A simplified procedure for the preparation of MHC/peptide tetramers: chemical biotinylation of an unpaired cysteine engineered at the C-terminus of MHC-I

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Abstract

Recently, a powerful approach for the detection of MHC/peptide-specific T cells has been made possible by the engineering of soluble-tetrameric MHC/peptide complexes, consisting of singly biotinylated MHC/peptide molecules bound to fluorescent-labeled streptavidin. These tetrameric molecules are thought to compensate for the low affinity and relative fast dissociation rate of the TCR/MHC-peptide interaction by increasing the avidity of this interaction, thus allowing the stable binding of MHC/peptide tetramers to TCR expressing cells. Here we describe a new more simplified procedure for obtaining MHC/peptide tetramers using the well-characterized H-2K(b)/VSV system. This procedure consists of the incorporation of an unpaired cysteine residue at the C-terminus of the H-2K(b) molecule, allowing site-specific biotinylation by a -SH-specific biotinylating reagent. The H-2K(b)/VSV tetramers bound only to hybridomas expressing H-2K(b)/VSV-specific TCRs. When coated on a plate, these tetramers were able to induce IL-2 release by those hybridomas. Furthermore, H-2K(b)/VSV tetramers bound to CTL populations obtained from mice immunized with VSV-peptide. The specificity of the binding was further refined by studying cross-recognition of VSV by CTL populations obtained from mice immunized with single amino acid substituted VSV peptide variants. H-2K(b)/VSV tetramers bound only to those CTL populations that cross-reacted with the wild-type VSV peptide. Our method provides a simple, efficient and inexpensive procedure for making MHC/peptide tetramers, a highly specific and very useful reagent with a number of important applications in basic and clinical T cell research.