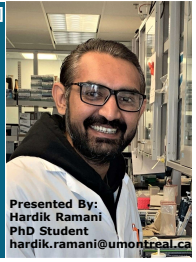
The 24th International AIDS Conference [EPA016]

Interleukin-32γ isoform induces the expression of a cardiotropic signature on a subset of memory CD4 T-cells with increased permissiveness to HIV-1 infection: A potential role in cardiovascular disease

H. Ramani [1,2], A. Gosselin[2], MA. Jenabian[3], M. Durand [2,4], Chartrand-Lefebvre[2,5], N. Chomont [1,2], JP. Routy[6], R. Thomas[7], B. Trottier[8], A. Landay[9], P. Ancuta[1,2], M. El-Far [2], and C. Tremblay [1,2] for the Canadian HIV and Ageing Cohort Study

1Département de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université de Montréal, QC, Canada, 2Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, Quebec, Canada, 3Department of Biological Sciences, Université du Québec, Montréal (UQAM), Montréal, QC, Canada, 4Département de Médecine, Faculté de Médecine, Université de Montréal, QC, Canada, 5Département de Radiologie, Radio-oncologie et Médecine Nucléaire, Faculté de Médecine, Université de Montréal, 6Research Institute of McGill University Health Centre, Montréal, QC, Canada, 7Clinique médicale l'Actuel, Montréal, QC, Canada, 8Centre de médecine urbaine du Quartier latin, Montréal, QC, Canada, 9Department of Internal Medicine, Rush University Medical Center, Chicago, IL, United States.



Background:

Chronic inflammation in HIV infection increases the risk of cardiovascular diseases (CVD), even under antiretroviral therapy (ART). We recently demonstrated that the chronic upregulation of the multi-isoform proinflammatory cytokine interleukin-32 (IL-32) is associated with increased CVD risk in people living with HIV (PLWH) receiving ART. IL-32 isoforms exhibit diverse roles in T-cell differentiation, functions, and migration. However, the effect of IL-32 on T-cell cardiotropism, a mechanism that contributes to plaque formation, remains unknown. Here, we investigated the impact of IL-32 isoforms on the induction of the cardiotropic signature c-Met+CCR4+CXCR3+ in memory CD4 T-cells in relationship with HIV-DNA persistence in these cells.

Methods:

Peripheral blood mononuclear cells (PBMCs) and plasma samples from ART-treated PLWH and non-infected control participants were obtained from the Canadian HIV and Aging Cohort Study (CHACS).

PBMCs from non-infected controls were stimulated with recombinant IL-32 isoforms (α, β, and γ) at 500ng/ml.

The expression of c-Met, CCR4, and CXCR3 was measured by flow cytometry on CD4 T-cell subsets from both IL-32-stimulated cells *in vitro* (5 days stimulation) and *ex vivo on* primary cells isolated from study participants.

Integrated HIV-DNA was quantified by Alu real-time PCR in sorted central memory CD4 T-cells from ART-naïve individuals (n=5, average CD4 counts: 557 cells/ml, and plasma viral load: 109,257 HIV RNA copies/ml).

Hepatocyte growth factor (HGF; the c-Met ligand) was quantified by ELISA in plasma.

Results:

Increased expression of CXCR3, CCR4 and c-Met in IL-32-stimulated memory CD4 T cells

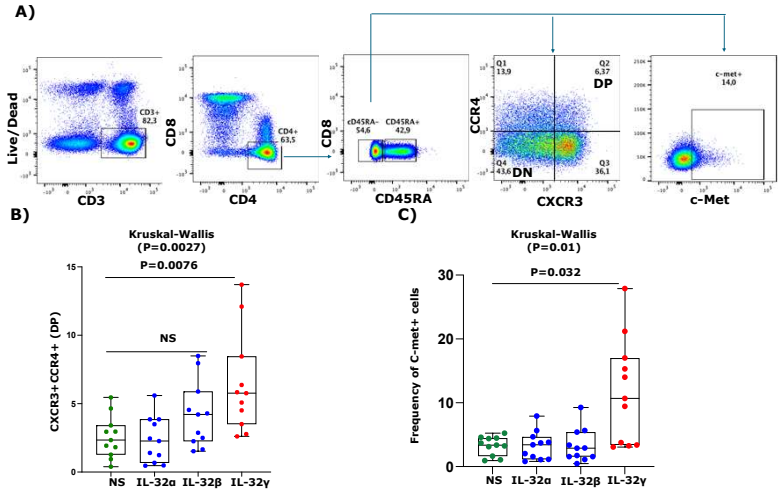


Figure 1: A) Representative flowcytometry dot blots showing the gating strategy on CXCR3+CCR4+ double positive (DP) and c-met positive memory CD4 T cells. B) PBMCs from uninfected individuals (n=11) stimulated with IL-32 isoforms α, β, and γ for 5 days and stained with CD3, CD4, CD8, CD45RA, CCR4, CXCR3 and c-Met Abs, then analyzed by flow cytometry. Left panel: graph analysis for the frequency of DP cells in IL-32-stimulated conditions. Right panel: Frequency of c-Met+ cells in IL-32-stimulated conditions. **DN:** double negative, **DP:** double positive, **NS:** non-stimulated cells. Data analyzed with the non-parametric test Kruskal-Wallis and Dunn's subtest.

CXCR3+CCR4+ double-positive CD4+ memory T-cells express the highest level of c-Met

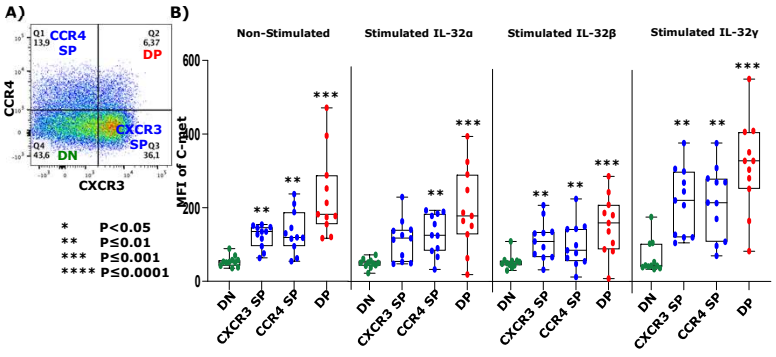


Figure 2: A) Shown is the the distribution of CXCR3 and CCR4 DP and DN cells on memory CD4+ T-cells subsets (CD4+CD45RA-). B) Graph figures showing the analysis of the expression of c-Met+ on memory CD4+ T cell subsets from PBMCs isolated from uninfected individuals (n=11) and stimulated with IL-32 isoforms for 5 days and then stained with CD3, CD4, CD8, CD45RA, CCR4, CXCR3 and c-Met Abs and analyzed by flow cytometry. Data analyzed with the non-parametric test Kruskal-Wallis and Dunn's subtest. **DN:** double negative, **SP:** single positive, **DP:** double negative

Central memory CCR4+CXCR3+ T-Cells harbor the highest levels of integrated HIV-DNA *in vivo*.

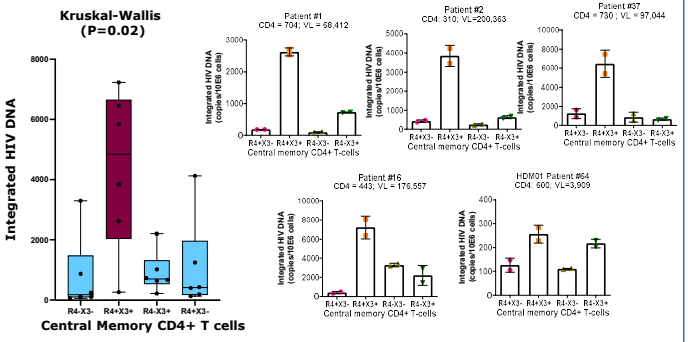


Figure 3: Polychromatic flow cytometry was used to sort central memory (CCR7+CD45RA-) CCR4+CXCR3-, CCR4+CXCR3+, CCR4-CXCR3-, and CCR4-CXCR3+ CD4+ T-cell subsets from PBMCs of recently HIV-infected treatment-naïve subjects with high plasma viral loads. Levels of integrated HIV DNA in these cells were quantified by real-time nested PCR. Left panel: Combined analysis for integrated HIV DNA from in the different memory subsets from five different HIV-infected individuals. Right panels: data in each individual. CD4 counts (cells/μl), and plasma viral load values (HIV RNA copies/ml) are indicated on top of the graphs.

ART-treated HIV+ individuals with subclinical CVD have the highest frequency of CXCR3+CCR4 DP memory CD4+ T cell populations suggesting increased cardiotropic T cells

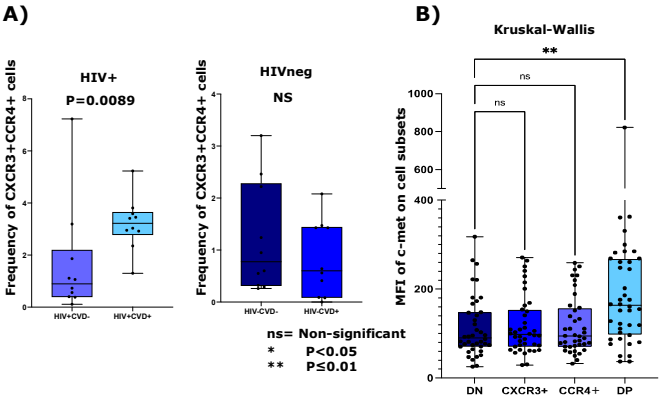


Figure 4: A) Co-expression of CCR4 and CXCR3 on memory CD4+ T cells from HIV+ individuals with/without subclinical CVD and and HIVneg controls with/without subclinical CVD (n=10/sub-group). Flow cytometry was used by a gating strategy as shown in Figures 1 and 2 to identify memory (CD4+CD45RA-) CCR4+CXCR3-, CCR4+CXCR3+, CCR4-CXCR3-, and CCR4-CXCR3+ CD4+ T-cell subsets from PBMCs of HIV+/HIVneg individuals with/without CVD *Ex vivo* (n=40, 10/group). B) Expression of c-Met+ in memory CD4+ T cells with/without expression CCR4 and/or CXCR3 (n=40). **DN:** double negative, **DP:** double positive, **NS:** non-significant.

Upregulation of the soluble ligand of the cardiotropic receptor c-Met (the hepatocyte growth factor HGF) in PLWH

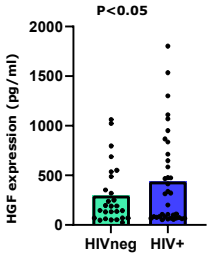


Figure 5: Comparison between the plasma levels of HGF from HIV+ compared to HIVneg individuals. Quantification of HGF was carried out by sandwich ELISA performed on plasma from ART-treated HIV+ (n=16) and HIVneg control group (n=20).

Conclusions:

IL-32 may likely contribute to heart inflammation by increasing the frequency of T-cells with cardiotropism that harbor proviral HIV-DNA. These cells may serve as a Trojan horse to bring HIV to the plaque-forming sites in heart arteries, which could further worsen the local inflammation. Thus, IL-32 might represent a potential therapeutic target in CVD in PLWH.