Human antitumor CD8$^+$ T cells producing Th1-polycytokines show superior antigen sensitivity and tumor recognition

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Broad application of TCR gene therapy will require a large repertoire of TCRs with high affinity and specificity for various tumor entities. Several approaches can be used to identify suitable TCR. We concentrated on RNA-loaded dendritic cells as antigen-presenting cells to prime T cells in vitro to different peptide-MHC (pMHC) epitopes. Regardless of approach, many T cell clones must be analyzed in a time-consuming and costly process, which is further complicated by variations in proliferation and survival of individual clones. Therefore we wished to identify functional or MHC-multimer binding parameters that would allow for rapid selection of cytotoxic T lymphocyte (CTL) clones with suitable characteristics. To define suitable parameters, twelve antitumor CD8$^+$ CTL clones of identical specificity but varying functional avidity for an identical pMHC ligand of the melanoma-associated antigen tyrosinase were compared in detail. Stronger TCR-pMHC interactions are reflected by greater capacity to bind MHC multimers and slower loss of bound multimers, thus these parameters seemed suitable to identify high-avidity CTL. Surprisingly, however, large disparities were found between CTL multimer binding, peptide sensitivity and tumor recognition, showing that with current technologies this was not a suitable parameter. In contrast, CD8$^+$ CTL with superior antigen sensitivity and tumor recognition were found to simultaneously secrete the CD4-associated T helper 1 (Th1) cytokines IFN-γ, IL-2 and TNF-α. Lower-avidity CTL did not show Th1-polycytokine production. Furthermore, high antigen sensitivity, superior tumor recognition as well as capacity for Th1-polycytokine secretion were transferred to

recipient lymphocytes using the TCR sequence of a high-avidity CTL clone. Thus, Th1-polycytokine secretion served as a suitable parameter to perform preliminary screening of clones to rapidly identify and select optimal CTL clones for further TCR evaluation.