IDENTIFICATION OF NEW TUMOR ASSOCIATED ANTIGENS AND THEIR USAGE FOR NEW THERAPEUTIC STRATEGIES BASED ON THE COMBINATION OF CHEMOTHERAPY AND IMMUNOTHERAPY FOR COLORECTAL CANCER PATIENTS

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The main general objective of this project was to characterize a new colorectal carcinoma (CRC) tumor-associated antigen (TAA) and validate a new therapeutic strategy combining chemotherapy and tumor vaccination for the treatment of cancer patients. To this purpose a strategic interaction between Drs. Proietti/Maccali at the ISS and the group of Drs. Rosenberg/Robbins at the NIH was established. A stage of Dr. Maccalli at the NIH allowed to carry out the first steps for the identification and the initial characterization of the CRC TAA named COA-1. A laboratory meeting with Dr. Robbins has been planned on May 24-25 2006 at the ISS, during the International Meeting on Immunotherapy of Cancer: Challenges and Needs, for discussing results and perspectives of this research project.

The specific objectives and the main results of the project are summarized below.

Pre-clinical studies have been set-up to verify the immunogenic potency of the new TAA COA; *in vitro* stimulation of PBMCs from DRβ1*0402+ or *1301+ CRC patients with the COA-1 derived HLA class II-restricted epitope have been carried out. COA-1 and tumor specific CD4+ T cells could be isolated only from patients with progressive disease (Duke's stage C and D) but not from patients with early stage tumor (Dukes' A and B) thus indicating that COA-1 is a relevant antigen for the anti-tumor immune response in CRC patients correlating with the progression of the disease.

Further results proved that COA-1 can represent an immunodominant antigen mediating an anti-tumor immune response in CRC patients. The laser scanning by confocal microscopy analysis, carried out on a panel of normal and tumor cell lines by using a specific polyclonal antibody directed to COA-1, showed a differential pattern of cellular distribution and of association with members of the cytoskeleton between normal and tumour cells. Furthermore, different mechanisms of cellular processing and trafficking of this TAA have been demonstrated in normal or tumour cell lines. The production of COA-1-specific monoclonal antibodies and of a recombinant-purified COA-1 protein has been essential to set up specific assays for the identification of the presence of COA-1 antigen and for the detection of natural anti COA-1 antibodies in CRC patients sera for predictive and diagnostic purposes. To this aim the collection of CRC patients samples is ongoing to perform extensive serological studies.

The second part of this project have been focused on the definition of the parameters crucial for combining chemotherapy with a peptide vaccine (mimicking relevant TAA) to achieve a secondary prevention against tumor recurrence in tumor resected patients. To this purpose, preclinical studies in murine models have been done to show the importance of chemotherapy treatment before tumor-immune lymphocyte administration in activating tumor-specific cytotoxic T cell expansion and migration into the tumor tissue. Real time PCR (RT-PCR) analyses have been extensively performed to characterize the cytokines induced by

cyclophosphamide administration in adoptively transferred mice, and their results showed an involvement of common gamma chain cytokines as well as hemopoietic factors which were produced shortly after chemotherapy administration causing a strong activation of tumor vaccine-induced immune responses. On the basis of these preclinical results, a clinical trial was set up to demonstrate the ability of a chemotherapeutic drug to enhance a tumor-specific immune response in patients treated with a tumor vaccine. This study, performed in melanoma patients, as a first model for a well antigenically characterized tumor, combined a standard dacarbazine (DTIC, a cyclophosphamide analogue) treatment with the vaccination with melanoma peptides (Melan A and gp 100) in association with Montanide and interferon alpha. The results obtained in 10 patients (five patients treated with vaccine alone and five patients treated with vaccine + DTIC) showed that DTIC strongly increased the number and the cytolytic activity of tumor-specific T lymphocytes, thus demonstrating, for the first time, the need of combining chemotherapy with a tumor vaccine to increase antitumor immune responses. Further clinical studies have been planned to combine chemotherapy and antitumor vaccination in CRC patients in view of obtaining high CRC-specific T lymphocyte numbers to be used for the ultimate lymphocyte transfer after a non myeloablative conditioning regimen in the same CRC patients.

Publications of the project

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Patents

Robbins PF, Rosenberg SA, Maccalli C, inventors; New colorectal cancer-associated antigen (COA-1). ISS-NIH patent no. 60/512,040; International PCT/EP2004/12087. 15 Oct 2004.