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Analysis of human T cell responses by using recombinant peptide-loaded MHC class I molecules

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The restimulation and *ex vivo* expansion of antigen-specific cytotoxic T cells from patients is still fraught with a number of severe technical problems, however, it is a most desirable goal in the light of impaired anti-tumor responses in many cancer patients or impaired anti-viral responses in immunosuppressed transplantation patients. In this study, we have developed novel tools for the antigen-specific stimulation and expansion of $CD8^+$ human T cells. We have engineered a panel of membrane-bound recombinant MHC class I molecules in which the ectodomain of HLA-A2 was covalently linked with human β_2-microglobulin through a flexible spacer, and β_2-microglobulin through another spacer with a peptide sequence known to bind to HLA-A2 and to be recognized by cytotoxic T cells. We incorporated several HLA-A2-restricted peptide epitopes derived from viral antigens and from tumor-associated antigens into such recombinant single-chain MHC class I trimers, termed pβA2. The covalent linkage of a peptide sequence to a MHC class I molecule enables the preferential binding and presentation of this *cis*-conjugated peptide epitope to T cells. Membrane-bound pβA2 were expressed on the surface of the human B-lymphoblastoid cell line C1R and successfully used for the activation of human T cells purified from the peripheral blood of healthy donors and cancer patients. Upon coculture with pβA2-bearing C1R transfectants, $CD8^+$ human T cells secreted IFN-γ which was detected on the single cell level by the IFN-γ capture assay. In $CD8^+$ T cells from a number of healthy blood donors, we often not only observed strong IFN-γ responses towards viral recall antigens from HCMV or EBV but also sporadic responses towards different tumor-associated self antigens (e.g., HER-2/neu, MUC-1, CEA, p53, NY-ESO-1) and HCV-derived epitopes. A larger subset of INF-γ secreting $CD8^+$ expressed the

memory T cell marker CD45RO. In addition to pβA2-transfected C1R cells, the human peptide transporter-deficient cell line T2 could be used for the activation of freshly isolated $CD8^+$ T cells after loading of T2 cells with synthetic HLA-A2 binding peptides. Activation of $CD8^+$ T cells from the same donor by pβA2-transfected C1R or peptide-loaded T2 cells yielded comparable results for most epitopes studied in IFN-γ capture assays. Using $CD8^+$ T cells purified from three cancer patients, we performed a comparative analysis using the IFN-γ capture assay with C1R/pβA2 transfectants one day after T cell purification on the one hand, and ELISPOT assays performed one week later using peptide-pulsed, GM-CSF matured, autologous dendritic cells as antigen presenting cells. For most T cell epitopes comparable results were obtained in terms of specificity and sensitivity, although for some epitopes differences were observed as well taht require further investigation. In conclusion, using C1R cells expressing recombinant MHC class I molecules covalently linked with a peptide epitope sequence of choice we established a novel system for a versatile and rapid restimulation of polyclonal memory T cells *ex vivo* which could be used in the clinical setting without the need of a time-consuming generation of autologous dendritic cells.

In a second approach we produced the same pβA2 molecules in a soluble, dimeric form by fusing the ectodomain of HLA-A2 to the Fc portion of human IgG1 or murine IgG2a. Such pβA2Fc proteins were purified from supernatants of transiently transfected HEK293T cells. After binding to magnetic beads or coating to tissue culture plates, recombinant pβA2Fc proteins as well as peptide-free βA2Fc molecules charged with synthetic peptides were successfully used for the selection of T cells, some of which could be expanded to T cell lines by repeated stimulation with peptides and interleukins. After further improvement of production yields and efficiencies of T cell binding *in vitro*, various pβA2Fc dimers developed by us could become versatile and comparatively inexpensive tools for future routine diagnostics of antigen-specific cytotoxic T cells from blood or tissues for which presently only MHC class I tetramers are available.