EPB093

IL-32 isoforms differentially impact coronary artery endothelium functions and potential to recruit inflammatory cells

R. Bunet[1,2], H. Ramani[1,2], M. Durand[2,3], C. Chartrand-Lefebvre[2,4], JP. Routy[5], R. Thomas[6], B. Trottier[7], P. Ancuta[1,2], A. Landay[8], M. El-Far[2] and C. Tremblay[1,2]

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

Background

HIV-induced inflammation leads to the premature development of cardiovascular diseases (CVD). We have previously shown that interleukin-32 (IL-32), a proinflammatory cytokine that is expressed in multiple isoforms (α, β, γ, D, ε, θ, ζ, η, and small/sm) with different inflammatory potential, is chronically upregulated in HIV-1 infection, even under ART and is associated with CVD. However, the mechanistic role of these different IL-32 isoforms in CVD is yet to be identified. In this study, we aimed to investigate the potential impact of the different IL-32 isoforms on primary coronary artery endothelial cells (pCAEC), the dysfunction of which is a major driver for atherosclerotic plaque formation.

Methods

Recombinant IL-32 isoforms (α, β and γ; the only commercially available isoforms) were used at 500ng/ml to stimulate primary CAEC (pCAEC/Creative Bioarray). pCAEC dysfunction in response to IL-32 stimulation was measured by studying the upregulation of VCAM-1 and ICAM-1 using flow cytometry, expression of the chemokines CCL2, CXCL1 and CXCL8 using both qRT-PCR (for gene expression) and ELISA for protein expression in supernatant as well as chemoattraction of monocytes.

Results

IL-32 isoforms showed differential effects on pCAEC. The following results are compared to non-treated cells. IL-32β and γ, but not IL-32α, significantly upregulated RNA expression of ICAM-1 (p=0.0028 and p= 0.0303, respectively) and VCAM-1 (p=0.0016 and p=0.0383, respectively) (**Figure 1A**), and surface protein expression: ICAM-1 (p<0.0001 and p=0.0005, respectively) and VCAM-1 (p<0.0001 and p=0.0007, respectively) (**Figure 1B**), as well as secreted proteins; ICAM-1 (p=0.0053 and p=0.0068, respectively) and VCAM-1 (p<0.0001 and p=0.0001, respectively) **(Figure 1C)**. Moreover, IL-32β and γ significantly upregulated RNA expression of the chemokines CCL2 (p<0.0001 and p=0.0012, respectively), CXCL1 (p=0.0001 and p=0.0023, respectively) and CXCL8 (p<0.0001 and p=0.0028, respectively) **(Figure 2A)** as well their respective protein expression: CCL2 (p<0.0001 and p=0.0132, respectively), CXCL1 (p<0.0001 and p=0.0198, respectively) and CXCL8 (p=0.030 and p=0.066 (NS), respectively) **(Figure 2B)**. Finally, supernatants from IL-32β and γ stimulated CAECs cells significantly attracted higher numbers of monocytes in transwell assays (p=0.0005 and p=0.0141, respectively) compared to non-treated or IL-32α-treated cells **(Figure 3A)**. The numbers of transmigrating cells were diminished upon the use of anatgonists to the CCL2, CXCL-1 and CXCL-8 receptors (CCR2 for CCL2 and CXCR2for CXCL-1 and CXCL-8); IL-32β (p=0.0018 for CXCR2 antagonist and p=0.0051 for CCR2 antagonist) and IL-32γ (p=0.0045 for CXCR2 antagonist and p=0.0078 for CCR2 antagonist) **(Figure 3B)**.

Conclusions

Our results suggest that the inflammatory IL-32β and IL-32γ isoforms induce coronary artery endothelial cell dysfunction and enhance their potential to recruit inflammatory cells such as monocytes. These IL-32 isoforms are upregulated in HIV infection and are likely contributing to endothelial cell inflammation and CVD and may represent a therapeutic target.

Main findings

Inflammatory **IL-32 isoforms β and γ** drive coronary artery endothelial cells :

- **Dysfunction (by upregulating VCAM-1 and ICAM-1)**,
- **Chemokines secretion (mainly CCL2, CXCL-1 and CXCL-8)**, and
- **Enhanced recruitment of monocyte**

IL-32-mediated upregulation of VCAM-1 and ICAM-1

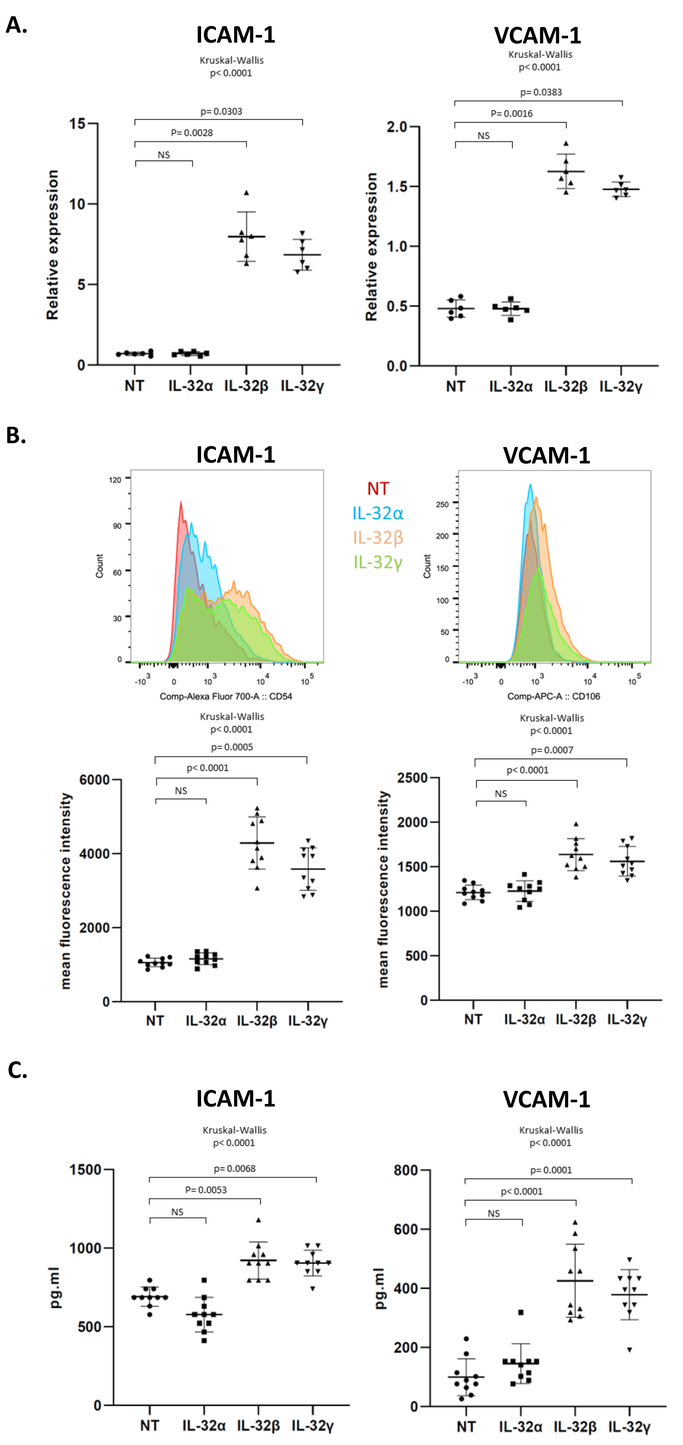

Figure 1 : A. Analysis of ICAM-1 (CD54) and VCAM-1 (CD106) RNA expression in primary CAECs showing the increased relative expression following stimulation with IL-32β and y but not IL-32α for 12h (n=6). **B.** Upper panels: Representative Flow cytometry histograms showing the Mean Fluorescent Intensity for ICAM-1 and VCAM-1 on CAEC cells following stimulation with IL-32α, β and y for 72h. Lower panels: Analysis from n=10 treatments. **C.** Analysis of ICAM-1 and VCAM-1 expression in CAECs supernatants following stimulation with IL-32α, β and y for 72h (n=10). Data analyzed with the non-parametric test Kruskal-Wallis and Dunn's subtest.

IL-32-mediated upregulation of CCL2, CXCL-1 and CXCL-8

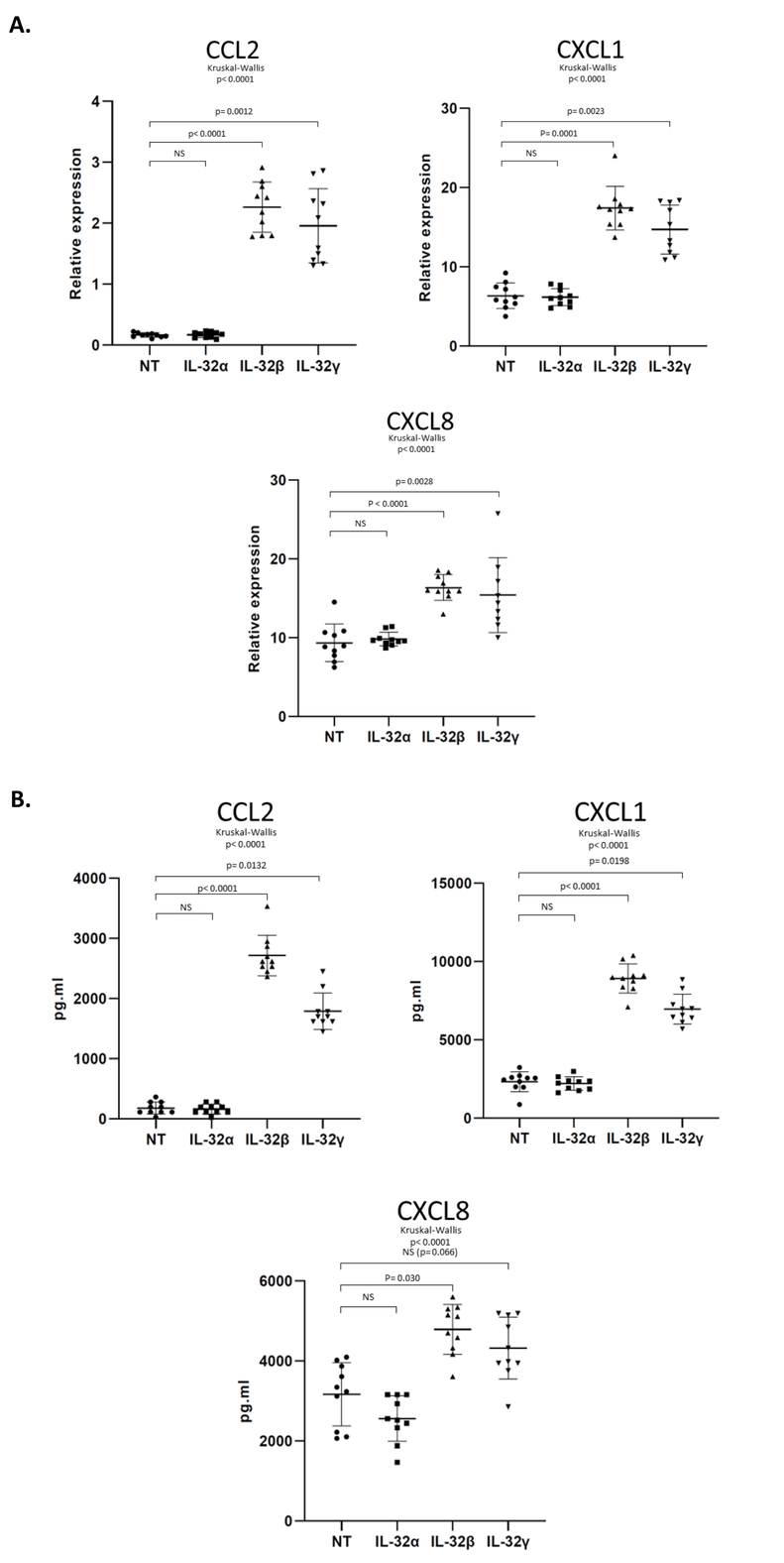

Figure 2 : A. Analysis of CCL2, CXCL1 and CXCL8 RNA expression in CAECs showing the increased relative expression following stimulation with IL-32α, β and y for 12h (n=10). **B.** Analysis for the production of CCL2, CXCL1 and CXCL8 proteins in CAECs supernatants following stimulation with IL-32α, β and y for 72h (n=10). Data analyzed with the non-parametric test Kruskal-Wallis and Dunn's subtest.

IL-32-mediated transmigration of monocytes

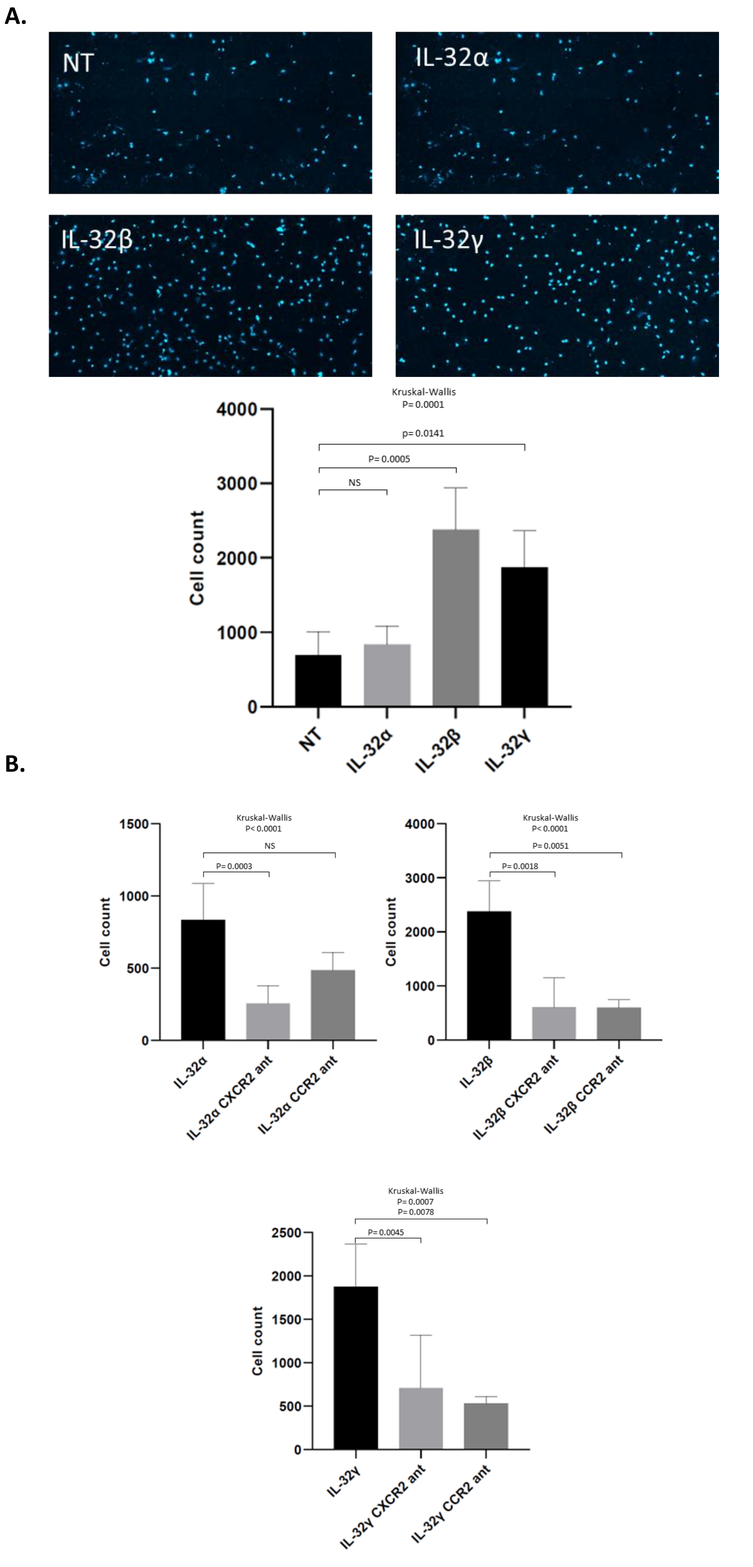

Figure 3 : A. Representative fluorescent images of monocytes nucleus stained with DAPI on transwell membranes (surface facing the lower chambers). Analysis of migrated cells number after migration towards CAECs supernatant treated with IL-32α, β and y (n=7). **B.** Analysis of migrated cells after treatment of monocytes with the CXCR2 and CCCR2 antagonists for each primary CAEC supernatants (IL-32α, β and y) (n=7).

Discussion

- IL-32 is known to be upregulated in multiple inflammatory conditions associated with increased risk for the development of cardiovascular diseases, such with chronic viral and bacterial infections, inflammatory bowel disease and Chronic obstructive pulmonary disease.
- Our previous studies demonstrated that IL-32 is persistently upregulated in both cells and plasma from people living with HIV (PLWH) and is associated with the presence of coronary artery and carotid artery atherosclerosis. However, whether IL-32 contributes to the pathogenesis of atherosclerosis is not yet clear.
- In the current study, we demonstrate that soluble IL-32 isoforms (IL-32β and y) have the potential to signal in coronary artery endothelial cells leading to their dysfunction and recruitment of monocytes, two conditions linked with the development of atherosclerosis. Given the persistent upregulation of circulating IL-32 in PLWH, these data may suggest IL-32 as a potential therapeutic target to limit CVD in this population.

Presented at AIDS 2022 – The 24th International AIDS Conference