INCREASED INFLAMMATORY $CX_3CR1^+GPR56^+CD57^+$ $CD4^+$ T CELLS IN FAT FROM HIV$^+$ DIABETICS

0672

Celestine N. Wanjalla[1,2], Wyatt J. McDonnell [9], Ramesh Ram[8], Abha Chopra[8], Rama Gangula[3], Shay Leary[8], Beverly O. Woodward[1,3], Mark A. Pilkinton[1,2], Mona Mashayekhi[4], Samuel Bailin[1], Curtis L. Gabriel[6], Alyssa M. Hasty[7], Simon A. Mallal[1-3, ,5,8], Spyros A. Kalams[1-3], John R. Koethe[1-3]

[1] Division of Infectious Diseases, [2] Center for Translational Immunology and Infectious Disease, [3] Tennessee Center for AIDS Research, [4] Division of Diabetes, Endocrinology and Metabolism, [5] VANTAGE, Vanderbilt University Medical Center, [6] Division of Gastroenterology, Hepatology and Nutrition, Vanderbilt University Medical Center[7] Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA[6] [8] Institute for Immunology and Infectious Diseases, Murdoch University, Perth, WA, Australia , [9]10X Genomics, Pleasanton, CA, USA

BACKGROUND

- Persons with HIV (PWH) are at higher risk of diabetes mellitus compared to the general population, which may be due, in part, to altered lipid metabolism and storage.
- **Compared to PWH without diabetes, we have shown that adipose tissue from diabetic PWH is enriched for CX_3CR1^+ $GPR56^+$ $CD57^+$ (i.e., 'CGC') $CD4^+$ T cells and a separate population of $CD69^+$ $CD4^+$ T cells.**
- CX_3CR1 and GPR56 expression are of interest because they are associated with anti-viral responses, and adipose tissue is a known reservoir of HIV and CMV.
- **We hypothesized that CGC$^+$ $CD4^+$ T cells are likely anti-viral and therefore would be present in higher proportions in the adipose tissue of PWH compared to HIV-negative.**

METHODS

- We performed subcutaneous abdominal liposuction and T cell isolation on 11 diabetic persons (6 PWH and 5 HIV-negative, all subjects CMV+), followed by flow cytometry phenotyping and single-cell sorting of memory T cells.
- Single-cell cDNA libraries were created using well-specific barcodes followed by 3' and 5' amplification and sequencing. Pooled data were demultiplexed and the transcriptome was linked to the indexed flow cytometry phenotype.
- Differential expression was done using *iiid* Visual Genomics Analysis Suite (VGAS) and pathway analyses were performed for immune genes in CGC and $CD69^+$ $CD4^+$ T cells in the HIV+ and HIV-negative participants.

RESULTS

T-Distributed Stochastic Neighbor Embedding (t-SNE) shows differences of $CD4^+$ T cell subsets between PWH and HIV-negative

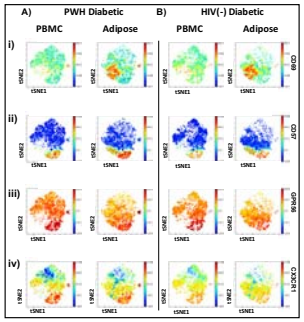
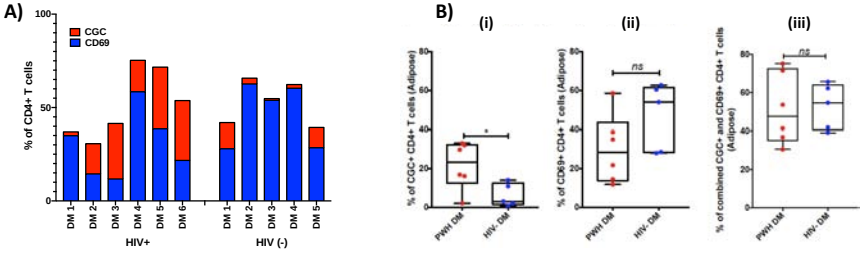


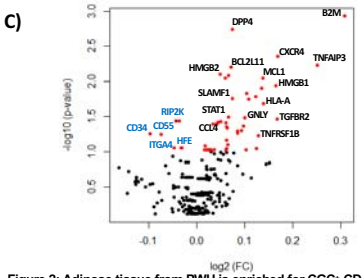
Figure 1. Concatenated viSNE plots showing the subpopulations of CGC$^+$ $CD4^+$ T cells and CD69+ CD4+ T cells in adipose and PBMCs of diabetic PWH and HIV negative. T-SNE analysis was done on Cytobank. Results are concatenated plots in diabetic PWH (n=6) (A) and HIV-negative (n=5). (B).

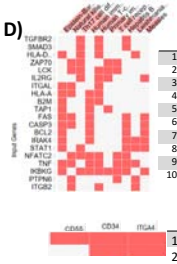
Compared to HIV-negative diabetics, the adipose tissue of diabetic PWH is enriched for potentially anti-viral *$CX3CR1^+GPR56^+CD57^+$ $CD4^+$ T cells* with an inflammatory RNA transcriptome

Adipose tissue in PWH is enriched for CGC$^+$ $CD4^+$ T cells while HIV-negative have predominantly tissue resident $CD69^+$ $CD4^+$ T cells. Differential gene expression reveals differences in top genes expressed.



ADIPOSE TISSUE: $CD4^+$ $CX3CR1^+$ T CELLS IN *DIABETIC PERSONS WITH HIV* VS. *DIABETIC HIV-NEGATIVE*



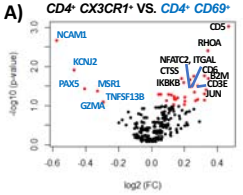


KEGG PATHWAY (PWH)	Adjusted p-value
1 Epstein-Barr virus infection	1.64E-15
2 Natural killer cell mediated cytotoxicity	3.93E-15
3 Th17 cell differentiation	6.12E-13
4 Human immunodeficiency virus 1 infection	3.33E-11
5 Human T-cell leukemia virus 1 infection	3.92E-11
6 Primary immunodeficiency	3.97E-10
7 T cell receptor signaling pathway	3.43E-10
8 Hepatitis B	8.04E-10
9 Leishmaniasis	8.62E-10
10 Measles	4.08E-09

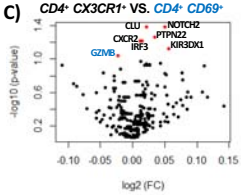
KEGG PATHWAY (HIV NEGATIVE)	Adjusted p-value
1 Hematopoietic cell lineage	0.001175
2 Cell adhesion molecules (CAMs)	0.1648

Figure 2: Adipose tissue from PWH is enriched for CGC$^+$ $CD4^+$ T cells, whereas HIV-negative adipose tissue is enriched for CD69$^+$ $CD4^+$ T cells. Bar charts showing distribution of CGC$^+$ (red) and CD69$^+$ (blue) $CD4^+$ T cells, CGC+ CD4+ cells are significantly lower in HIV-negative persons, $p < 0.05$ (t-test) (A). Boxplots showing percentages of CGC$^+$ $CD4^+$ and CD69$^+$ $CD4^+$ T cells in diabetic PWH and HIV-negative (B). Volcano plot showing differential gene expression of adipose tissue CD4+ CX3CR1+ T cells from diabetic PWH (n=2, cells – 133) versus HIV-negative (n=5, cells = 185) (C). Enriched KEGG pathways of top differentially expressed genes (Kruskal Wallace, $p < 0.1$) (D)

ADIPOSE TISSUE PWH
$CD4^+$ $CX3CR1^+$* VS. *$CD4^+$ $CD69^+$



ADIPOSE TISSUE HIV-NEGATIVE
$CD4^+$ $CX3CR1^+$* VS. *$CD4^+$ $CD69^+$



B)

KEGG PATHWAY (CD4 CX3CR1+) - PWH	Adjusted P-value	Genes
T cell receptor signaling pathway	5.7E-07	IKBKB;JUN;NFATC2;CD3E;RHOA
Human immunodeficiency virus 1 infection	1.2E-05	IKBKB;JUN;NFATC2;CD3E;B2M
Epstein-Barr virus infection	1.2E-05	IKBKB;JUN;ITGAL;CD3E;B2M
Th1 and Th2 cell differentiation	1.8E-05	IKBKB;JUN;NFATC2;CD3E
Th17 cell differentiation	2.3E-05	IKBKB;JUN;NFATC2;CD3E
C-type lectin receptor signaling pathway	2.4E-05	IKBKB;JUN;NFATC2;RHOA
Human cytomegalovirus infection	4.0E-04	IKBKB;NFATC2;B2M;RHOA
Fluid shear stress and atherosclerosis	1.8E-03	IKBKB;JUN;RHOA
Non-alcoholic fatty liver disease (NAFLD)	3.5E-02	IKBKB;JUN
mTOR signaling pathway	3.5E-02	IKBKB;RHOA

Figure 3: Top genes expressed by adipose tissue CX3CR1$^+$ vs. CD69$^+$ $CD4^+$ T cells. Heat map showing differential gene expression (DGE) between CX3CR1$^+$ and CD69$^+$ $CD4^+$ T cells in **diabetic PWH** (n=5 PWH) (A). Volcano plot showing DGE in **5 HIV-negative persons** (B). KEGG pathway of top genes expressed by CX3CR1$^+$ over CD69$^+$ $CD4^+$ T cells in PWH. None of the other groups has significant gene pathways. DGE – Kruskal-Wallis, genes with $P < 0.05$ used for KEGG pathway analysis on Enrichr.

CONCLUSIONS

- Adipose tissue of diabetic PWH is enriched for an inflammatory population of potentially anti-viral $CD4^+$ T cells expressing CX_3CR1, GPR56 and CD57 (CGC) compared to HIV-negative diabetics
- Adipose tissue serves as a reservoir for HIV, CMV, and possibly other viruses. Based on surface marker expression and RNA transcriptome, these CGC$^+$ $CD4^+$ T cells may represent a local anti-viral response. CD69+ CD4+ T cells are more likely to be infected by HIV.
- Future studies will determine if the proportion of CGC$^+$ $CD4^+$ T cells correlate with the HIV reservoir adipose tissue, and whether these cells have T-cell receptors specific for viral antigens and could impair adipocyte function due to their proinflammatory phenotype.

Acknowledgements

This study was funded by NIH grants R01 DK112262, R56 DK108352, the Tennessee Center for AIDS Research grant P30 AI110527, and CTSA award No. KL2 TR002245

CONTACT: Celestine N. Wanjalla, Vanderbilt University Medical Center
Email: celestine.wanjalla@vumc.org