MASSIVE ENDOCYTOSIS MECHANISMS ARE INVOLVED IN CD169-MEDIATED UPTAKE OF HIV-1 BY DENDRITIC CELLS.

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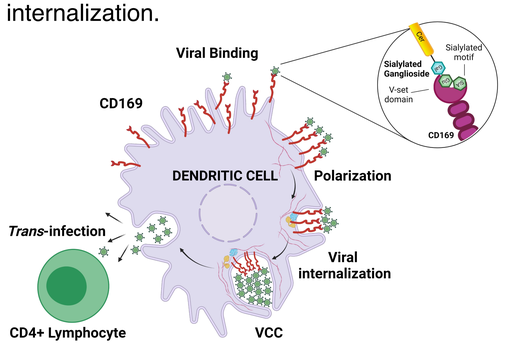
BACKGROUND

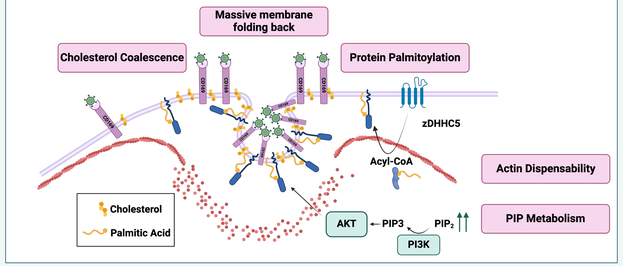
- HIV-1 enter Dendritic Cells (DCs) and remain infectious in a structure named Viral-Containing Compartment (VCC).
- CD169/Siglec-1 attachment receptor recognizes gangliosides on the viral envelope and triggers viral internalization.



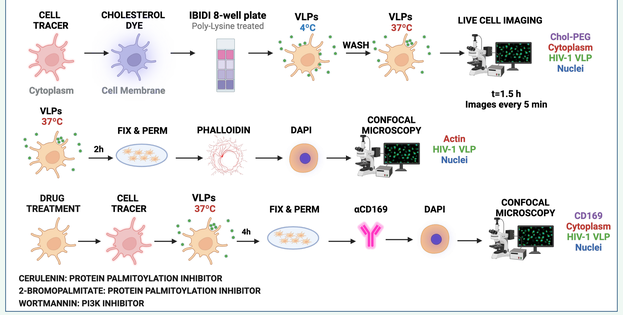
HYPOTHESIS

- The specific cellular endocytic mechanism responsible for VCC formation process remains unknown.
- Previous data indicates clathrin independence, cholesterol dependency, and transient deregulation of cortical actin.

Enveloped viruses could exploit Massive Endocytosis (MEND) machinery for uptake in DCs

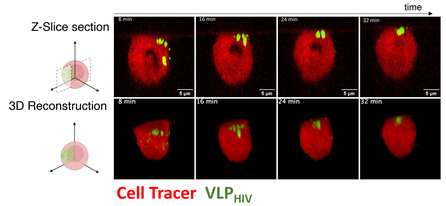


METHODS

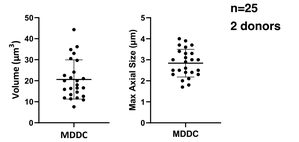


RESULTS

Classical endocytosis processes do not participate in VCC formation



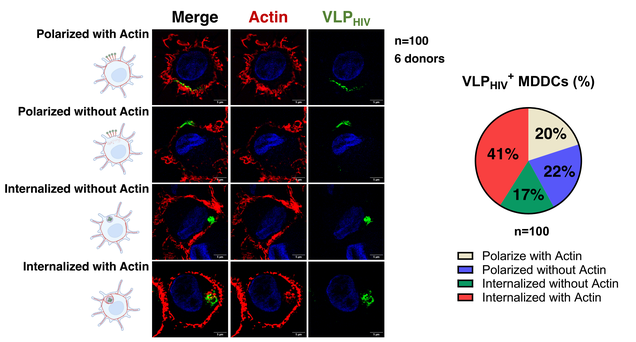
VCC formation dynamics by live confocal microscopy.



Volume and maximal axial size of VCCs.

- **Internalization of VLP_{HIV} occurs as a single-entry process into one VCC with an average volume of 20μm³.**
- **Size and dynamics of VCC formation do not resemble conventional endocytosis processes.**

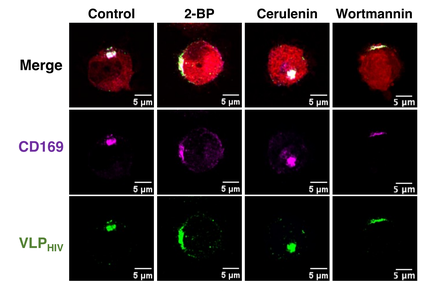
Actin cytoskeleton is present in VCC structure



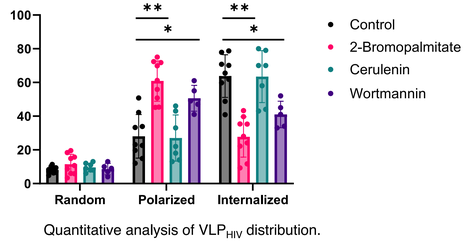
Quantitative analysis of MDDCs with different polarization and internalization phenotypes.

- **Actin cytoskeleton is involved at early stages of VLP_{HIV} polarization and in the final phase to maintain fully formed VCC.**
- **VCCs co-localization with actin reveals that actin cytoskeleton is required to maintain VCC structure.**

Protein Palmitoylation and PI3K inhibition arrest VCC formation



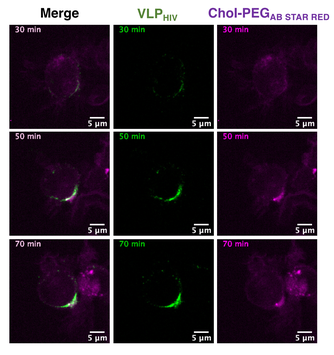
Representative images of MDDC pulsed with VLP_{HIV} upon treatment with 2-BP, cerulenin or wortmannin



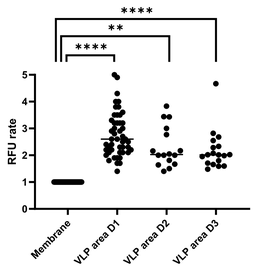
Quantitative analysis of VLP_{HIV} distribution.

- **VLP_{HIV} internalization in MDDCs is arrested at polarization stage in the cell membrane when inhibiting protein palmitoylation and PI3K activity.**

Cholesterol coalescence occurs in VCC formation



Dynamics of cholesterol accumulation during VLP_{HIV} polarization



Quantification of cholesterol fluorescence intensity in cellular membrane.

- **Cholesterol migrates during VLP_{HIV} polarization and accumulates in VCC.**
- **Cholesterol concentration at VLP polarization region is compared to the rest of cell membrane.**
- **Cholesterol coalescence reinforce the idea that MEND mechanisms are essential for VCC formation in myeloid cells.**

CONCLUSIONS

MASSIVE ENDOCYTOSIS

- **Clathrin independent**
- **Massive membrane folding back**
- **Actin independent**
- **PIP regulation dependency**
- **Protein palmitoylation in cell membrane**
- **Cholesterol coalescence dependency**
- **Large cytoplasmic calcium elevation**

VCC FORMATION

- ✓ **Clathrin independence**
- ✓ **VCC volume: 20 μm³**
- ✓ **Actin independent at specific VCC stage**
- ✓ **PI3K activity dependent**
- ✓ **Protein palmitoylation dependent**
- ✓ **Cholesterol coalescence in VCC area**
- • **Changes in intracellular calcium?**

TAKE HOME MESSAGE

Our data suggest that MEND mechanisms participate in VCC formation upon VLP interaction to CD169 receptor.

Blocking VCC formation offers potential cross-protection against enveloped viral infections that enter myeloid cells via CD169 and can disseminate in the body.

ACKNOWLEDGMENTS

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