KNOCKDOWN OF CCR5 EXPRESSION IN ANTIGEN-SPECIFIC CYTOTOXIC CD4 T CELLS

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Background: CCR5+ cytotoxic CD4 T cells may be critical to control of persistent viruses in immune cells, but are susceptible to HIV-1 infection. We investigated use of gene therapy with short-hairpin (sh) RNA to knockdown CCR5 in recall antigen (Ag)-specific CD4 T cells in vitro.

Methods: 30-40x10^6 PBMC from healthy HIV-uninfected adults were incubated for 48 hr with CMV lysate. Non-Treg Ag-specific CD4 T cells were identified as CD25+CD134+(OX40+) and CD39neg. Cell sorted highly purified OX40+ cells were transduced with a lentiviral vector containing shRNA specific for CCR5 under control of the H1 promoter, and GFP under control of the ubiquitin C promoter, in the presence of IL-2 for 3 days, and analysed by fluorescence microscopy. After 3 weeks incubation in IL-2 containing medium in the presence of feeder cells, GFP+ cells were cell sorted, further expanded and phenotyped for markers of cytotoxic T lymphocytes by flow cytometry.

Results: 10,000-30,000 OX40+CD39neg Ag-specific CD4+ T cells were isolated by cell sorting. 3-10% of purified Ag-specific cells were transduced with GFP, as measured by fluorescence microscopy. Autologous monocyte-derived dendritic cells (Mo-DC) gave best overall results as feeder cells during expansion with IL-2, and 4-6 weeks was required to obtain ≥ 2 million GFP+ Ag-specific CD4+ T cells. Using flow cytometry, GFP+ CD4+ T cells were CCR5-negative, and had a Granzyme B+ cytotoxic phenotype.

Conclusion: Using the OX40 assay, Ag-specific CD4+ T cells can be isolated for genetic modification of CCR5 expression via stable transduction with lentiviral shRNA. Cell therapy with in vitro expanded Ag-specific T cells, such as for EBV and CMV, has proved beneficial following hematopoietic stem cell transplant. Our results suggest that CCR5-negative Ag-specific cytotoxic CD4+ T cells may also be produced in vitro for possible augmentation of immunity, including to HIV-1 itself, in HIV+ subjects.

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