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Flow-based differentiation between latently HIV-1-infected single cells expressing Gag mRNA alone or in conjunction with Gag protein following latency reversal.

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Background: Current antiretroviral treatments cannot eradicate HIV-1 infection due to a pool of persisting latently infected cells. Reactivation of the latently infected cells, using for example HDAC inhibitor, has been suggested as an approach to reduce the HIV-1 reservoir. However, it remains unclear in how many latently infected cells reactivation occur, and whether reactivation leads to production of viral RNA alone versus production of viral proteins or viruses. We aimed to develop an approach to evaluate the molecular kinetics of HIV-1 latency reactivation on the single cell level to distinguish cells in which only viral mRNA is expressed from cells in which viral proteins or novel viruses are produced.

Methods: J89 cells were used as a HIV-1 latency reactivation model, and treated with different concentrations of hTNFα cytokine for defined time points ranging from 1 hr to 24 hrs. Combined intracellular staining for p24 Gag protein and Gag mRNA was performed, using a newly established technique that allows for simultaneous detection of mRNA targets and intracellular proteins. HIV-1 p24 Gag protein production and p24 Gag mRNA synthesis was quantified simultaneously on the single cell level using multiparameter flow cytometry.

Results: Following stimulation of J89 cells with 1 ng/mL of hTNFα for 6h, moderate HIV-1 Gag mRNA expression was detected, accompanied with almost no intracellular Gag protein detection. Higher concentrations of hTNFα (10ng/ml) resulted in elevated expression of HIV-1 Gag mRNA as well as intracellular Gag protein synthesis. After 24h stimulation with 10ng/ml of hTNFα, three distinct populations were identifiable by flow cytometry: only HIV-1 Gag mRNA positive cells, HIV-1 Gag mRNA and Gag protein double-positive cells, and cells only expressing Gag protein, but no HIV-1 Gag mRNA anymore.

Conclusions: We here describe a novel method allowing for the first time to simultaneously quantify the kinetics of HIV-1 mRNA and HIV-1 protein synthesis upon latency reactivation. This approach will enable the phenotypic characterization of latently infected cells at different stages of latently reversal and the identification of surface markers that render these cells as targets for innate and adaptive immune responses.