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Inducing HIV-1 in latently infected T cells with an autologous full length HIV-1 representing the majority intrapatient virus population

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Background: Complete eradication of HIV with antiretroviral drugs is almost insurmountable, as the virus persists in cellular reservoirs as latent proviral integrants. Most approaches to induce the latent HIV-1 pool involve some type of cell activation through mitogens, cytokines/chemokines, or HDAC inhibitors to up-regulate gene expression, which by default may also activate HIV-1 mRNA expression from latent proviruses. Prior to treatment, HIV-1 primarily infects HIV-specific CD4+ T cells that then transitions to the latently infected memory T cell population. Thus, we propose that the most effective and specific activator of latently infected T cells is the patient´s HIV-1 quasipecies prior to treatment.

Methods: The entire HIV-1 genome and population was amplified from multiple plasma samples prior to HAART. The near full length (nfl) HIV-1 genome was RT-PCR amplified as a full fragment, in overlapping halves or thirds are recombined into a yeast-based vector via homologous recombination/gap repair. Through a positive/negative selection system, yeast colonies are grown as population for subsequent proviral plasmid purification and transfection into 293T cells to produce a replication incompetent vector based on the intrapatient HIV-1 population prior to treatment.

Results: The autologous HIV-1 vector, a NL4-3-based vector, and a cocktail of flu/tetanus/CMV antigens were loaded onto the patient DCs and then co-cultured with isolated T cells. DCs and T cells were obtained from patients after 3 years on stable HAART. In three different patients, the autologous HIV-1, present by DCs, induced at least 30-fold higher HIV-1 production from the T cells than did the NL4-3 and 100-fold higher than the Flu/TT/CMV cocktail. In contrast, gamma interferon ELISPOTS on the DC-antigen-T cell cocultivations revealed 10- to 100-times more spot forming units with the Flu/TT/CMV antigen cocktail than with the autologous HIV-1 vector.

Conclusion: These findings suggest that the entire intrapatient HIV-1 population in a safe, replication incompetent vector may be the best and most specific stimulus to drive HIV-1 out of the latent T cell pool. We suspect that most latently infected T cells are also HIV- specific in antigen recognition and that during active virus replication the majority of the acquired immune response was focused on HIV-1.