Lipidomic Fingerprinting To Identify HIV Reservoirs *In Vitro* and *In Vivo*

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Background:

One of the most important questions in designing cure-based strategies in HIV research is the origin(s) of the reservoir. It is well established that HIV rebounds upon withdrawal of Highly Active Antiretroviral Therapy (HAART) leading to viral breakthrough and HIV envelopes contain lipids *originating from the membrane of the virus producing cells.* Thus, an innovative highly sensitive, clinical sample-validated Virus Lipidomic Mapping (VLM) assay was developed to accurately quantify lipid compositions of HIV-1 virions (< 5 copies/mL) and host cells, allowing virus to be mapped to host cell origin.

Methods:

Primary human CD4$^+$ T cells or monocytes were isolated (healthy buffy coats; magnetic sorting; n = 12). HIV-1_{BaL} was used to infect CD4$^+$ T cells or differentiated macrophages (MΦ). HIV from CD4$^+$ T cell or MΦ origin (n = 4) was captured from viremic plasma of HIV$^+$ individuals with magnetic nanoparticles (MNP) coupled to Ab against T cell or MΦ-specific proteins incorporated into virions followed by virus purification and exosome removal. Lipids were extracted from cells; profiles, abundance and ratios (cells and virus) were determined by LC-MS/MS (Q-Exactive Plus).

Results:

For all donors, the cellular lipid profiles for CD4$^+$ T cells were distinct from MΦ lipid profiles. Virions grown in each cell type contained unique lipids that match unique lipids on host cell origin. Distinctive profiles for MΦ cellular and virion lipids included monoetherphosphatidylcholine (MePc), ceramides (Cer), glucosylceramide (CerG1), diacylglycerol (DG), phosphatidylinositol (PI), phosphatidylcholine (PC, phosphatidylethanolamine (PE), sphingomyelins (SM), and triglyceride (TG); unique profiles for T-cell virion and cellular lipids include subspecies SM(d35) and CerG1(d18). For all donor samples, virions that carried specific T-cell proteins showed unique lipid profiles compared to virions that carried MΦ-specific proteins; Cer (d17, G1d18), DG(53.1), MePC(31), PC(34), PE(42), PI(18), SM(d35), and ChE(18).

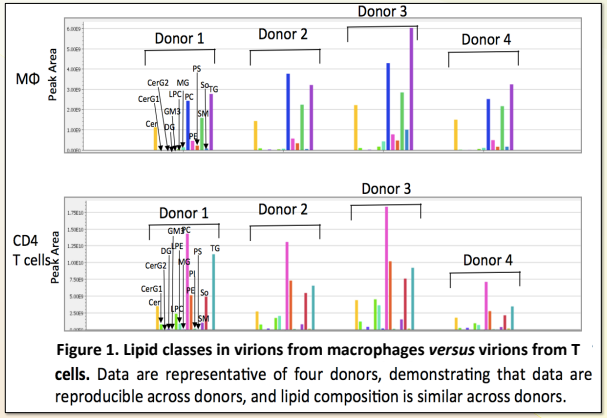

Figure 1. Lipid classes in virions from macrophages *versus* virions from T cells. Data are representative of four donors, demonstrating that data are reproducible across donors, and lipid composition is similar across donors.

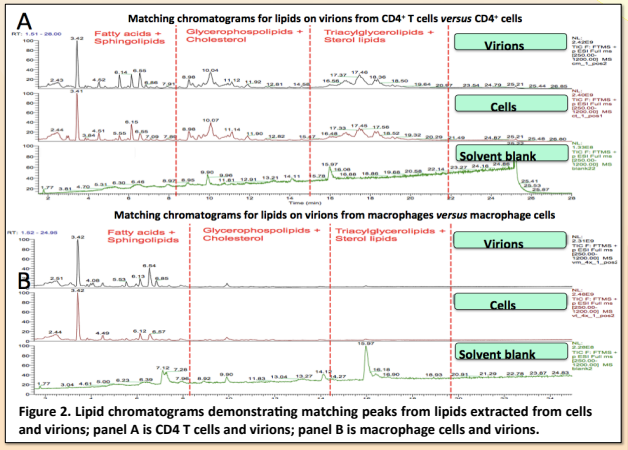

Figure 2. Lipid chromatograms demonstrating matching peaks from lipids extracted from cells and virions; panel A is CD4 T cells and virions; panel B is macrophage cells and virions.

Table 1. Unique lipidomic profile (class/species/subspecies) of virions from macrophages or CD4$^+$ T cells.

Lipids	Cell Type	
	Macrophages	CD4 T cells
SM (d16:0/20:3	❖	
Cer (d14:0/18:1+O)	❖	
Cer (d16:0/16:0)	❖	
Cer (d22:2/18:0)	❖	
Cer (d22:2/8:0)	❖	
PI(16:1/18:1)	❖	
LPE(20:3)		❖
PC(16:0/19:0)		❖
PC(16:0/17:0)		❖
PC(18:0e/16:0)		❖
PC(18:0e/20:4		❖
PE(18:0p/16:0)		❖
PE(18:0/20:3)		❖
PE(20:PS(20:2/20:2)		❖
SM(d16:0/22:0		❖
SM(d16:0/19:0)		❖
SM(d16:0/22:3)		❖
SM(d16:0/26:2		❖
SM (d16:0/18:4)		❖
SM (d16:0/28:3)		❖
SM(d36:1)		❖
SM(d35:1+pO)		❖
Cer (d14:1/16:0)		❖
Cer (d14:1/18:0)		❖
Cer (d16:0/14:0)		❖
Cer (d20:1/18:0)		❖

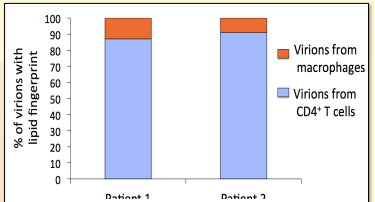

Figure 3. Two patient plasma samples from HIV$^+$ individuals containing virions originating fro both T cell and macrophage cellular origins.

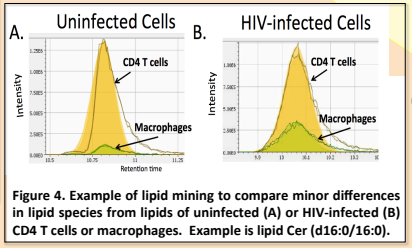

Figure 4. Example of lipid mining to compare minor differences in lipid species from lipids of uninfected (A) or HIV-infected (B) CD4 T cells or macrophages. Example is lipid Cer (d16:0/16:0).

Conclusions:

For the first time, the VLM method:

1) Identified host cell origin of persistent HIV-1 *in vivo*, even with low-prevalence populations of mixed virions.
2) Determined unique lipid profiles for virions from T-cells *versus* MΦ, which match cellular lipid profiles on host cell origin.

This information provides a foundation for cure-based strategies to identify and eliminate key cells harboring persistent HIV not eliminated by HAART.

Acknowledgements

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