Cancer Immunity, Vol. 3 Suppl. 1, p. 15 (6 February 2003)

Comprehensive approaches to monitor CD8+ T-cell responses against tumor antigen NY-ESO-1

Sacha Gnjatic

Ludwig Institute for Cancer Research, New York, NY

Abstract

The development of tumor immunology and of its vaccine applications depends on how good our knowledge of immune responses to tumor antigens is. Throughout more than a decade, the identification of short peptides from tumor antigens presented on HLA class I molecules to CD8+ T cells has been fostering critical advances. Today, it is possible to use comprehensive monitoring approaches, without having to define specific peptides or being restricted by particular HLA context, by taking advantage of naturally processed epitopes. Examples below explain how to rely on antigen presenting cells (APCs) to select T cell epitopes from NY-ESO-1, our model tumor antigen, provided in different forms.

NY-ESO-1 recombinant adenovirus or vaccinia virus can be used to transduce APCs, which in turn process and present peptides from NY-ESO-1 as a result of interaction with polymorphic molecules (TAP to HLA) specific to each individual. When used to stimulate CD8+ T cells, these transduced APCs can recall pre-existing memory responses and expand NY-ESO-1 repertoire for facilitated analysis.

Another approach consists of using recombinant soluble protein as a source of antigen. One obstacle to overcome is that proteins, when given exogenously, do not typically enter the HLA class I presentation pathway, except in dendritic cells (DCs) in a process known as cross-presentation. We compared the abilities of human monocyte-derived DCs and DCs derived *in vitro* from CD34-positive stem cells to present NY-ESO-1 epitopes to MHC class I-restricted CD8+ T cells. Although monocyte-derived DCs did not efficiently cross-present free NY-ESO-1 protein, IgG-immune complexes containing NY-ESO-1 were avidly presented after uptake by Fcgamma receptors. In contrast, CD34-derived DCs were unable to process either soluble or immune complexed NY-ESO-1, although they efficiently presented preprocessed NY-ESO-1 peptides. This difference did not necessarily correlate with endocytic capacity. Thus, when conditions are gathered for cross-presentation, uptake of antigen in complex with specific antibody is a reliable way to detect antigen-specific CD8+ T-cell responses in any HLA context.

Finally, long peptides, or polypeptides, are an alternative for full-length antigens while still useful in a general strategy for monitoring T-cell responses. To address the antigenicity of long peptides, we analyzed two synthetic 30-mer peptides from NY-ESO-1, polypeptides 80-109 and 145-174, for their capacity to be processed by APCs and to stimulate CD8+ T cells. By incubating APCs with polypeptides at different temperatures or in the presence of protease inhibitors, we found that NY-ESO-1 polypeptides were rapidly internalized by B cells, T2 cells, or PBLs, and submitted to cellular proteolytic action to yield nonamer epitopes presented by HLA class I. Polypeptides were also immunogenic *in vitro* and stimulated the expansion of CD8+ T cells against naturally-

processed NY-ESO-1 epitopes in the context of 3 different HLA class I alleles. Polypeptides can thus serve as exogenous antigens that are cross-presented on HLA class I without requiring the action of professional antigen presenting cells.

In conclusion, when T-cell epitopes are not strictly defined, comprehensive strategies with full-length antigen or polypeptides may give us information on the naturally selected repertoire to a tumor antigen and guide us in our search for more effective vaccination approaches.