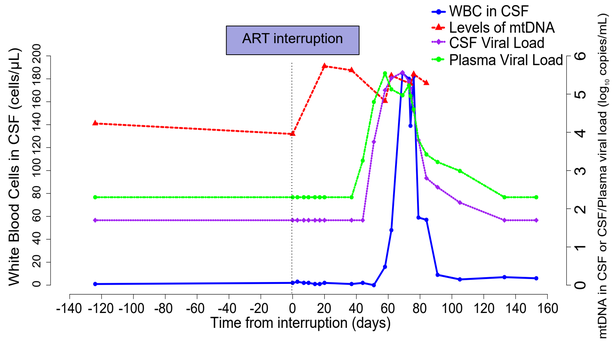


Figure 2: In all cases when ART is interrupted, mtDNA levels in the CSF rise before plasma HIV RNA levels, CSF RNA levels, and CSF white blood cell (WBC) count.

Figure 3: mtDNA in CSF rise simultaneously with inflammation markers

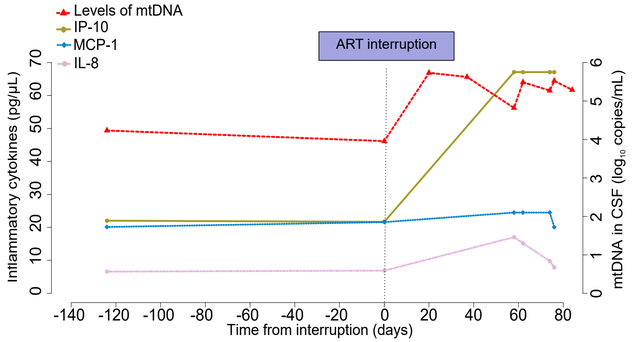


Figure 3: In all cases when ART is interrupted, mtDNA levels rise simultaneously with all markers of inflammation.

Table2: mtDNA and CSF VL predicts pleocytosis and inflammation

A.

Outcome \ Predictors	mtDNA	CSF VL	Interaction
WBC	0.0	<0.01	<0.01
IP-10	0.01	<0.01	0.03
MCP-1	0.16	0.06	0.09
MIP-a	0.02	<0.01	-
TNF-a	0.01	<0.01	<0.01
IL-2	0.0	<0.01	0.06
IL-8	0.5	0.1	0.32
IL-6	0.19	0.21	0.32
sCD14	0.4	0.36	0.57

B.

Outcome \ Predictors	mtDNA	Plasma HIV RNA	Interaction
WBC	0.07	0.03	0.01
IP-10	0.09	0.19	0.05
MCP-1	0.92	0.62	0.83
MIP-a	0.03	<0.01	-
TNF-a	0.16	0.25	0.11
IL-2	0.93	0.9	0.7
IL-8	0.59	0.99	0.66
IL-6	0.7	0.67	0.53
sCD14	0.23	0.24	0.14

Table 2: In a multivariate mixed-effect regression model, mtDNA and CSF RNA levels could predict levels of pleocytosis and other markers of neuroinflammation better that plasma HIV RNA levels. The p-values of each predictor (mtDNA and HIV RNA levels) and the p-value associated with interaction term are shown for each multivariate model.

Conclusions

- ❖ Levels of mtDNA were significantly higher in individuals with AIDS (**Figure 1**).
- ❖ Higher levels of mtDNA were associated with more inflammation in the CNS and blood; however, this association appears to be different in individuals with AIDS compare to those without AIDS for some inflammatory markers (**Figure 1**).
- ❖ Altogether, these data suggest that mtDNA may be a biomarker of inflammation despite successful viral suppression, but this inflammatory mechanism may be different in subjects with AIDS.
- ❖ Increased levels of cell-free mtDNA in CSF coincided with an increase in levels of inflammatory cytokines, and preceded both HIV rebound and pleocytosis (**Figure 2-3**).
- ❖ Pleocytosis and other markers of inflammation can be predicted by the levels of mtDNA and HIV RNA in CSF better than by HIV RNA in blood plasma (**Table 2**).
- ❖ These data suggest that cell-free mtDNA may be a trigger for neuroinflammation either on its own or in conjunction with HIV RNA.

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