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Journal Title: Journal of Virology
Volume: Volume 89, Number 5
Publisher: American Society for Microbiology | 2015-03-01, Pages 2985-2985
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JVI.03352-14
Permanent URL: https://pid.emory.edu/ark:/25593/q45c1

Final published version: http://dx.doi.org/10.1128/JVI.03352-14

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Accessed March 15, 2018 6:36 AM EDT
Can HIV-1 Entry Sites Be Deduced by Comparing Bulk Endocytosis to Functional Readouts for Viral Fusion?

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A recent Journal of Virology article by Herold and colleagues (1) addresses the controversial issue of the HIV-1 entry pathways. Two lines of evidence led the authors to conclude that internalized viruses do not contribute to productive HIV-1 entry into lymphoid cells. First, a dominant-negative dynamin mutant blocked HIV-1 uptake but not fusion. Second, preincubation at 22°C allowed virus endocytosis while preventing fusion/infection. Subsequent fusion induced by raising the temperature could be fully blocked by membrane-impermeable peptide inhibitor T20, demonstrating that productive endocytosis did not occur at 22°C. The latter result fully agrees with our data showing that HIV-1 engages CD4 and coreceptors on the cell surface upon incubation at reduced temperatures (2, 3). However, we interpreted the subsequent fusion induced by shifting to 37°C as synchronized endocytosis followed by fusion with endosomes, since the virus escape from the low-temperature block was delayed compared to escape from a T20-like peptide (2).

The key question is whether bulk virus uptake faithfully reports productive entry. Since the majority of internalized viruses are degraded, comparison of bulk endocytosis and viral fusion/infectivity could be misleading. Indeed, CD4-mediated HIV-1 uptake appears to occur independently of coreceptor binding or the ability to fuse with HeLa cell-derived and lymphoid cells (1, 4, 5). Thus, only a fraction of CD4-bound viruses may acquire a requisite number of coreceptors prior to being internalized. Based upon apparent colocalization of intracellular HIV-1 with CD4, these viruses were considered productively internalized in the study of Herold and colleagues (1), but colocalization does not demonstrate the formation of functional prefusion complexes with CD4 and coreceptors. Furthermore, HIV-1 uptake and intracellular trafficking upon prolonged incubation (5 to 16 h) at 22°C is likely perturbed. Considering that endocytosis is a highly dynamic process, which is grossly altered by various interventions, including reduced temperature (6–10), productive HIV-1 uptake could be inhibited at 22°C.

Additional concerns are related to the HIV-1 endocytosis assay employed in the study of Herold and colleagues (1). Viruses located inside the peripheral F-actin layer were scored as internalized. However, the limited resolution of optical microscopy and the complex shape of the plasma membrane (PM) confound the identification of particles near the PM as internalized. If peripheral viruses colocalized with the actin layer were not scored, early endocytic events could be rejected, while viruses residing in late endosomes/lysosomes—likely destined for degradation—would be favored. It is therefore surprising that this assay was not validated using conventional techniques, such as removal of noninternalized viruses by trypsin (4, 5). Another missing critical control in this study (1) is using the above method to demonstrate a correlation between uptake and infection for viruses known to enter via endocytosis, such as vesicular stomatitis virus (VSV).

We believe that it is time to move from counting internalized HIV-1 in fixed cells to direct functional approaches. Visualization of single viral lipid/content transfer events under physiological conditions (2, 3) can reveal the HIV-1 entry sites in lymphocytes and macrophages. This technique distinguishes between virus fusion with the PM and endosomes based on the extent of dilution of the viral lipid marker.

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Editor: R. W. Doms
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For the author reply, see doi:10.1128/JVI.03376-14.
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