

## DIFFERENTIAL EFFECT OF Gag DETERMINANTS ON HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 RESISTANCE TO PROTEASE INHIBITORS AND REPLICATIVE CAPACITY

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Human Immunodeficiency type 1 (HIV-1) resistance to protease inhibitors results from gradual accumulation of mutations in the viral protease (PR). Resistance is also accompanied by emergence of mutations in the Gag polyprotein at the NC-SP2 and the SP2-p6 cleavage sites, which have been found to partly compensate for the loss of viral replicative capacity (RC) that can result from mutations in PR. In this study, we examined the extent that changes in Gag selected under selective pressure by protease inhibitors *in vivo* could also exert a direct impact on resistance, and delineated the role of different domains of Gag in this effect. We constructed NL4-3-derived molecular clones carrying highly mutated PR and RT plasma virus sequences from six patients having experienced failure of multiple lines of treatment, and combined these sequences with different segments of homologous Gag sequences, which all carried cleavage site mutations A431V or I437V in NC-SP2-p6. We found that both resistance and RC were highly dependent upon the presence of patient-derived Gag sequences. These effects were essentially carried by the NC-SP2-p6 domains, with little or no effect exerted by MA and CA domains. In absence of PR mutations, Gag sequences alone were able to mediate perceptible effects on resistance and, for some of them, notable increases in RC. Mutagenesis of NC-SP2 cleavage site mutations A431V and I437V back to wild-type produced strong losses in resistance. The impact of these reversions on RC, however, was generally modest, suggesting the participation of other changes in the NC-SP2-p6 region. Western blot analyses of NC and CA cleavage in mutant viruses assembled in the presence or in the absence of protease inhibitor strongly emphasized the importance of the NC-SP2 cleavage event as a rate-limiting step in HIV-1 replication and resistance. We conclude that the principal determinants of Gag coevolution with PR in treated patients lie within the NC-P2-PR region and that the primary function of mutations in the NC-SP2 cleavage site is to increase resistance.