

## POSTER 37

### CYCLOSPORINE SENSITIVE RESTRICTION TO HIV-1 IN HeLa CELLS

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We have isolated HeLa cell subclones that have increased resistance to HIV-1 and designated them HeLa.HR cells. HeLa.HR cells were present at a frequencies of  $1 \times 10^{-6}$  or greater in HeLa cell parental populations. These cells resisted infection by various HIV-1, but not Mo-MLV or SIV, vectors pseudotyped with VSV-G. HIV-1 displaying HIV-1 Env was also restricted in HeLa.HR cells engineered to express CD4. Real-time PCR analysis revealed that the block in HeLa.HR cells occurs prior to or during the initiation of HIV-1 reverse transcription. The presence of an early block and difference between HIV-1 and SIV susceptibility prompted us to examine whether HIV-1 with CA substitutions could evade the restriction. Notably, HIV-1 isolates with CA substitutions that preclude the interaction of host cell cyclophilin A (CypA) were not impaired in their ability to infect HeLa.HR cells. Consistent with this observation, treatment of cells with cyclosporine A (CsA), a competitive inhibitor of CypA, also rescued HIV-1 infectivity. Furthermore, depletion of cellular CypA by RNA interference made HeLa.HR cells permissive to wild-type HIV-1 infection, providing additional evidence that the CypA interaction with HIV-1 CA underlies this restriction. Microarray analysis indicated that many CypA related genes had increased expression in HeLa.HR cells. Notably, an shRNA targeting a subset of CypA pseudogenes rescued HIV-1 infection without reducing endogenous CypA expression in HeLa.HR cells. Based on these data, we are determining whether a CypA-like protein or CypA-derived fusion protein is enriched in HeLa.HR cells compared to parental HeLa cells and accounts for their differing susceptibility to HIV-1.