Impaired response of memory Treg to HDL is associated with high cardiovascular disease risk in persons with HIV

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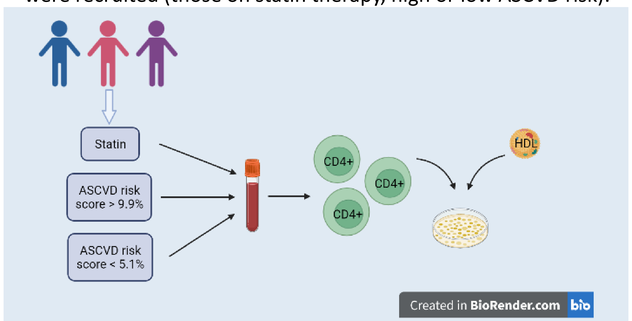
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BACKGROUND

Cardiovascular disease (CVD) is one of the main causes of death in persons with HIV (PWH) who are on effective antiretroviral treatment (ART). Inflammation is a common link between CVD and chronic HIV infection with evidence of unstable atherosclerotic lesions and more rapid progression of disease in PWH. High-density lipoproteins (HDL) have been shown to afford protection from CVD, but the mechanisms of action remain unclear. HDL has widely known anti-inflammatory effects on endothelial cells and macrophages beyond their cholesterol efflux capacity. *Importantly, our published and unpublished data have revealed a novel connection between HDL and regulatory T cells (Treg)*. Notably, comparing Treg from PWH and seronegative individuals, we found that binding and internalization of HDL promotes Treg survival, particularly that of memory and effector memory Treg, the most suppressive Treg subsets. Mechanistically, HDL interactions appear to blunt oxidative stress. *As Treg have also been implicated in CVD protection, our data suggest a novel mechanism by which HDL may protect against CVD*. This mechanism has not yet been studied in the context of HIV infection. Thus, the aims of this project were to: 1**) evaluate whether increased risk of CVD in PWH is associated with decreased circulating memory Treg populations; 2) establish whether HDL can blunt oxidative stress in Treg from PWH, and if their effect is similar in patients with high and low CVD; 3) determine the effect of statin treatment on these parameters.**

METHODS

- Matching triplets of PWH were recruited based upon age (+/- 10 years), sex, smoking status and cardiovascular risk. Three groups were recruited (those on statin therapy, high or low ASCVD risk):



- Reactive oxygen species (ROS) production were evaluated by flow cytometry in Treg ($CD127^{low}CD25^{high}FOXP3^{high}$) subsets: naïve ($CD45RA^{high}$, $HLA\text{-}DR^{low}$), memory ($CD45RA^{low}$, $HLA\text{-}DR^{low}$) and effector memory ($CD45RA^{low}$, $HLA\text{-}DR^{high}$)

RESULTS

Regulatory T cells can be classified as Naïve, Memory and Effector memory

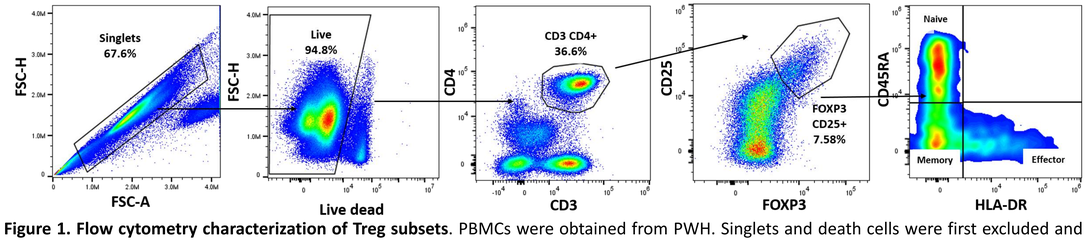


Figure 1. Flow cytometry characterization of Treg subsets. PBMCs were obtained from PWH. Singlets and death cells were first excluded and within CD4+ T cells, Treg were identified as CD4+CD25hiFoxP3+. Within Treg, three subsets were identified: naïve (CD45RA+HLA-DR-), memory (CD45RA-HLA-DR-) and effector memory Treg (CD45RA-HLA-DR+).

PWH with high CVD risk tend to have decreased absolute number of CD4+ and FOXP3+ cells in circulation

Naïve Treg frequency remains higher in PWH with low CVD risk while effector Treg frequency remains higher in PWH under statins treatment

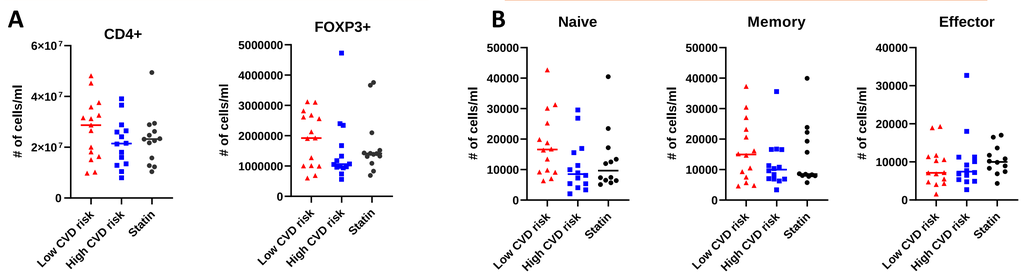


Figure 2. CD4+ and FOXP3+ T cells frequency seem to decrease in PWH with high CVD risk. PBMCs from PWH under statins treatment (n=15), and with low (n=15) or high CVD risk (n=15) were stained for different markers to determine the frequency of **A.** CD4+, FOXP3+ **B.** Naïve ($CD45RA^{high}$, $HLA\text{-}DR^{low}$), memory ($CD45RA^{low}$, $HLA\text{-}DR^{low}$) and effector memory ($CD45RA^{low}$, $HLA\text{-}DR^{high}$) Treg by flow cytometry.

HDL decrease ROS production in memory and effector memory Treg from PWH

Memory Treg from PWH with high CVD risk were less responsive to HDL

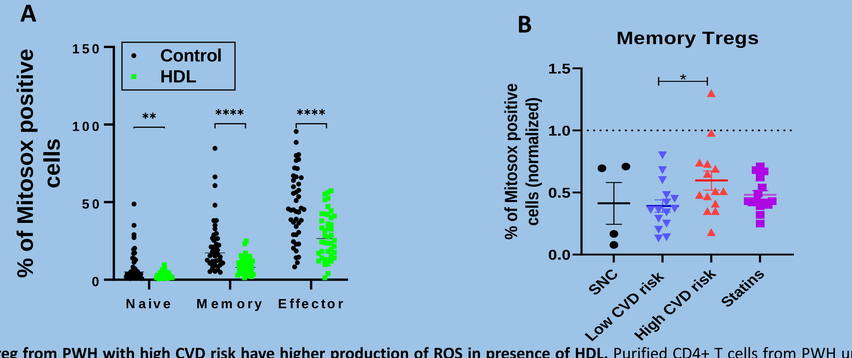


Figure 3. Memory Treg from PWH with high CVD risk have higher production of ROS in presence of HDL. Purified CD4+ T cells from PWH under statins treatment (n=15), and with low (n=15) or high CVD risk (n=15) were incubated with HDL for 24h in X-VIVO medium. Percentage of ROS production (Mitosox staining) was evaluated in **A.** naïve ($CD45RA^{high}$, $HLA\text{-}DR^{low}$), memory ($CD45RA^{low}$, $HLA\text{-}DR^{low}$) and effector memory ($CD45RA^{low}$, $HLA\text{-}DR^{high}$) by flow cytometry. Black dots indicate the control (cells from PWH without HDL) and green dots indicate the cells from PWH treated with HDL (obtained from seronegative controls (SNC)). Asterisks indicate significant differences (**p<0.005, ****p<0.0001) in two-way ANOVA test **B.** Data are normalized versus the level of Mitosox expression in cells without HDL treatment (control, dotted line). SNC: response to HDL from seronegative control memory Treg. Asterisks indicate significant differences (* p<0.05) in one-way ANOVA test.

Decreased proportion of Memory Treg tends to correlate with less response to HDL

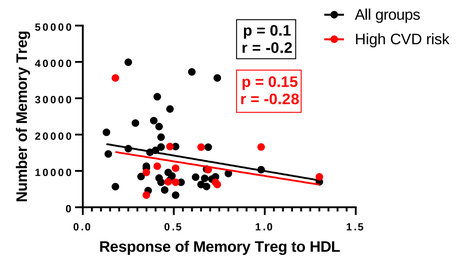


Figure 4. Decreased number of Memory Treg correlates negatively with the response to HDL in PWH. Spearman correlation between Memory Treg frequency and their response to HDL. Black dots display the data for all the three groups: statins, high and low CVD risk. Red dots display the data for high CVD risk

Table 1. Characteristics and Demographics of Study Participants

Parameter	Statin (N=15)	High CVD Risk (N=15)	Low CVD Risk (N=15)
Median Age (yrs)	60	61	50
Sex			
Male	87%	80%	73%
Female	13%	20%	27%
Median CD4 count (cells/mm³)	623	588	676
Median ASCVD PCE	12.7%	13.2%	3.9%
Total Cholesterol (mg/dL)	186	187	193
HDL (mg/dL)	49	48	49
LDL (mg/dL)	105	115	123

CVD=Cardiovascular disease; ASCVD=Atherosclerotic cardiovascular disease pooled risk equation score; Note: All participants had HIV RNA < 200 copies/mL. All comparisons P>0.05.

CONCLUSIONS

Our results suggest that there is a trend towards lower Treg numbers in PWH with high CVD risk, not prescribed statins. Statin treatment seems to increase the number of effector memory Treg in PWH. Like in non-HIV persons, HDL decreases oxidative stress in Treg from PWH, but they are less efficient in the case of memory Treg from PWH with high CVD. These data suggest that intrinsic defects impair memory Treg homeostasis in these patients, a mechanism that could be drive the inflammation-induced increased CVD risk observed in many HAART-treated PWH.

ADDITIONAL KEY INFORMATION

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