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Entry of Human Immunodeficiency Virus Type 1 in T-cells

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Human Immunodeficiency Virus type 1 is the most extensively studied pathogen of mankind. Despite all collective efforts that contributed to the transformation of HIV-1 infection from a death sentence to a chronic condition, a lot of aspects of HIV-1 cell biology and pathogenesis remain enigmatic. The focus of the present work was to re-visit early events of the HIV-1 life-cycle, namely attachment, binding and entry. A detailed understanding of how HIV-1 *really* enters its target cells is not necessarily required to develop medically effective preventive strategies against HIV-1. To date, however, the development a prophylactic vaccine, which would have to target HIV-1 before it integrates into the host genome, has not been successful. This can partly be explained by the high mutation rates of HIV-1 and immune escape mechanisms like shielding of the envelope glycoprotein by extensive glycosylation. Nevertheless, a clear and unambiguous characterization of HIV-1 entry on a cellular, viral and molecular scale seems to be a valuable starting point to design future vaccines and targeted therapies.

HIV-1 enters the cytoplasm by fusion of viral and cellular membrane. Fusion is mediated by binding the viral glycoprotein to the viral receptor CD4 and a co-receptor. Three aspects of specific HIV-1 entry were addressed in the present study. First, it was shown that X4-tropic HIV-1 envelope glycoproteins did not induce calcium signaling in either primary or immortalized CD4⁺ T-cells, although the physiological CXCR4 ligand SDF-1 α yielded strong calcium responses. Hence, Env-mediated calcium signaling is dispensable for HIV-1 infection. Second, Env-induced morphological alterations of the actin cytoskeleton at the level of viral attachment sites could not be observed with state-of-the-art microscopy (total internal reflection microscopy and spinning disk confocal microscopy) and nanoscopy (stimulated emission depletion microscopy) methods. These methods could, however, demonstrate that HIV-1 particles do not randomly bind to the target cell surface. Instead, they bind the cell membrane at the vicinity of an actin cable. This observation was not dependent on the viral glycoprotein. Future studies are needed to elucidate whether the viral envelope composition dictates binding to actin-cable associated plasma membrane domains. Third, this study addressed the question whether the portal for productive HIV-1 infection in T-cells is the plasma membrane or the endosome. Since the HIV-1 fusion process does not require a low pH trigger, the plasma membrane was considered a possible fusion site. Endocytosis, contrarily, was thought to result in non-productive particle degradation. It has been shown, however, that HIV-1 can productively enter epitheloid reporter cell lines *via* endocytosis. Recently, endocytosis was suggested to be the exclusive entry pathway also in lymphoid cell lines. The present work demonstrated that binding and endocytosis in T-cells was dependent on the viral receptor, but not the co-receptor. Endocytosed particles were co-localizing with

the viral glycoprotein and CD4. Blocking endocytosis by overexpression of dominant-negative dynamin-2, however, revealed that endocytosis was not required for either cytoplasmic entry or productive infection. Flow cytometry analyses of SupT1/CCR5 cells showed the same fusion efficiency and infection for NL4-3-based HIV-1 in the presence of either wild-type or dominant-negative dynamin-2. This observation was virus-dose independent and was unaffected by the presence of the viral protein Nef.

Furthermore, uncoupling fusion and endocytosis by incubating HIV-1 particles with cells at 22°C allowed HIV-1 endocytosis, but inhibited HIV-1 fusion. Subsequent addition of the membrane-impermeable fusion inhibitor T-20 only blocked fusion at the plasma membrane, whereas previously endocytosed particles could not be reached. Switching the temperature to 37°C enabled the exclusive quantitation of fusion and infection from endocytosed HIV-1 particles. Fusion and infection were scored by flow cytometry after endocytosis at 22°C for 5 or 16 h. The positive control in which no T-20 was added and experiments with inhibitors of endosomal acidification revealed that incubation at 22°C for prolonged times did not interfere with infectivity *per se*. However, addition of T-20 after allowing endocytosis at 22°C reduced fusion and infection to levels of a negative control in which T-20 was present throughout the experiment. These results could be confirmed for both SupT1/CCR5 and CEM-ss T-cell lines. Furthermore, primary CD4⁺ T cells exhibited the same phenotype, and this was also true for HIV-1 particles pseudotyped with primary Env isolates. Collectively, these experiments demonstrated that productive infection in T-cells was not mediated by endosomal fusion. Although CD4-mediated endocytosis occurs in T-cells, productive infection requires fusion at the plasma membrane.