3D in SEM, (S)TEM, Ion Imaging, incl. FIB-SEM and SBF-SEM

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3D visualization of HIV-1 in mature dendritic cells.

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Dendritic cells are potent antigen-presenting cells and play a unique role in initiating the primary immune response. HIV-1 has developed strategies to subvert the dendritic cell antiviral activity. HIV-1 virions specifically bind SIGLEC-1 on the mature dendritic cell (mDC) surface and are captured and stored in these cells *via* a non-infectious pathway. Particles stored in mDCs can subsequently be transferred to susceptible T-cells in a process called *trans*-infection. Light microscopy revealed captured HIV-1 Gag virus like particles (VLPs) accumulating in a large, apparently intracellular sac-like compartment [1]. The nature of this compartment is so far unknown. Here we investigated the DC ultrastructure using scanning transmission electron tomography (STEM) and focused ion beam milling combined with scanning electron tomography (FIB/SEM). These methods allow 3D analysis of cellular structures with comparable resolution as transmission electron microscopy (TEM) [2].

Peripheral blood mononuclear cells were isolated from blood of HIV-1-seronegative donors and $CD14^+$ populations were cultivated in the presence of granulocyte-macrophage colony-stimulating factor and interleukin-4 to differentiate DCs. Maturation was induced by stimulation with lipopolysaccharide. mDCs were incubated for 5 h with 50-100 ng HIV-1 VLPs fluorescently labeled by enhanced green fluorescent protein (eGFP) inserted into the structural Gag polyprotein ($VLP_{HIV\text{-}Gag\text{-}eGFP}$). Subsequently, cells were seeded on top of 160 µm thick carbon-coated, glow-discharged and PEI-coated sapphire discs 30 min before high pressure freezing (HPF010, Bal-tec). The frozen samples were freeze substituted (AFS2, Leica, Germany) in acetone, uranyl acetate, osmium tetroxide and 5% of water and embedded in Epon. STEM tomography and FIB/SEM tomography were carried out as described earlier [2, 3]. In addition, mDCs were seeded on glass coverslips and fixed with PFA for confocal imaging.

Confocal microscopy analysis (SP2, Leica, Germany) of mDCs showed that internalized $VLP_{HIV\text{-}Gag\text{-}eGFP}$ accumulated in a large, apparently intracellular compartment (Figure 1A). The captured $VLPs_{HIV\text{-}Gag\text{-}eGFP}$ within this compartment could also be visualized in 80 nm thick sections by TEM (EM10, Zeiss, Germany) (Figure 1B). Although, the ultrastructure of the compartment is perceptible by TEM analysis, the dimensions of the storage compartment as well as its potential connection to the plasma membrane remain unclear. Hence, we applied STEM tomography of 1 µm thick sections using a Titan microscope (FEI, Eindhoven, The Netherlands) to investigate the 3D architecture of the capture compartment. Accumulation of $VLP_{HIV\text{-}Gag\text{-}eGFP}$ within an intracellular compartment could be visualized over the entire tomographic reconstruction (Figure 1 C), but the size of the storage compartment extended the thickness of the respective section. For a more complete reconstruction, FIB/SEM was performed using a Helios Nanolab 600 (FEI, Eindhoven, The Netherlands). Preliminary results obtained on a single cell revealed well-resolved $VLP_{HIV\text{-}Gag\text{-}eGFP}$ in close proximity to the plasma membrane (Figure 1D), but the particular cell chosen for analysis did not display uptake of HIV-1 particles into an intracellular compartment.

We show here that STEM tomography and FIB/SEM approaches can be successfully used to analyze the architecture of the HIV-1 storage compartment within mDCs. In our further experiments we will characterize the ultrastructure of this compartment and investigate its potential connection to the plasma membrane.

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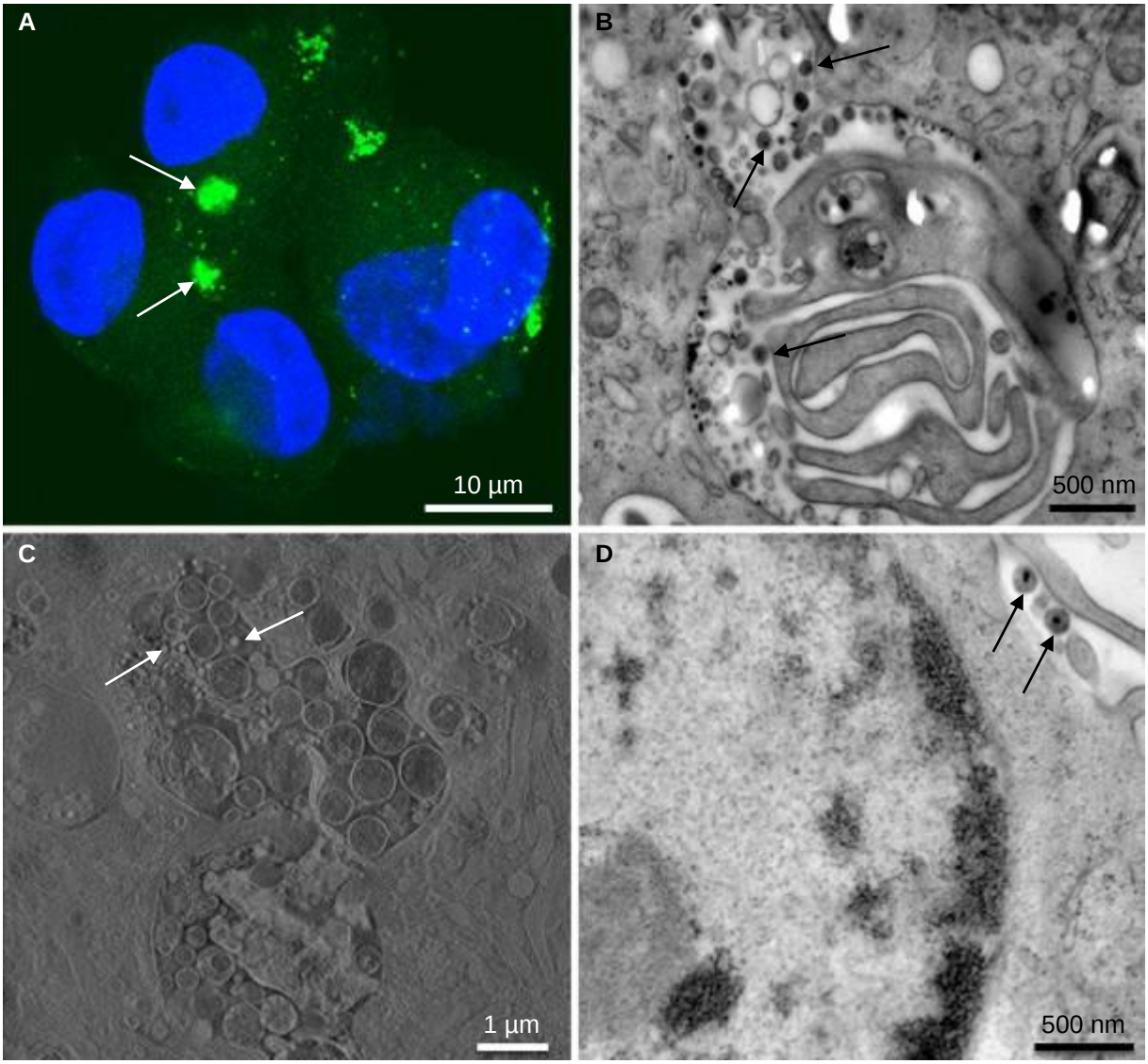

Figure 1. mDCs with internalized $VLP_{HIV\text{-}Gag\text{-}eGFP}$. (A) Confocal image of a z-stack displaying the captured $VLP_{HIV\text{-}Gag\text{-}eGFP}$ aggregated within an intracellular compartment (green fluorescent signal) (white arrows) besides a DAPI-stained nucleus (blue fluorescent signal). (B) TEM image of a 80 nm section of a mDC. A compartment including $VLP_{HIV\text{-}Gag\text{-}eGFP}$ (black arrows) is visible within the cell. (C) Virtual section of a STEM tomogram of a 1 µm thick section. A compartment with stored $VLP_{HIV\text{-}Gag\text{-}eGFP}$ (white arrows) can be visualized in the tomographic reconstruction. (D) FIB/SEM image of a $VLP_{HIV\text{-}Gag\text{-}eGFP}$ incubated mDC. Well-resolved $VLPs_{HIV\text{-}Gag\text{-}eGFP}$ (black arrows) can be found in close proximity to the plasma membrane.