

INTERACTIONS AMONG HTLV-1 ENVELOPE AND CELL PROTEINS AND THEIR IMPACT ON VIRUS INFECTION AND RESTRICTION

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The fact that HTLV-1 Env may be specifically adapted for cell-to-cell infection is supported by data showing that VLPs containing HTLV-1 Env were efficient in cell-to-cell infection but very poor for cell-free infection; the opposite was true for VSV-G pseudotyped VLPs. In addition to binding to its receptor on target cells and mediating fusion of virus and cell membranes, HTLV-1 Env may also play a role in initiating adhesion events between effector and target cells to facilitate polarized transmission of the virus. To better understand the mechanisms that control HTLV-1 Env trafficking and activity, we have examined two protein-protein interaction motifs in the 27-amino acid cytoplasmic domain of Env. One is a YSLI sequence, which matches consensus YXX Φ motifs that are known to interact with various adaptor proteins; the other is an ESSL sequence at the C-terminus of Env, which matches the consensus PDZ-binding motif. Mutations that disrupt the YXX Φ motif result in increased Env accumulation in cells, increased cell-cell fusion activity, and increased incorporation of Env into virus particles. Silencing of AP2 and AP3 expression with siRNA had effects similar to mutation of the YXX Φ motif. Interestingly, mutations that destroy the YXX Φ motif abolished virus infection by either cell-free or cell-associated routes, despite the presence of Env in virus particles. Mutation of the PDZ-binding motif greatly diminished Env accumulation in cells, but mutations that disrupted both YXX Φ and PDZ-binding motifs restored Env expression to wt levels. The data are consistent with a model in which the low affinity YXX Φ motif in Env interacts weakly with AP2 and initiates internalization of Env from the cell surface; the PDZ-binding motif may then cause a delay in endocytosis of Env in the cell-cell contact area through interaction with PDZ-protein, maintaining polarity in cell-to-cell virus transmission.

It is known that some viral Env proteins not only usurp cellular trafficking proteins but also counteract proteins restricting viral release like BST2/Tetherin. HTLV-1 Env does not overcome BST2/Tetherin restriction and it is unlikely that HTLV-1 encodes accessory proteins that counteract BST2/Tetherin. Cotransfection of cells with HTLV-1 vectors and BST2-expression plasmid resulted in potent inhibition of VLP release and hence ablation of cell-free infection. To test the effects of BST2/Tetherin on HTLV-1 cell-to-cell infection, varied amounts of BST2 expression plasmid were cotransfected with HTLV-1 vectors into Jurkat cells, which were then cocultured with Raji cells. At levels of BST2/Tetherin expression that abolished HTLV-1 Gag release into the culture medium, there was only a modest decrease in cell-to-cell infection. These data indicate that cell-to-cell transmission may allow HTLV-1 to circumvent BST2/Tetherin restriction.