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Vaccination with T cell-defined antigens: biological basis and clinical applications

Giorgio Parmiani
Unit of Immunotherapy of Human Tumors, Istituto Nazionale Tumori, Milan (Italy)

The extraordinary development in the molecular characterization of tumor antigens recognized by T cells, both in MHC class I- and class II-restriction, is providing a large array of potential vaccines to be tested in the clinics. Several peptide antigens, particularly in melanoma, have been used to immunize metastatic patients after in vitro evaluation of their immunogenicity, as detected by induction of cytotoxic T cells from stimulated peripheral blood lymphocytes (PBL) of cancer patients. We have recently identified new antigens/epitopes in melanoma, like the differentiation antigens gp100/Cw8, TRP-2/Cw8, gp100/A3 and the melanoma-specific antigens TRP2-INT2/A68. They were tested for in vitro immunogenicity and in vivo recognition by tetramers staining of patients' PBL. High frequency of CTL precursors to these antigens was found in blood of 30-40% of patients. However, an analysis of anti-MART1 CTL distribution in vivo in untreated metastatic stage III patients suggested that, even when infiltrate metastatic lesions, T cells were unable to significantly destroy tumor cells.
The first generation of peptide-based trials in melanoma, colon and prostate cancer has provided conflicting results. In fact, while in some studies significant and durable clinical responses were observed (e.g. with the MAGE-3.A1 peptide in melanoma patients), other trials resulted in only 10-30% response rate, despite the use of autologous, peptide or tumor lysate pulsed dendritic cells to vaccinate patients.
More recently heat-shock proteins, which can bind a full repertoire of epitopes present in cancer cells, have been used in clinical trials of metastatic patients. We have vaccinated metastatic melanoma patients with autologous, HSPPC-96 (Oncophage) and obtained an increased anti-melanoma specific T cell response in 50% of patients that was associated with a clinical response. Results of this vaccination trial will be discussed.

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Rolf Kiessling, Jan-Alvar Lindencrona, Jehad Charo, Christina Hising, Giuseppe Masucci, Max Petersson, Jonas Bergh, Lars Franksson.
Department of Oncology, Karolinska institutet.

Title; Specific vaccination based on the Her2/neu proto-oncogene.

The HER-2/neu (HER-2) proto-oncogene product is overexpressed in various human cancers, associated with poor prognosis. Recent results in clinical studies based on the humanized anti-HER-2 antibody have shown promising results. Our efforts to design new therapeutic strategies targeting HER-2 expressing tumors will be discussed, focusing on T cell based immunotherapy. HER-2 derived peptide epitopes with high affinity for MHC class I have been characterized and tested for their ability to induce cytotoxic T –cell responses which also can recognize HER-2 overexpressing tumors. Plasmid DNA (pDNA) constructs based on the full-size HER-2 gene or on a "string-of-beads" construct derived from mini-genes coding for known HER-2 epitopes are being tested in HLA A2 transgenic mice, in combination with cytokine genes and co-stimulatory molecules. Two Phase I clinical trials in patients with advanced ovarian and breast cancer are being initiated, one based on 3 different CTL epitopes and the other on a full-length HER-2 pDNA construct, both in combination with GM-CSF and IL-2.

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Applications of CpG oligonucleotides in cancer therapy

Gunther Hartmann, MD
Department of Internal Medicine, University of Munich, Germany

In 1890, the New York surgeon William B. Coley learned of a case of spontaneous regression of metastatic sarcoma following infection of the tumor margin. Now it is known that DNA is critical for the anti-tumor activity of bacterial extracts. Bacterial DNA is detected by the vertebrate immune system based on the presence of unmethylated CG dinucleotides within certain flanking bases (CpG motifs). CpG oligonucleotides (CpG ODN) provide a potent stimulus for dendritic cells and thus represent strong immune adjuvants which promote the development of a Th1 response. CpG ODN improve protein and peptide cancer vaccines including dendritic cell-based strategies. Furthermore, CpG ODN potentiate the therapeutic effect of anti-tumor antibodies and upregulate target proteins such as CD20 in human B cell malignancies. In a first clinical trial, CpG ODN demonstrated excellent adjuvant characteristics to promote antibody production induced by a hepatitis B vaccine. CpG ODN are currently under clinical develop-ment for immunotherapy of cancer. A novel type of CpG-containing sequences is characterized by the induction of large amounts of type I interferon (IFN-α) and thus mimicks viral infection. CpG-induced IFN-α is produced by a rare cell population in peripheral blood, the plasmacytoid dendritic cell. IFN-α-inducing CpG ODN demonstrate profound effects on innate and acquired immunity. Therapeutic strategies which include CpG ODN with distinct sets of biological activities may open new avenues in the field of cancer immunotherapy.

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IDENTIFICATION OF NEW IMMUNOGENIC ANTIGENS IN ACUTE AND CHRONIC MYELOID LEUKEMIAS (AML / CML) FOR THE DEVELOPMENT OF VACCINES

Michael Schmitt, University of Ulm, 3rd Dept Internal Medicine, Ulm, Germany.

For the development of immunotherapies for cancer, identification of tumor/leukemia-associated antigens (TAA/LAA) as target structures is a pivotal step. The method of serological screening of cDNA expression libraries (SEREX) has been employed to characterize TAA in solid tumors. We used SEREX for AML and CML to identify LAA. Serological screening of expression libraries prepared from a patient with AML and from the CML cell line K562 yielded different immunogenic antigens: 1) Receptor for hyaluronic acid mediated motility (RHAMM) mediates migration, transformation and metastatic spread of fibroblasts. 8/19 (42%) of AML and 3/10 (30%) of CML patients, whereas 0/12 of healthy volunteers showed positive serological reactions. 12/29 (41 %) of AML and 5/10 (50%) of CML samples showed high mRNA expression, but neither 12 PBMN samples nor eight CD34 separated cell samples from healthy volunteers. In western blot analysis, patients with mRNA expression of RHAMM were also positive on the protein level, but none of the healthy volunteers. 2) Positive serum reactivity to a gene encoding the c-myc associated zinc finger (MAZ) protein, was found in 35% of AML patients, 40% of CML patients and 25% of healthy volunteers. 15/34 (44%) of AML patients, but only 8% of controls showed high mRNA expression of MAZ in RT-PCR. Newly characterized immunogenic antigens like RHAMM, MAZ or MPP11 are potential targets for vaccination therapies in AML or CML.