Quantitation of MHC tetramer-positive cells from whole blood: evaluation of a single-platform, six-parameter flow cytometric method.

BACKGROUND: Quantitation of antigen-specific T cells provides an insight into the development and dynamics of T-cell responses in tumor immunology and infectious diseases. Soluble major histocompatibility class I tetramers are widely used to monitor immune responses; however, variations due to handling and analysis are likely to confound comparisons between different experiments and laboratories.

METHODS: Whole blood from healthy donors was stained with HLA-A*0201/tetramers specific for an epitope of phosphoprotein 65, the immunodominant antigen in cytomegalovirus infection. With the help of Trucount tubes, a single-platform, four-color flow cytometric assay was established to obtain absolute counts of tetramer-positive cells. Various staining and gating strategies were evaluated. RESULTS: The no-wash method was a quick and straightforward procedure for the quantitation of tetramer-positive events from whole blood. The level for background staining was low. This information about the intra-assay-related variation and the physiologic variation will allow validation and interpretation of data in future studies. CONCLUSIONS: The method is highly reliable and can be standardized for multiple experiments. It is therefore suitable for the direct ex vivo analysis of antigen-specific T cells in a variety of clinical settings.
such as infectious, autoimmune, or neoplastic diseases and can be implemented as a tool for multicenter studies.