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Isolation and Characterization of an HIV-1 Variant with Reduced Sensitivity to an Aminoalcohol Protease Inhibitor

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Human immunodeficiency virus (HIV) encodes for a protease which is essential for production of infectious virus and therefore represents an important target for antiviral therapy. Development of viral resistance to an aminoalcohol protease inhibitor was studied by serially passaging HIV-1 RF in MT-2 cells in the presence of increasing concentrations of drug. After 11 passages, an HIV variant emerged that showed a 15-fold increase in the IC_{50}. This HIV variant displays cross-resistance to a structurally similar aminoalcohol inhibitor and low level resistance to A77003 (Abbott's protease inhibitor), but remains sensitive to R031-8959 and SC52151 (Roche's and Searle's inhibitors, respectively). The protease genes from protease-resistant and wild type HIV-1 RF strains were PCR-amplified, cloned and sequenced. All resistant clones (18/18) contained A71T and V82A mutations. To determine the effect of these mutations on protease activity and viral resistance, *E. coli* expression clones and proviral HIV clones containing the single mutations A71T and V82A, or double mutations A71T/V82A were constructed. Protease carrying either the V82A or A71T/V82A mutations displayed resistance to the inhibitor, cross-resistance to structurally similar inhibitors and low level resistance to Roche's protease inhibitor. In drug sensitivity assays, a 3 and 6-fold level of resistance was detected with recombinant virus containing V82A and A71T/V82A, respectively, however, virus containing the A71T change remained susceptible to the protease inhibitor. These results indicate that the V82A alteration mediates the protease inhibitor resistance.

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