An Immunogical Signature for Subclinical Atherosclerosis in HIV-1 infection

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Background

The immunological mechanisms underlying the increased incidence of cardiovascular disease (CVD) and atherosclerosis in HIV-infected individuals remain unclear.

Persistent inflammation, microbial translocation and immune dysfunction occurring in HIV infected subjects may contribute to CVD establishment.

These alterations are driven by loss of Th17 cells in the gut, monocytes activation and dendritic cells dysfunction.

Chemokine receptors expression is critical to the migrations of those cells into atherogenic plaques.

The objective of this study is to understand the immunological mechanism driving coronary atherosclerosis in HIV infected individuals.

Material and Methods

Study Participants

Blood was available from HIV-infected on ART (HIV+) and uninfected (HIV-) participants belonging to the Canadian HIV and Aging cohort. Inclusion criteria for this study were: subjects without clinical manifestations or diagnosis of coronary artery disease and intermediate risk level of developing cardiovascular disease based on 10-years Framingham risk score at the moment of the recruitment. Exclusion criteria were renal impairment and hypersensitivity to contrast agents.

ELISA

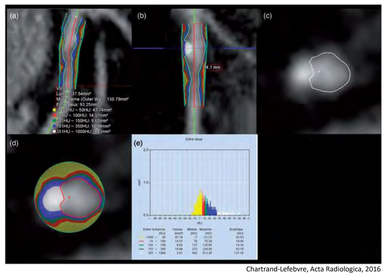
Markers of microbial translocation (LPS biding protein, LBP), mucosal damage (Intestinal-type fatty acid-biding protein, I-FABP), immune activation (sCD14), and chemokines (CCL20, CX3CL1, CCL25, MIF) were quantified in plasma by ELISA as was described by the provider (R&D Systems, Minneapolis, MN).

Flow Cytometry

Flow cytometry identified subsets of CD4+ T-cells (Th17 and Tregs), monocytes (classical, intermediate, non-classical), and plasmacytoid dendritic cells (pDC) and measured expression of chemokine receptors involved in atherosclerotic plaque formation (CCR2, CCR6, CCR9, CX3CR1). Cells were analyzed by fluorescence-activated cell sorting (FACS) using BD-LSRII cytometer, BD-Diva (BD Biosciences) and FlowJo (Tree Star, Inc., Ashland, Oregon, USA) softwares. All Abs were titrated for an optimal noise/ratio, and Ab cocktails were validated by comparing single with multiple staining. Positive gates were placed based on fluorescence minus one controls. Backgating strategy was used to confirm the gates.

Computed Tomography Angiography Scan

Study participants underwent Computed Tomography Angiography Scan (CTA-Scan) to determine total plaque volume (TPV), low attenuated plaque volume (LAPV, high-risk atherosclerotic lesions) and Coronary Artery Calcium Score (CACS) as was described previously. Participants were categorized according to total plaque volume in low plaque volume (<100 mm^3) or high plaque volume (≥100 mm^3) to subsequent comparison between groups.



Chartrand-Lefebvre, Acta Radiologica, 2016

Statistical Analysis

Shapiro-wilk test was used to determine the normal distribution of the continues variables. Categorical variables were represented as proportions. Data were presented as means with standard deviation, or median with interquartile range (IQR) according to normality. Comparison between 2 groups were performed using Student's t-test, Mann-Whitney U test or chi-square as appropriate. Kruskal-Wallis and Dunns post-test were applied to compare differences between more than 2 groups. Statistical analysis were performed using the GraphPad Prism 7 and SPSS25 software.

Results

Table 1. Characteristic of HIV-infected and non-HIV infected individuals

	HIV-infected individuals (n=68)	Uninfected Individuals (n=23)	P-Value
Demographics			
Age (years)	55±7	57±7.8	0.27
Male %	98.5	82.6	**0.014**
Waist Circumference (cm)	93 (87.75,103)	96 (91,100)	0.373
BMI (kg/m2)	24.54 (22,28)	25.95(.24.57,27.65)	0.165
Framingham Score	9 (6,14)	8 (8,13)	0.985
Diabetes, %	8.8	0	0.33
Hypertension, %	33.8	17.4	0.188
Personal History of Cardiovascular Disease, %	9.2	0	0.33
Family History of Cardiovascular Disease, %	33.3	17.4	0.188
Pack of Cigarettes/year	12 (0,26)	0.8 (0,5.1)	**0.049**
Current Statin Treatment, %	17.6	21.7	0.75
Coronary Artery Disease Parameters			
Total Plaque Volume	66.1(0,688)	49.7(0,210)	0.263
Low attenuated plaque volume	22.1(0,188.31)	10.5(0,101)	0.319
Coronary Artery Calcium Score	7(0,408)	11.3(0,69.5)	0.649
HIV disease parameters			
Duration of HIV infection(years)	18(11,24)	NA	NA
Duration of Antiretroviral therapy (years)	14.8(5.6,19.6)	NA	NA
Under viral suppression	95.3	NA	NA
Laboratory Parameters			
White blood cells	5.5(4.5,7)	6(5.25,7)	0.204
Lymphocytes	1.8±0.71	NA	NA
CD4	545.28±254	NA	NA
CD8	770.2±334	NA	NA
CD4/CD8 ratio	0.73(0.46,1.02)	NA	NA
Nadir CD4	190(103,320)	NA	NA
Hemoglobin	143.97±12.34	146.76±10.705	0.35
D-Dimer	3.06(2.59,3.6)	3.04(2.78,4.24)	0.495
Fibrinogen	390(185,390)	334(284,523)	0.22
LDL	2.6(2.04,1.4)	3.09(2.23,3.97)	0.207
HDL	1.2(1.01,1.35)	1.36(1.29,1.58)	**0.002**
Insulin	88(49,135)	74.5(38.5,92.5)	0.242
Serology			
Hbs Ag	12.1	4.8	0.44
Anti-Hbs Ag	54.7	47.1	0.59
Anti-HCV	15.3	11.1	0.44
RNA HCV	6.8	16.7	0.411
CMV-IgG	90.5	27.3	**0.001**

Table 2. Comparison between HIV-infected individuals with High and Low plaque Volume

	High plaque Volume (n=33)	Low plaque Volume (n=34)	P-Value
Demographics			
Age (years)	56.25±6.48	53.98±7.5	0.191
Male %	100	97.1	1
Waist Circumference (cm)	90(85.5,106)	94(89,102)	0.63
BMI (kg/m2)	24.05(20.96,18.54)	25.29(22.48,27.82)	0.423
Framingham Score	11(6.5,19.5)	8(5.75,13)	**0.031**
Diabetes, %	15.2	2.9	0.101
Hypertension, %	42.4	25.7	0.201
Personal History of Cardiovascular Disease, %	15.6	3	0.105
Family History of Cardiovascular Disease, %	42.4	25.7	0.105
Pack of Cigarettes/year	18.4(1.16,30.6)	3.8(0,15.75)	**0.009**
Current Statin Treatment, %	27.3	8.6	**0.059**
Coronary Artery Disease Parameters			
Total Plaque Volume	688(408.54,934)	0(0,20)	0.0001
Low attenuated plaque volume	188.31(104.98,346.53)	0(0,4.62)	0.0001
Coronary Artery Calcium Score	412(100.33,596.47)	0(0,0)	0.0001
HIV disease parameters			
Duration of HIV infection(years)	19(15,24)	14(5.5,25)	0.07
Duration of Antiretroviral therapy (years)	15.8(12.3,20.8)	10.4(3.5,16.7)	**0.026**
Under viral suppression	100	90.3	0.108
Laboratory Parameters			
White blood cells	5.7(4.7,7.8)	5.4(4.3,6.6)	0.264
Lymphocytes	1.8(1.5,2.25)	1.8(1.45,2.4)	0.954
CD4	597.09±279.51	504.09±223.3	0.641
CD8	790.45±329.9	747.05±65.03	0.18
CD4/CD8 ratio	0.76(0.49,1.05)	0.65(0.29,1.0)	0.195
Nadir CD4	220(102.5,255)	174(103,280)	0.446
Hemoglobin	144.7±11.29	143.29±13.38	0.498
D-Dimer	3.4(2.7,4.1)	2.9(2.29,3.20)	**0.005**
Fibrinogen	340(207.5,430)	270(175,355)	0.149
LDL	2.39(1.92,3.4)	2.92(2.2,3.4)	0.108
HDL	1.16(1.06,1.33)	1.26(0.98,1.38)	0.598
Insulin	97.5(51.2,145)	78(39,118.5	0.181
Serology			
Hbs Ag	12.5	11.8	1
Anti-Hbs Ag	54.8	54.5	1
Anti-HCV	24.1	6.7	0.08
RNA HCV	12.5	0	0.239
CMV-IgG	87.5	93.5	0.672

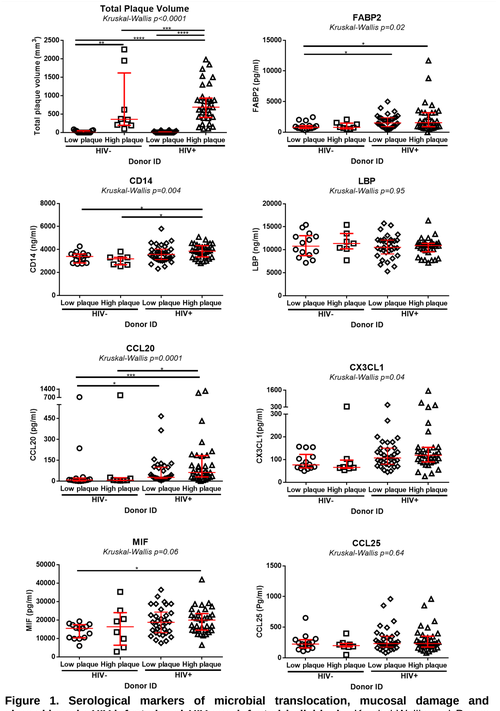


Figure 1. Serological markers of microbial translocation, mucosal damage and chemokines in HIV-infected and HIV-non infected individuals. Kruskal-Wallis and Dunns post-test were applied to compare differences between groups. P-Values are indicated on the graphs (*, p<0.1; **, p<0.01; ***, p<0.001; ****, p<0.0001)

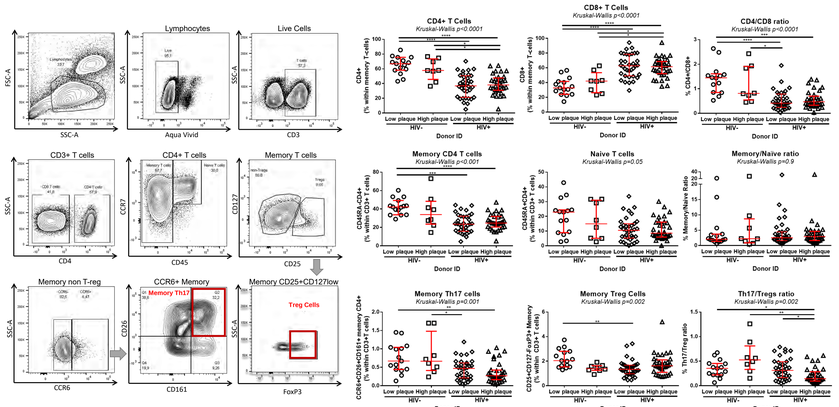


Figure 2. Altered Memory Th17 and Treg cells frequencies in HIV infected individuals with subclinical coronary artery disease. PBMCs were available from HIV infected and non-infected individuals. Cells were stained with T cell subsets surface markers. Frequencies of CD4, CD8 (CD4-), naive (CD3+CD4+CD45RA+), memory (CD3+CD4+CD45RA-), memory Th17 (CD3+CD4+CD45RA-CD25-FoxP3-CCR6+CD26+CD161+) and Tregs (CD3+CD4+CD45RA-CD25+CD127lowFoxP3+) were determined within CD3+ T cells. The CD4/CD8 ratios, memory/naive ratios, and Th17/Treg ratios were also calculated. Subjects were stratified based on their HIV status and total coronary plaque volume (TPV; low plaque volume <100 mm3 and high plaque volume >100 mm3). Kruskal-Wallis and Dunns post-test were applied to compare differences between groups. P-Values are indicated on the graphs (*, p<0.1; **, p<0.01; ***, p<0.001; ****, p<0.0001)

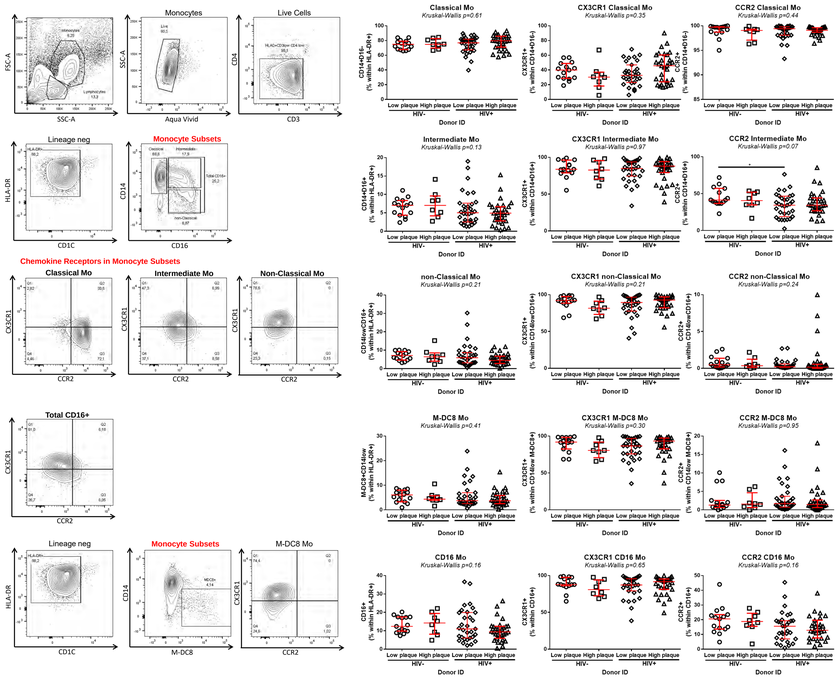


Figure 3. Frequency of Monocyte cell subsets cells subsets in HIV-infected and non-infected individuals. PBMCs were available from HIV infected and non-infected individuals. Cells were stained with monocyte subsets surface markers. Frequencies of Classical (CD14+CD16-), non-Classical(CD14lowCD16+), intermediate(CD14+CD16+), total CD16(CD14low and high CD16+) and M-DC8 (CD14low M-DC8+) monocytes were determined within HLA-DR+ cells. Cx3CR1 and CCR2 expression was also evaluated in each monocyte subset. Subjects were categorize according to HIV status and total plaque volume(low plaque volume <100mm3 and high plaque volume >100mm3). Kruskal-Wallis and Dunns post-test were applied to compare differences between groups. P-Values are indicated on the graphs (*, p<0.1; **, p<0.01; ***, p<0.001; ****, p<0.0001)

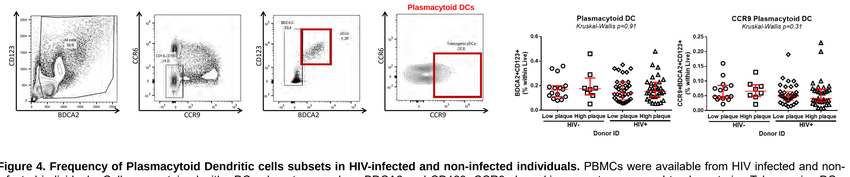


Figure 4. Frequency of Plasmacytoid Dendritic cells subsets in HIV-infected and non-infected individuals. PBMCs were available from HIV infected and non-infected individuals. Cells were stained with pDCs phenotype markers BDCA2 and CD123. CCR9 chemokine receptor was used to characterize Tolerogenic pDCs. Subjects were categorize according to HIV status and total plaque volume (low plaque volume <100mm3 and high plaque volume >100mm3). Kruskal-Wallis and Dunns post-test were applied to compare differences between groups. P-Values are indicated on the graphs (*, p<0.1; **, p<0.01; ***, p<0.001; ****, p<0.0001)

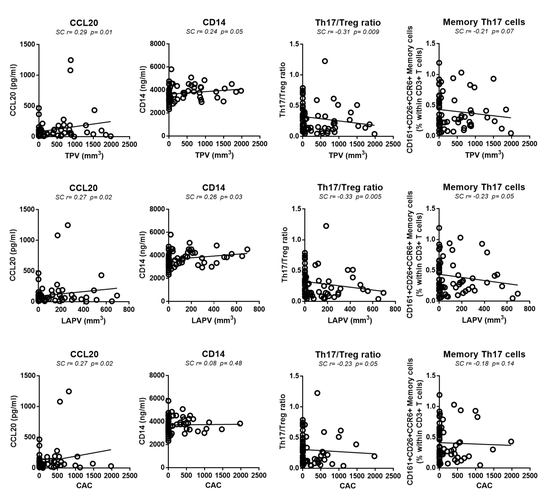


Figure 5. Correlation between different soluble markers and T cell subsets with coronary artery plaque measurements. Spearman correlation (SC) was applied to determine the relationship between CCL20, CD14, frequency of Th17 and Th17/Treg ratio and coronary artery plaque measurements among HIV+ subjects. TPV, total plaque volume; LAPV, low attenuated plaque value; CAC, coronary artery calcium score.

Results Summary

HIV+ (n=68; infection time: 16±8 years; ART duration: 14.8±8 years; CD4 counts: 545±254 cells/µl; undetectable viral load 95.3%) *vs.* HIV- (n=23) were similar in age (55±7 *vs.* 57±8 yrs) and Framingham scores (9 [6,14] vs. 8 [8,3]). HIV+ *vs.* HIV- had higher TPV (66.1 [0,688] *vs.* 48.7 [0,210]), LAPV (22.1 [0,188.31] *vs.* 10.5 [0,101]), but not CACS (7 [0,408] *vs.* 11.3 [0,69.5]. In addition HIV+ individuals consumed more cigarettes (p=0.049) and had lower levels of HDL (p=0.002) **(Table 1)**.

HIV+ subjects with high TPV *vs.* low TPV had higher Framingham Score (p=0.031), cigarette consumption(p=0.009), longer duration of ART(p=0.026) and higher levels of D-dimer (p=0.005). There were no differences in HDL levels (p=0.59) **(Table 2)**.

Marker of microbial translocation (sCD14) was higher among HIV+ subjects with high TPV compared to all HIV- subjects, and the marker of mucosal damage (I-FABP) was higher among all HIV+ compared to HIV- with low TPV. Between the different chemokines, HIV+ subjects with high TPV showed the highest levels of CCL20 compared to other groups **(Figure 1)**.

The frequency of CD4 T cells was lower in HIV+ subjects compared to all HIV- and the frequency of memory CD4 T cells was also lower but only compared to HIV- with low TPV. Th7 cells were depleted in HIV+ compared to HIV- subjects. Interestingly Th17/Treg ratio was the lowest among HIV+ subjects with high TPV compared to HIV+ with low TPV and HIV- with low and high TPV **(Figure 2)**.

There were no differences in the frequencies of monocytes subsets among the different groups except for a lower frequency of CCR2 in HIV+ with low TPV *vs.* HIV- with low TPV **(Figure 3)**.

Among HIV+, CCL20 levels positively correlated with TPV (p=0.01, r=0.29), LAPV (p=0.02, r=0.27), and CACS (p=0.02, r=-0.27); CD14 levels positively correlated with TPV (p=0.05, r=0.24),and LAPV (p=0.03, r=-0.26); Th17 frequency negatively correlated with LAPV (p=0.05, r=-0.23); and Th17/Treg ratio negatively correlated with TPV (p=0.04, r=-0.27), LAPV (p=0.05, r=-0.26), and CACS (p=0.03, r=-0.29); and Th7/Tregs ratios negatively correlated with TPV (p=0.009, r=-0.31), LAPV (p=0.005, r=-0.33), and CACS (p=0.05, r=-0.292) **(Figure 5)**.

Conclusion

The paucity of blood Th17 cells, together with the over representation of Tregs and decreased Th17/Treg ratios, correlated with subclinical coronary artery disease in HIV+ individuals on ART. This suggests a link between the "leaky gut" caused by Th17 depletion and the development of HIV-associated CVD.

Acknowledgemetns

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