

Poster presentation

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PI6-27. Optimization of the expansion of antigen-specific CD8+ T-cells for use in a viral suppression assay

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Background

CD8+ T-cells have long been shown to be important for control of HIV-1 infection, though it is clear that not all CD8+ T-cell responses are equal. For the best chance of producing a successful vaccine, it is crucial to define which responses lead to better disease outcomes. Many studies have correlated HIV-specific T-cell responses with slower HIV disease progression; however, a causal relationship has not been clearly defined. Tetramers to antigen-specific cells allow enumeration, characterization and separation of cell populations, allowing comparison of their viral suppressive capabilities in downstream assays.

Methods

Tetramers were used to enumerate HIV-1 antigen-specific CD8+ T-cells in PBMCs from HIV infected subjects, on a BD LSR-II flow cytometer. A protocol for expansion of these epitope-specific cells was developed in control samples using the CMV peptide NLVPMVATV due to its immunodominance in North America. Cells were stimulated for 10 days with varying concentrations of IL-2, IL-7 and IL-15 along with the tetramer-matched peptide to elucidate optimal expansion conditions.

Results

Initially, ~0.5%–1.5% of CD8+ T-cells bound to HIV-1 specific tetramers in HIV-1 infected subjects. These low cell numbers identified a need for expansion of the cells for use in downstream assays. In our protocol, addition of

IL-15 and peptide results in an increase of tetramer+ cells from 3.5% to 85% of total CD8+ T-cells, while IL-2 and IL-7-induced responses were less robust. Increasing the concentrations or adding multiple cytokines at once decreased the population of tetramer+ T-cells to less than 1%.

Conclusion

These results indicate that antigen-specific T-cells can be expanded to high levels for use in subsequent assays, such as a study of their HIV-1 suppressive capabilities. This will provide a direct link between specific CD8+ T-cells and viral control, which will be key in determining which responses are most likely to be effective in an HIV-1 vaccine.