00647 - PLASMA INFLAMMATORY BIOMARKER SIGNATURE ASSOCIATED WITH CVD IN HIV INFECTION

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ABSTRACT

Background:
We have recently shown that a specific isoform of the human proinflammatory cytokine IL-32 (IL-32D) is selectively upregulated in HIV+ individuals with cardiovascular disease (CVD). Here we extend our studies to screen for other inflammatory markers that could be combined with IL-32 to enhance the prediction of CVD in HIV infection.

Methods:
Using the MesoScale Technology, we measured 84 inflammatory and anti-inflammatory factors in plasma from n=79 HIV+ aviremic males participating in the Canadian HIV and Aging Cohort Study (n=49 CVD+ with coronary artery atherosclerosis measured by cardiac computed tomography and n=30 CVD-) and HIVneg controls (n=25 CVD+ and n=24 CVD-). We used a generalized linear regression algorithm (glmnet) with Lasso regularization and Leave-One-Out cross validation to predict the presence of coronary artery atherosclerosis in the study participants.

Results:
Alongside with the upregulation of IL-32D, we observed an HIV-specific signature characterized by higher plasma levels of IL-18, VEGF-A, FGF23, FLT3L and FSH in HIV+ individuals with CVD (univariate analysis, p=0.0016, 0.0053, 0.0386, 0.0075 and 0.049, respectively) combined with lower levels of TNF-related apoptosis inducing ligand (TRAIL), IFNb and IL-3 (p=0.0149, 0.0093 and 0.0288, respectively). By integrating IL-32D expression with these modulated factors in a multivariate analysis, CVD was predicted as a binary outcome (presence/absence) with a misclassification error of 34%. The prediction model was independent of age, statin treatment and smoking. Given the growing evidence for the atheroprotective role of TRAIL-expressing monocytes, we tested the functional link between IL-32 and TRAIL. We show here that primary human monocytes treated with IL-32 down-regulate TRAIL expression, acquire an M1 activated phenotype (CD206negCD163negCD80+TRAILneg) and produce inflammatory cytokines such as IL-6 and TNFα.

Conclusions:
Here we report a specific plasma inflammatory signature that holds promise to predict CVD in HIV+ individuals independently of traditional risk factors. Moreover, the functional link between IL-32 and TRAIL and their opposite effects on monocyte functions further highlight the key role of IL-32 in CVD and its potential as a therapeutic target in HIV infection.

Study population and objectives

Variable	HIVneg Male CVD- (n=24)	HIVneg Male CVD+ (n=25)	*P* value	HIV+ Male CVD- (n=30)	HIV+ Male CVD+ (49)	*P* value
Age (Years)	53.04±6.39	55.81±7.47	NS	52.48±6.27	55.55±6.54	0.037
Framingham Risk score	10.21±4.36	11.65±4.34	NS	9±4.14	10.69±5.66	NS
Statin Treatment	2 (8.3%)	5 (20%)	NA	4 (13.3%)	19 (38.7%)	
Smoking						
Never smoked	15 (62.5%)	12 (48%)		13 (43.3)	13 (26.5%)	
Current smoker	2 (8.3%)	4 (16%)		5 (16.6%)	20 (40.8%)	
Past smoker	7 (29.1%)	9 (36%)		12 (40%)	16 (32.6%)	
Body Mass Index (BMI)	26.87±3.68	27.02±5.68	NS	25.8±3.16	24.26±4.84	NS
Duration of infection (Years)	NA	NA	NA	14.07±8.06	18.36±7.63	0.015
Viral load (Copies/ml)	NA	NA	NA	< 40	< 40	NS
Nadir CD4 count (cells/mm³)	NA	NA	NA	169±164	199±145	NS
CD4 count (cells/mm³)	NA	NA	NA	526±257	594±270	NS
CD4/CD8 ratio	NA	NA	NA	0.85±0.41	0.82±0.44	NS

Table 1: Demographic and clinical parameters of study participants. Numbers are shown in Mean ± SD. NA: non-applicable.

- ❑ ***To screen for CVD biomarkers in our Canadian cohort of HIV and aging***
- ❑ ***To study the link between IL-32 expression and modulation of these biomarkers***
- ❑ ***To evaluate the predictability of CVD by IL-32 levels in combination with new biomarkers***

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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Upregulation of specific IL-32 isoforms in HIV^{neg} and HIV^{+} individuals with CVD

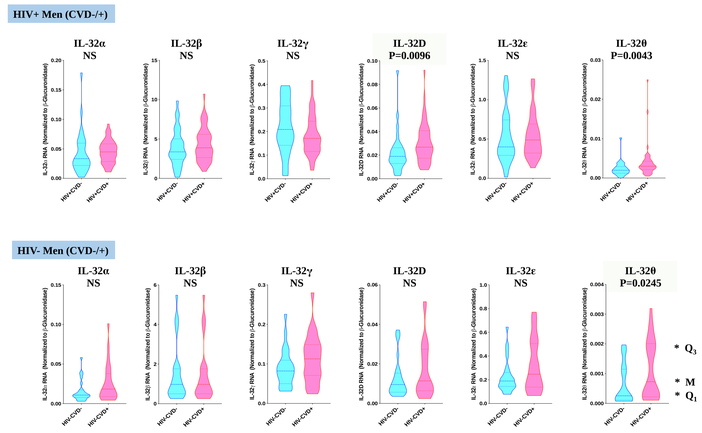

Figure 1. Relative expression of IL-32 isoforms in HIV^{neg} and HIV^{+} individuals (cART-treated). Shown are normalized data for quantitative RT-PCR on RNA extracted from peripheral blood mononuclear cells (PBMCs) of HIV^{+} individuals with (n=49) and without (n=30) coronary artery atherosclerosis measured by cardiac computed tomography (upper panels) and HIV^{neg} individuals (lower panels). *P* values are calculated by the non-parametric Mann-Whitney test. M: Median, Q1: first quartile, Q3: third quartile.

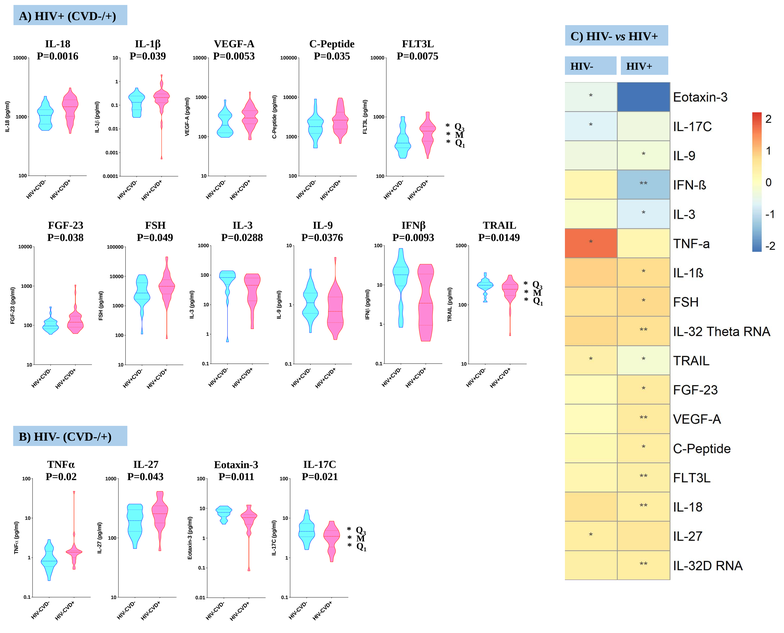

Figure 2: Significantly upregulated or down-regulated proteins in plasma from HIV+ **(A)** and HIV- **(B)** individuals with or without CVD (CVD+/CVD-) (Soluble proteins measured by MesoScale assays). **(C)** Heatmap showing fold change of significantly modulated proteins in CVD+ relative to CVD- in both HIV+ and HIV- individuals. *P* values are calculated by the non-parametric Mann-Whitney test.

Glmnet prediction model

Marker	Coef
IL32D RNA	6.234126
Statin treatment	0.758134
Age	0.034532
TSLP	0.021859
Smoking	0.014683
Ghrelin	0.002891
IL12/IL23p40	0.001673
VEGFA	0.000849
FGF23	0.000796
IL-18	0.00064
FLT3L	0.000321
BAFF	0.000277
FSH	3.28E-05
BDNF	5.97E-07
TRAIL	-0.00135
Eotaxin	-0.00158
IFNβ	-0.00214
IL-1α	-0.01694
IFNγ	-0.02689

Features are ranked by regression coefficients

Table 2: Predicting the presence/absence of the coronary artery atherosclerosis (calculated with total plaque volume, TPV) in HIV+ individuals using IL32 isoforms in combination with inflammatory plasma biomarkers.

Binary Outcome:
- **Presence of TPV (n=49) *versus* absence of TPV (n=30)**

Predictors:
- **IL-32 isoforms (IL-32α, β, γ, D, ε and θ) measured by qRT-PCR**
- **Plasma Biomarkers (84) measured by MesoScale technology**
- **Demographics and clinical predictors (Age, Statin, LDL, HDL, smoking)**

Classifier:
- **Glmnet, a generalized linear regression algorithm with Lasso regularization using Leave-one-out cross validation**

Model Performance:
- **Misclassification error = 0.34**

Tight correlation between IL-32 expression and IL-18 (a negative regulator of the atheroprotective TRAIL expression)

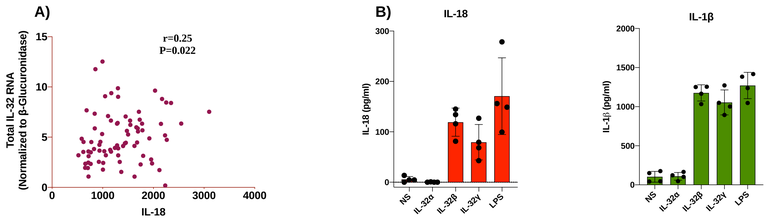

Figure 3: Correlations between IL-18 plasma levels and cell-associated IL-32 total RNA from HIV+ samples A) and impact of IL-32 on IL-18 and IL-1β in monocytes (B). Spearman test was used to assess the significance of correlations.

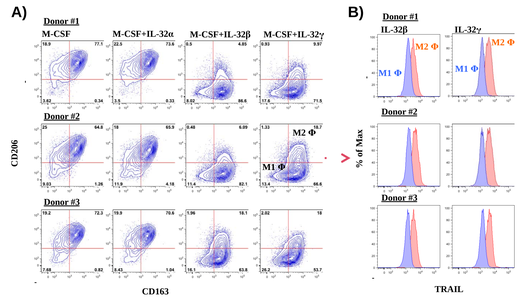

Figure 4: Impact of IL-32 on monocyte differentiation to macrophage-like cells. A) Monocytes isolated from PBMCs of HIV^{neg} donors and stimulated with M-CSF with or without IL-32 isoforms (α, β and γ) and incubated for 5 days. M-CSF alone or in combination with IL-32α induces M2-like phenotype (CD206+CD163+) whereas IL-32β and γ induce M1-like cells (CD206-CD163-). **B)** Expression of TRAIL on both M1-like and M2-like cells treated with IL-32β and γ (both isoforms induce downregulation of TRAIL on M1-like cells).

CONCLUSIONS

- ❖ **All IL-32 isoforms are significantly increased in PBMCs from HIV^{+} cART-treated individuals compared to HIV^{neg} controls. However, only IL-32D and IL-320 were differentially expressed in individuals with coronary artery atherosclerosis.**
- ❖ **We identified a novel plasma biomarker signature consisting of upregulation of IL-18, VEGF-A, FLT3L, FSH, FGF-23, IL-1β and C-peptide combined with down-regulated IFNβ, TRAIL, IL-3 and IL-9. These biomarkers were modulated in HIV+ individuals with and without CVD but not in HIV^{neg} individuals (HIV-specific signature).**
- ❖ **In a multivariate analysis, we confirm that IL-32D, combined with these biomarkers with the exception of C-peptide, IL-1β and IL-9 can predict CVD in HIV+ individuals independently of age, smoking and Statin treatment.**
- ❖ **Interestingly, IL-32RNA positively correlates with plasma IL-18. In line with this, IL-32 protein induces IL-18 expression in monocytes *in vitro*. IL-18 was previously shown to mediate down-regulation of TRAIL expression in atheroprotective macrophage. Here we show that this mechanism is likely IL-32-dependent.**

Conflict of Interest: We declare that we do not have any conflict of interest.

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