Title: *In vivo* and *in vitro* evidence for infection of naïve CD4 T cells with CCR5-tropic HIV.

Authors: Charlotte Kearns, Marilia Pinzone, Alfonso Oceguera, Ashley Ginda, Una O’Doherty.

Historically, the field of HIV research has largely ignored the viral reservoir in naïve CD4+ T cells, due to lower HIV DNA levels compared to memory subsets. Our analysis of proviral sequences in naïve T cells reveals unique proviral sequences suggesting that the naïve reservoir is distinct from the memory reservoir. The naïve reservoir represents a unique hurdle because it is persistent, diverse, and resistant to immune clearance. It may serve to replenish the more differentiated memory reservoir. Curiously, we detected CCR5-tropic HIV in naïve cells in Persons Living with HIV (PLWH) using computer algorithm to determine tropism. This result raises the question of how those cells are infected, as naïve T cells appear to lack the CCR5 coreceptor. We first wanted to determine tropism phenotypically *in vivo* and then explored mechanisms of naïve infection *in vitro*. We tested the *in vivo* tropism by stimulating sorted naïve CD4 T cells to release virus at limiting dilution from PLWH on antiviral therapy. The tropism of HIV+ supernatants were determined by infecting cells engineered to express CCR5 or CXCR4. We next investigated *in vitro* conditions that promote naïve infections. We infected mixtures of CD4 subsets, as well as pre-sorted naïve cells alone with a CCR5-tropic HIV utilizing fibroblast reticular stromal cells to preserve the naïve phenotype. We showed CCR5-tropic HIV can be isolated from naïve CD4 T cells (CD95-,CD45RA+,CCR7+,CD27+) of PLWH by performing infection studies with out-growth virus in the presence and absence of CCR5 inhibitors. We also found CCR5-tropic infection occurred *in vitro* in phenotypically naïve cells when CD4+ T cells were infected in bulk, but not when naïve cells were pre-sorted and then infected. Thus, memory T cell reversion or transient upregulation of CCR5 expression may provide a mechanism for CCR5-tropic naïve infection which may be promoted by cellular interactions that occur in the lymph node milieu. We present *in vivo* data that CCR5-tropic infection occurs in naïve cells. We present an infection model that promotes reversion. This model can be utilized to explore mechanisms that underlie naïve infection and for preclinical studies to probe the naïve reservoir.