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CELL-TO-CELL HIV-1 TRANSMISSION THROUGH A CLATHRIN-DEPENDENT ENDOCYTOTIC PATHWAY

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BACKGROUND: In the course of HIV infection and dissemination, the contact between HIV-1 producing cells and target primary T CD4+ cells, through a structure called the virological synapse, may induce the uptake of viral particles by target cells in a viral envelope gp120-CD4 receptor dependent manner while being coreceptor independent.

METHODS: Coculture of infected and non-infected CD4 T cells, flow cytometry analysis, confocal microscopy and western blot detection of expressed proteins.

RESULTS: Internalized virus particles were localized in large intracellular vesicles in cocultures of primary T CD4+ cells with T cells persistently infected by either one of three HIV-1 isolates, namely, NL4-3, Bal and a clinical isolate CI-1-SI. Since non-stimulated T CD4+ cells have a very low metabolism, they only express residual levels of the early endosomal marker EEA-1 or the late endosomal marker CD63. Conversely, HIV-1infected cells are strongly CD63 positive. Coculture of infected and uninfected cells led to the translocation into T CD4+ cells of the HIV-1 Gag antigen and the CD63 marker, as seen by immuno-confocal microscopy. Internalized virus colocalised with the clathrin-mediated endocytosis EEA-1 marker in primary CD4 T+ cells but not with the Caveolin-1 marker. CD63 staining was observed, by flow cytometry, in T CD4+ lymphocytes after cell-to-cell contacts and viral transmission. Moreover, CD63 was present in the transferred viral particles, explaining the presence of a late endosomal marker in EEA-1 positive, Gag positive endosomes. Analysis of CD4 and HIV envelope subcellular localization revealed their colocalization with Gag in the polarized phenotype, confirming the dependence on CD4 and the transmission of complete viral particles.

CONCLUSIONS: Taken together these results suggest that HIV-1 cell-to-cell transmission probably occur through a caveolin-independent but clathrin-dependent

endocytic process leading to the formation of endosomal vesicles containing complete HIV-1 particles.

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