

**POSTER 48****THE CAPSID-SPACER PEPTIDE 1 GAG PROCESSING INTERMEDIATE IS A DOMINANT-NEGATIVE INHIBITOR OF HIV-1 MATURATION**

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The HIV-1 maturation inhibitor bevirimat disrupts virus replication by inhibiting the cleavage of the capsid-spacer peptide 1 (CA-SP1) Gag processing intermediate to mature CA. The observation that bevirimat delays but does not completely block CA-SP1 processing suggests that the presence of uncleaved CA-SP1 may disrupt the maturation process *in trans*. In this study, we validate this hypothesis by using a genetic approach to demonstrate that a noncleavable CA-SP1 mutant exerts a dominant-negative effect on maturation of wild-type (WT) HIV-1. To determine to what extent CA-SP1 interferes with HIV-1 maturation, we coexpressed WT Gag and a Gag mutant (CA5, obtained from HG Kräusslich) unable to be cleaved at the CA-SP1 junction due to two amino acid mutations (L363I and M367I). We observed that cotransfection of WT and CA5 molecular clones at ratios of 1:1, 2.5:1, and 5:1 resulted in the formation of virions that contained ~60%, ~40%, and ~30% CA-SP1, respectively. Virus produced from WT and CA5 cotransfections were used to determine whether CA-SP1 imposes a dominant-negative effect on HIV-1 infectivity. Our results showed that virions from WT/CA5 mixtures were markedly less infectious than pure WT particles. The 1:1 WT/CA5 mixed virions exhibited only ~16% infectivity, suggesting that the phenotype of the CA5 mutant is dominant. In contrast, a CA-SP1 mutant containing only the L363I mutation was a very weak inhibitor of the WT when supplied *in trans*, despite the fact that the L363I mutant was non-infectious. We also observed that WT/CA5 mixed virions displayed a profound delay, relative to the WT, in replication in Jurkat T cells. CA5 virions, or WT virions produced in the presence of bevirimat, are characterized by an acentric core accompanied by a crescent-shaped zone of electron density when visualized by transmission electron microscopy, while mature WT virions contain mainly conical cores. Our results show that increased levels of uncleaved CA-SP1 in virions were accompanied by a reduction in the percentage of virions containing conical cores. These data underscore the importance of full CA-SP1 processing for HIV-1 maturation and highlight the therapeutic potential of inhibitors that target this Gag cleavage event.