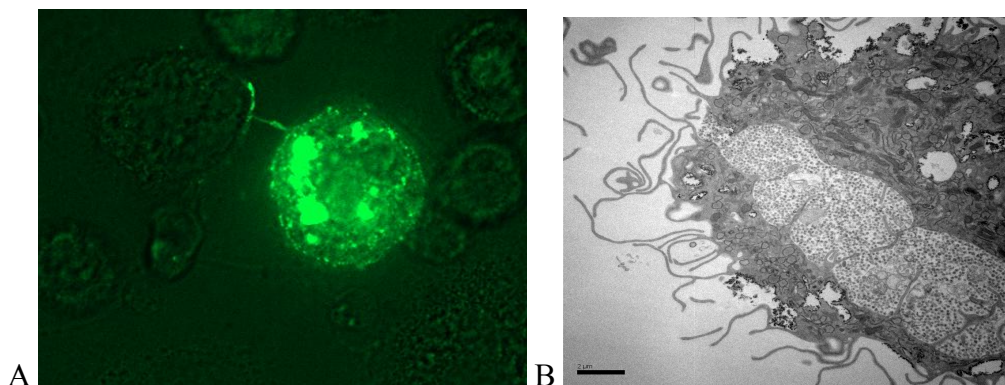


HIV-1 Particle-containing Compartments Identified by Confocal and Electron Microscopy in Human Monocyte-derived Macrophages

Hin Chu*, Jaang-Jiun Wang, and Paul Spearman[#]

Division of Infectious Diseases, Department of Pediatrics and Department of Microbiology and Immunology, Emory University and Children's Healthcare of Atlanta, GA, USA
2015 Uppergate Drive, Atlanta, GA 30322, USA
j.wang@emory.edu

T-cells and macrophages are the two major cell types targeted by HIV-1 infection in human body. It is generally accepted that HIV-1 particles assemble on the plasma membrane (PM) of T-cells and in the endosomal compartments of monocyte-derived macrophages (MDMs). However, recent debates argue that the assembly of HIV-1 in macrophage occurs really on invaginated PM, which appears internally localized, in macrophage. The mechanisms of budding of viruses from endogenous vacuoles and direct transfer of viral particles from infected macrophages to nearby healthy cells remain to be clarified. In this study, the virus-containing compartments (VCC) were evaluated with 3D confocal correlated with electron microscopy (EM). Our confocal microscopy results demonstrate that a significant amount of HIV-1 Gag were colocalized with LE/MVB markers such as CD63 and CD81 in the VCC infected MDM. A significant population of the VCC was accessible to the LE marker antibodies only after cell permeabilization. Tracers such as cationized ferritin (CF), ruthenium red (RR) and BSA-gold conjugates were applied to identify early endosomes (EE) and late endosomes/multi-vesicular bodies (LE/MVB). Our EM analysis showed that CF/RR-labeled virus particles on the surface of MDM were polarized, forming viral synapses, toward uninfected cells. The internalized virus particles were stored in EE, which is BSA-gold/CF/RR positive. In LE, which also containing viruses, was CF/RR-positive only. Channels of CF connecting LE and PM were observed, but no BSA-golds were found in the channels. Sorting of CF/RR from BSA-gold occurred in the sorting endosomes in MDM. Altogether, our data suggests that heterogeneous populations of VCC exist in HIV-1 infected MDM, such that some of the VCC originate from endocytosis, and are completely inaccessible from the PM. Some other virus-containing compartments are endogenous in nature and are occasionally connected to the PM. Characterization of the nature of the virus-containing compartments in infected MDM is important for future AIDS treatments. (Supported by grant EECRC 2009)



The major reservoir of HIV-1 in the body after HIV-1 infection is macrophage. The green deposit area in the cell (A) correlated to the virus-containing vacuoles indicated the storage system in macrophage (B).