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IL-18 and IL-3 In Extracellular Vesicles: Biomarkers for a Durable Elite Control

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BACKGROUND

Elite controllers (EC) with a durable control of HIV-1 replication may represent a model of functional cure. Extracellular vesicles (EVs) have emerged as a mechanism for intercellular communication by targeted delivery of cytokines. We evaluated the cytokine profile associated with EVs in well-characterized cohorts of people living with HIV (PLWH) with different virological control status, including durable and transient EC.

METHODS

✓ Plasma samples from 120 donors were included and divided into 5 groups defined as:

- 30 antiretroviral therapy (ART)-naïve (median 7 days after HIV diagnosis).
- 30 ART-treated with nondetectable viremia (median time on ART 9 years).
- 30 EC who controlled viremia for a median of 14.4 years [15 transient controllers (TC) who ultimately lost virus control, and 15 persistent controllers (PC) who sustained virus suppression].
- 30 HIV-uninfected controls.

✓ Levels of 39 pro-inflammatory markers in and on EVs isolated by ExoQuick from stored plasma were quantified using a multiplexed bead-based Luminex assay.

✓ Random forest, principal component analysis, and decision trees were performed to identify specific cytokines as a signature of each study group.

Table 1. Main characteristics of the study population.

	Controls (N=30)	ART-Naïve (N=30)	ART-exposed (N=30)	PC (N=15)	TC (N=15)	*p-value Global*	*p-value PC vs. TC*
Age (years)	36.0 [29.0-41.0]	41.0 [32.0-52.0]	52.0 [43.0-56.0]	54.1 [48-58]	48.4 [39-52]	<0.001	n.s
Gender (male)	50 (15)	71 (22)	66.7 (20)	53.3 (8)	46.7 (7)	n.s	n.s
Year of diagnosis	NA	2017 [2017-2018]	2004 [1997-2011]	1998 [1991-2006]	1993 [1985-2003]	<0.001	n.s
Days since HIV diagnosis	NA	7 [0-64]	4803 [2316-7373]	4427 [1971-7054]	5783 [2075-8697]	<0.001	n.s
HIV viral load (copies/mL)	NA	27450 [9700-110000]	ND	ND [25-50]	ND [37-50]	<0.001	n.s
CD4 (cells/µL)	842 [712-1018]	456 [267-657]	827 [609-1071]	855.9 [426-1290]	833.7 [640-1038]	<0.001	n.s
CD8 (cells/µL)	474 [404-653]	983 [730-1284]	824 [512-933]	1075 [575-1724]	913.9 [637-1094]	<0.001	n.s
CD4/CD8 ratio	1.6 [1.4-2.1]	0.4 [0.2-0.7]	1 [0.8-1.3]	1.1 [0.56-1.3]	1 [0.5-1.5]	<0.001	n.s
Platelet (cells/µL)	246 [211-275]	219 [186-257]	212 [192-245]	n.a	n.a	n.s	n.a
Neutrophils (cells/µL)	3.40 [2.70-4.73]	2.64 [1.91-3.21]	3.67 [2.68-4.90]	n.a	n.a	0.002	n.a

Data are expressed as median [interquartile range], %(n). NA: not applicable; ND: not detectable; n.s: not significant. Kruskal-Wallis H and Chi-squared test were used for comparisons between groups.

Higher levels of **IL-18** associated with **Extracellular Vesicles** (EVs) were identified as a **biomarker** for the **HIV Elite Controller** population and higher levels of EV **IL-3** best distinguished Persistent Controllers from Transient Controllers.

RESULTS

Figure 1. Heat Map: relative cytokines levels in EVs.



✓ Overall, the median levels of cytokines were 1.33 fold higher for PLWH than for the HIV uninfected control group. Within PLWH, EC showed the highest levels of cytokines (1.11 fold higher than for ART-exposed and 1.32 higher than for ART-naïve). Within the EC the levels of cytokines were 1.36 higher for PC than for TC.

Table 2. Specific cytokines in EVs as potential biomarker of PLWH groups.

Groups (median)	Cytokines	AUC	Sen/Spe (%)	Cut-off pg/mL
PLWH vs. HIV-				
1.15 vs. 0.26	IL-18	0.741	70/77	0.489
EC (↑) vs. ART-exposed				
3.98 vs. 0.15	IL-18	0.942	73.3/100	2.23
EC (↑) vs. ART-naïve				
3.98 vs. 1.56	IL-18	0.746	73.3/66.7	2.192
14.08 vs.7.67	MIG	0.741	70.0/76.7	10.49
3.33 vs. 2.00	IL-22	0.705	83.3/56.7	2.23
0.39 vs. 0.23	MIP-1β	0.701	73.3/66.7	0.31
TC vs. PC (↑)				
2.32 vs. 4.50	IL-3	0.824	73.3/86.7	3.97
3.16 vs. 9.09	TRAIL	0.813	86.7/73.3	3.71
80.56 vs. 97.94	INF-λ	0.782	66.7/93.3	81.41
10.35 vs. 17.00	MIG	0.707	93.3/60.0	10.87

✓ Higher levels of EV IL-18 best distinguished PLWH and uninfected controls.

✓ In the context of supressed viremia, higher levels of EV IL-18 also distinguished Elite Controllers from ART-exposed with a sensitivity of 73.3% and a specificity of 100%.

✓ Within Elite Controllers, higher levels of EV IL-3 best distinguished transient and persistent controllers. (**Figure 2**).

CONCLUSIONS

✓ EC showed higher levels of EV-associated cytokines compared with other PLWH groups.

✓ EV-associated cytokine levels were higher for EC with durable control of HIV-1 replication (PC) than for those without (TC).

✓ Higher levels of IL-18 associated with EVs were identified as a biomarker for the EC population in persons with virologic control and IL-3 best distinguished the PC phenotype from TC.

✓ The role of EV cytokines, intercellular communication and endogenous control of HIV expression should be investigated further.

ADDITIONAL KEY INFORMATION

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