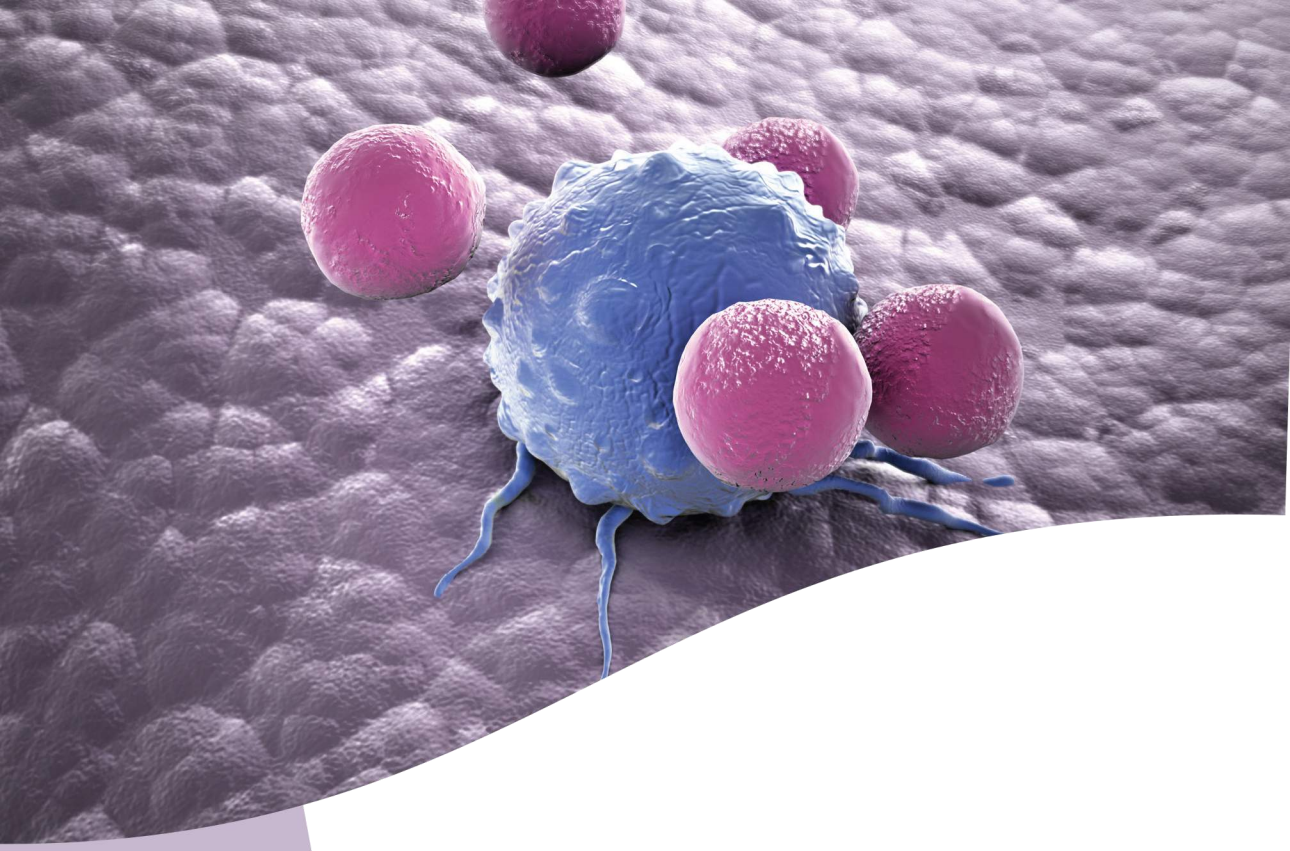

Immune monitoring in

Cancer Research

MABTECH

Despite much progress, the development of new and improved cancer therapies remains one of the greatest medical challenges. In addition to the continuous refinement of already well-established treatments with surgery, radiation and chemotherapy, immunological approaches have been gaining in interest and efficacy and are becoming important alternatives or adjuncts to conventional therapy for several types of tumors. These strategies all rely on the existence of specific antigens selectively expressed or strongly upregulated in the tumors compared to normal tissue. Antigens such as these are referred to as tumor-specific or tumor-associated antigens and can serve as targets both for antibodies and cell-mediated cytotoxicity.

Vaccine monitoring

Due to its excellent properties, the ELISpot assay has become widely used for monitoring vaccine-induced T-cell responses to a variety of antigens with demonstrated overexpression in several types of tumors (e.g. MAGE, NY-ESO-1, Gp100, PSA, tyrosinase and survivin). An example of this is shown in Fig. 1 where induction of antigen-specific T cells were revealed in an IFN-γ ELISpot following vaccination with the tumor-associated antigen, survivin. Given its high sensitivity, it has also been possible to detect T-cell responses to several of these antigens in tumor patients prior to vaccination and, by screening against individual peptides, define the most immunogenic epitopes.

Methods of tumor-targeted immunotherapy

- Administration of tumor reactive antibodies conjugated with e.g. toxins
- Therapeutic vaccination with tumor antigens
- Adoptive transfer of *in vitro*-expanded tumor-infiltrating lymphocytes (TIL)
- Blocking/breaking of immunological tolerance to "release" existing or vaccine-induced anti-tumor responses

Sensitive detection of tumor-reactive T cells

As a consequence of antigen recognition, T cells will be stimulated to produce cytokines and to express activation markers. This induction of cytokines is made use of in the ELISpot assay, which captures and detects secreted cytokine from individual cells. Capable of detecting a few activated cells in 100,000, the ELISpot has been shown to be one of the most sensitive methods making it ideal for studying tumor-specific T cells, which are typically found in very low frequencies. The more recently developed FluoroSpot assay builds on the same principle as the ELISpot but by utilizing fluorescent detection it has the capacity to detect several cytokines simultaneously. This allows for a better characterization of the T cells involved and requires fewer cells.

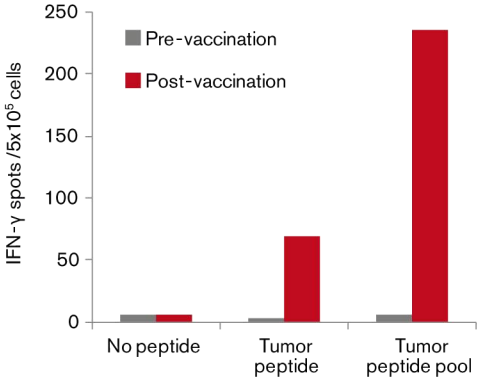

Figure 1. Example data from a tumor patient vaccinated with a pool of five peptides derived from the tumor-associated antigen survivin. The figure shows IFN-γ ELISpot responses by PBMC (5x10^5 cells/well) to a single tumor peptide and to the tumor peptide pool. PBMC were drawn before vaccination and after onset of therapy. Data kindly provided by Dr. V. Lennerz, Johannes Gutenberg University, Mainz, Germany.

Furthermore, ELISpot screening for T-cell reactivity against peptides eluted from the MHC molecules of donors' tumor cells has allowed the identification of new target antigens potentially suitable for development of new vaccines.

In the great majority of these studies, IFN-γ has been used as the marker for T-cell activation. However, analytes like Granzyme B and Perforin, expected to better reflect the killing capacity of the T cells, have also been employed successfully. With the introduction of the FluoroSpot assay, it has become possible to more effectively investigate the cytokine-secreting profiles of the tumor-directed T cells and to analyze polyfunctional T cells (see Fig. 2). Given the "self" nature of many tumor antigens, there is also an interest to investigate regulatory T cells characterized by their secretion of cytokines such as IL-10 and TGF-β.

Tumor-primed T cells

The ELISpot and FluoroSpot assays can also be used to measure and characterize tumor-reactive T cells in several other situations. These include the analysis of tumor-specific T cells in *in vitro*-expanded TIL cultures and the monitoring of T-effector cells in patients treated with the anti-CTLA-4 antibody, Ipilimumab. By blocking the activation of regulatory T cells this antibody can help enhance both naturally existing T cells with tumor specificity as well as those induced by vaccination.

Other immune cells

Although T cells are believed to be the primary effector cells in the defense against tumors, other cells play pivotal roles and may, like T cells, be investigated with the ELISpot and FluoroSpot techniques. Thus, secretion of anti-tumor specific antibodies can be assessed at the level of individual B cells and the cytokine profiles of dendritic and other antigen-presenting cells, critical for the activation of T cells, can be determined. Given the role of these latter cells in regulating the magnitude and types of T cells generated, this type of functional phenotyping may be of great use and importance both in conventional vaccination and particularly with vaccines based on the administration of tumor antigen-loaded dendritic cells.

ELISpot and FluoroSpot in Cancer Research

- Detection and analysis of tumor-specific T cells
- Identification and epitope mapping of tumor antigens
- Monitoring of vaccine-induced T- and B-cell responses

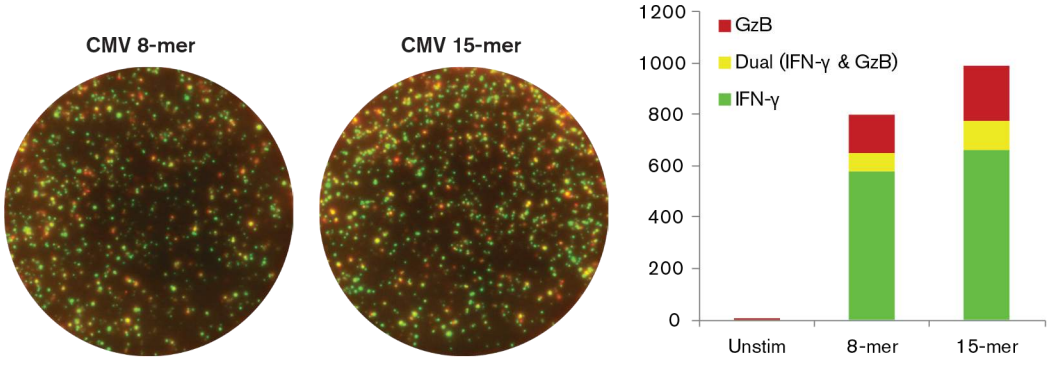

Figure 2. FluoroSpot analysis of IFN-γ and Granzyme B secretion in human PBMC (125,000 cells/well) treated with two different CMV peptides for 40h.

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