

PE25

Minimal HIV-I Gag epitope presentation in a T-cell line during reactivation

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Background: “Shock and kill” methods are being tested as a strategy to cure HIV infection. Various agents can reactivate latent provirus; however, immune-mediated killing of these cells appears to be inefficient. To investigate whether this is due in part to poor antigen presentation, we developed a reporter T cell assay to detect HIV epitopes on latent cells following reactivation.

Methods: A latent Jurkat-GFP (J-Lat) cell line stably expressing HLA-A*02+ was constructed and used as target cells. HIV was reactivated using anti-latency agents (TNF α and HDAC inhibitors). Enhancers of antigen processing (IFN γ and ATRA) were also tested. Effector T cells were generated by transfection of Jurkats with TCR α/β specific for the A*02-restricted Gag FK10 epitope, CD8 α and NFAT-driven luciferase reporter plasmids. Reactivation of J-Lat cells was measured by assessing GFP and Gag-p24 expression using flow cytometry. Live GFP+ and GFP-negative target cells were collected by FACS and FK10 presentation on these cells was detected following co-culture with TCR+ effector cells as an increase in luciferase signal.

Results: Co-culture of FK10-pulsed J-Lat-A*02 targets with TCR+ effectors resulted in a dose-dependent increase in luciferase signal. Anti-latency agents reactivated ~5% to 40% of live J-Lat cells, versus TNF α (30%) and DMSO (0%), and expression of Gag-p24 correlated with higher GFP fluorescence. Despite robust Gag expression, no difference in TCR-driven luciferase signal was observed between GFP+ and GFP-negative J-Lat targets. Addition of IFN γ enhanced the ability of TNF α -treated J-Lat target cells to induce luciferase signal; and a further increase in signal was observed when IFN γ and all-trans retinoic acid (ATRA) were added in combination. However, these effects were not observed when HDACi-treated target cells were used.

Conclusions: These results indicate that J-Lat cells present endogenous viral peptides poorly, but this activity could be enhanced by IFN γ and ATRA. Lack of TCR-mediated stimulation by HDACi-treated target cells, even in the presence of IFN γ and ATRA, suggests that these drugs further impaired peptide presentation. Altered antigen presentation intrinsic to latent cells/cell lines or as a side-effect of anti-latency drugs should be considered as a potential barrier to HIV eradication.