POSTER 30

MECHANISM OF ANTI-HIV-1 ACTIVITY OF THE CHOLESTEROL-BINDING COMPOUND AMPHOTERICIN B METHYL ESTER: INHIBITION OF ASSEMBLY/RELEASE EXHIBITS Vpu-DEPENDENCE

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Plasma membrane cholesterol plays an important role in HIV-1 assembly and virus entry. We previously reported that a cholesterol-binding compound, amphotericin B methyl ester (AME), potently inhibits viral entry and that single amino acid substitutions (P203L and S205L) in the cytoplasmic tail (CT) of the transmembrane Env glycoprotein gp41 confer resistance to AME. We have shown that the gp41 CT in AME-resistant virions undergoes cleavage mediated by the viral protease (PR) (Waheed et al., PNAS 2007). Interestingly, we observed that this gp41 CT cleavage is inhibited by mutations in PR that confer resistance to PR inhibitors. These results identify a novel mechanism of escape from a cholesterol-binding HIV-1 entry inhibitor via cleavage of gp41 by PR.

In addition to its effect on virus entry, AME also inhibits virus particle production ~5 fold with no significant effect on Gag binding to the plasma membrane, Gag association with lipid rafts, or Gag multimerization. Our EM results suggest that AME does not have a significant effect on the pinching-off of budding particles but significantly alters virion morphology. We analyzed the involvement of viral proteins other than Gag in the ability of AME to disrupt virus production. Interestingly, we found that the viral accessory protein Vpu is required for AME-imposed inhibition of viral production. Consistent with this observation, we observed that AME does not inhibit the release of retroviruses (e.g., SIVmac239 or MLV) that do not encode Vpu. Furthermore, the release of a Gag chimera bearing the Fyn membrane-targeting signal [Fyn(10)fullMA] is sensitive to AME whereas the Vpu-defective derivative of this clone (Fyn(10)fullMAdelVpu) is insensitive. We demonstrated that the ability of Vpu to counter the activity of the host restriction factor CD317/BST-2 (tetherin) is markedly reduced by AME. Our recent studies indicate that tetherin is degraded by Vpu expression. We are currently investigating the cell-surface localization of tetherin and its colocalization with HIV-1 Gag in the presence of AME when Vpu is coexpressed. These results support the concept that Vpu represents a potential target for the development of novel antiretrovirals.