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Immunological monitoring: Lessons from natural and vaccine-induced responses to Melan-A/MART-1

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Abstract

It is widely accepted that cancer immunotherapy will be most effective in patients with early disease or minimal residual disease. The initial evaluation of various vaccine-based strategies in such patients cannot rely on usual clinical end points, such as tumor regression or time to recurrence, because of the number of patients and years required to achieve meaningful results. Following the identification of T cell-defined tumor antigens, it has been proposed to use immunologic assays that enable quantitative and qualitative monitoring of antigen-specific T-cell responses for the evaluation of new immunotherapeutic strategies in phase I clinical trials. Recent advances in immunological monitoring now allows direct detection and functional characterization of antigen-specific T cells. In particular, the use of soluble, fluorescently labeled HLA class I/peptide complexes, commonly referred to as tetramers or multimers, allows direct enumeration by flow cytometry of antigen-specific CD8+ T cells provided their frequencies are >1 in 10,000 cells. Moreover, by combining tetramers with appropriate antibodies, it is possible to obtain a direct assessment of the surface and functional phenotype of antigen-specific CD8+ T cells at the single cell level. Finally, following tetramer-based cell sorting of antigen-specific CD8+ T cells, further characterization, including T-cell receptor usage, functional potential and assessment of proliferative history, can be carried out in the absence of irrelevant cells.

In the past few years, our laboratory has applied these assays to assess the number as well as the quality of CD8+ T cells directed against the melanocyte/melanoma differentiation antigen Melan-A/MART-1 in HLA-A2 positive melanoma patients before and after peptide-based vaccination. In contrast to other tumor antigens, Melan-A has two features that makes this antigen a unique model to monitor antigen-specific T-cell responses: (i) it contains a single immunodominant peptide presented by HLA-A2, and (ii) the majority of HLA-A2 healthy individuals exhibit a relatively high number (up to 1 in 1,000 cells) of circulating CD8+ T cells that stain specifically with HLA-A2/Melan peptide multimers. Remarkably, this high frequency is not generated through peripheral T cell expansion, but reflects the thymic output of a high number of specific T cells that are maintained in a naïve state throughout life. In contrast, although the mean frequency of Melan-A multimer+ T cells in peripheral blood of HLA-A2 melanoma patients is not significantly different from that found in healthy individuals, a distinct, albeit variable, proportion of these cells display an activated/memory phenotype. In addition, in such patients, the frequency of Melan-A multimer+ T cells in metastatic lymph nodes is very high (up to 1 in 10 CD8+ T cells) and close to 100% of these cells exhibit an activated/memory phenotype, thus demonstrating that strong tumor antigen-specific responses may naturally occur in cancer patients.

Based on these findings, we have recently applied the assays mentioned above to the longitudinal analysis of Melan-A specific CD8+ T cells in melanoma patients enrolled in Melan-A peptide-based vaccine trials. The contrasting results obtained in two patients, which illustrate the usefulness of incorporating detailed immunological monitoring in clinical trials of antigen-specific immunotherapy, will be discussed.

References

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