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

Susanne Wilde\(^1\), Daniel Sommermeyer\(^2\), Matthias Leisegang\(^2\), Bernhard Frankenberger\(^1\), Barbara Moetter\(^1\), Wolfgang Uckert\(^2,3\) and Dolores J. Schendel\(^1,4\)

\(^1\)Institute of Molecular Immunology, Helmholtz Zentrum München, German Research Center for Environmental Health, D-81377 Munich, Germany

\(^2\)Max-Delbrück-Center for Molecular Medicine, D-13125 Berlin, Germany

\(^3\)Institute of Biology, Humboldt-University, D-10115 Berlin, Germany

\(^4\)Clinical Cooperation Group ‘Immune Monitoring’, Helmholtz Zentrum München, German Research Center for Environmental Health, D-81377 Munich, Germany

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-\(\gamma\), IL-2 and TNF-\(\alpha\). 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.