Dissection of melanoma-specific T-cell immunity by use of combinatorial encoding of MHC multimers

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Background. Fluorescently labeled multimeric complexes of peptide-MHC, the molecular entities recognized by the T-cell receptor, have become essential reagents for detection of antigen-specific $CD8^+$ T cells by flow cytometry. Here, we present a method for "high-throughput" parallel detection of antigen-specific T cells by combinatorial encoding of MHC multimers, and we demonstrate its use in a large scale T-cell screening project.
Tumor-infiltrating lymphocytes isolated from patients with metastatic melanoma and expanded *in vitro* using high-dose IL-2 have been used for adoptive transfer with impressive clinical results; however, the antigen specificities of the T cells in these cellular products have not been determined. Using a compilation of all described melanoma-associated antigens and the combinatorial encoding of MHC multimers technology, we dissected the composition of melanoma-restricted T-cell responses in tumor-infiltrating lymphocyte cultures. With previous technologies, it has not been possible to make a screen of this magnitude; however, this innovative technology has enabled large screenings of antigen-specific T-cell responses.

Methods. Peptide-MHC complexes are produced by UV-mediated MHC peptide exchange, where a UV-sensitive ligand is exchanged with a peptide of choice. Peptide-MHC complexes are multimerized in the form of streptavidin-fluorochrome conjugates. Eight different fluorochromes are used for generation of MHC multimers (PE, APC, PE-Cy7, quantum dot (Qdot)585, Qdot605, Qdot625, Qdot655 and Qdot705) and, by a two-dimensional combinatorial matrix, these eight fluorochromes are combined to generate 28 unique two-color codes. By use of combinatorial encoding, 27 different T-cell populations can be detected in a single sample. The sensitivity of the method is as low as 0.002% of $CD8^+$ T cells, due to a very strict gating strategy.
Other flow cytometry-based methods were used for further T-cell characterization.

Results. Using this platform, we screened 63 tumor-infiltrating lymphocyte cultures for T-cell reactivity against 175 melanoma-associated epitopes and detected in total 90 responses against 18 different epitopes—predominantly from differentiation antigen origin. Notably, the majority of these responses were of low frequency. We observed a large variation in the T-cell specificities detected in cultures established from different fragments of resected melanoma lesions. Finally, it was evident that although these defined T-cell populations contributed to tumor recognition, the majority of the tumor-reactive TILs remained unspecified.

Conclusions. We hereby present a tool to dissect the melanoma-restricted reactivity in tumor infiltrating lymphocytes that can be used also post-transfer to dissect T-cell responses associated with clinical efficacy. The technology can also be used for T-cell epitope discovery, and for the monitoring of $CD8^+$ immune responses during cancer and infectious disease, or following immunotherapy.