Evaluation of a panel of selective inhibitors of PKCθ or Lck for controlling HIV-1 replication in CD4+ T cells

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Background: PKC theta (θ) is selectively expressed on CD4+ T lymphocytes and is activated through TcR/CD28 engagement at the immunological synapse. PKCθ activation is mediated by the lymphocyte-specific protein tyrosine kinase (Lck), which is required for PKCθ translocation to the plasma membrane. This initiates a cascade of events that culminates in the activation of essential factors for HIV-1 replication such as ERK, AP-1 and NF-κB. We reported previously that blocking PKCθ selectively by rottlerin reduces viral replication in T lymphocytes and that mRNA interference for PKCθ provided a refractory state to HIV-1 infection. Now we analyzed the effect on viral replication of a panel of 10 selective PKCθ or Lck inhibitors and proved that blocking Lck also reduced HIV-1 replication.

Methods: 10 selective PKCθ or Lck inhibitors were used to evaluate their ability to control HIV-1 infection in PBLs and MT-2. Inhibition of viral replication was determined by infecting with NL4.3-renilla (X4) and BX08-renilla (R5) strains. IC₅₀ and CC₅₀ were estimated using GraphPad Prism software (sigmoidal dose-response formula). Inhibition of NF-κB was monitored by transient transfection of κB-LUC expression vector. Proviral integration was analyzed by quantitative Alu-LTR PCR. Released IL-2 was assessed by ELISA.

Results: all PKCθ or Lck specific inhibitors reduced HIV-1 (X4- and R5-tropic) replication in PBLs and MT-2. Specifically, CGX0471 and CGX1079 (CompleGen) inhibited HIV-1 replication (NL4.3wt) more than 14-fold in MT-2 (IC₅₀=3.67µM and 2.28µM, respectively) and more than 16-fold in PBLs (IC₅₀=11.18µM and 12.86µM, respectively) (R²=0.98; CC₅₀>50µM). Both compounds reduced NF-κB activity and the decrease of IL-2 release to the culture medium was assessed. Lck inhibitor II (Merck) also reduced HIV-1 replication in PBLs (IC₅₀=1.19µM; CC₅₀>37µM) and MT-2 (IC₅₀=15µM; CC₅₀>100µM), as well as IL-2 release, NF-κB activity and proviral integration.

Conclusions: PKCθ is essential for HIV-1 replication in CD4+ T-lymphocytes. Specific inhibition of PKCθ and Lck in CD4+ T-cells delayed HIV-1 transcription and proviral integration. Since both kinases are expressed primarily in T-lymphocytes, the use of specific inhibitors as adjuvant of cART during acute infection may reduce the pool of activated CD4+ T-cells, the viral production and the size of the reservoir, without causing general immunosuppression.