Understanding Natural Killer Cell Control of HIV Infection

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Natural killer (NK) cells are innate lymphocyte responders that rapidly and robustly kill HIV-infected cells. They show great promise in establishing early control of infection in a vaccine or therapeutic setting where adaptive immune responses have proven to be too little and too late. Before this response can be elicited in a vaccine or therapeutic setting, NK cell recognition of HIV-infected cells must be understood at a detailed level. However, NK cells are a highly heterogeneous cell population, capable of a vast variety of functions and possessing a complex mosaic of surface receptors. The limits of fluorescence cytometry have prevented the simultaneous examination of all of these traits. To address this question, we propose the use of a novel technology, Cytometry by Time-Of-Flight (CyTOF). CyTOF uses rare metal isotope-labeled rather than traditional fluorophore-conjugated antibodies, with detection via mass spectrometry. Because it is not subject to spectral constraints, CyTOF can detect up to 40 cellular markers simultaneously, a considerable advance from the traditional fluorescence cytometry limit of 12-18. We have designed and validated a panel of antibodies for CyTOF to measure NK cell phenotype, and propose to add additional antibodies to measure NK cell functions during killing of HIV-infected cells. We will use this panel in conjunction with an established *in vitro* viral suppression assay, in which NK cells from healthy donors recognize and kill autologous HIV-infected CD4+ T cells. The highly parametric, deep profiling capabilities of CyTOF, applied to this specific, controlled interrogation of anti-HIV NK cell function, will allow us to gain profound insight into which NK cells are actively recognizing and killing HIV-infected cells.

Results will be analyzed using a variety of complementary statistical techniques. Once this analysis has defined specific subsets of NK cells that appear to be involved in killing HIV-infected cells, we will complement these findings using *in vitro* functional assays to define precisely how the NK cells are recognizing and killing HIV-infected cells. We will use assays that classically examine single receptors or functions, but can be adapted to simultaneously interrogate multiple receptors or functions in a combinatorial manner. By combining the novelty of CyTOF with the reliability of established techniques, our innovative approach aims to understand which NK cell subsets to prime and which functions to stimulate in order to purposefully elicit an anti-HIV response in a vaccine or therapeutic setting. Our findings will therefore inform the development of innovative new approaches to vaccination to protect Californians from HIV.