CLINICAL TRIALS USING IFN-α AS A VACCINE ADJUVANT: NEW STRATEGIES FOR THE MOLECULAR MONITORING OF THE IMMUNE RESPONSE

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The main general objective of this project was to define immunotherapy protocols based on the new concept of using IFN-α as and immune adjuvant, developing innovative methodologies suitable for predicting and monitoring the immunological and clinical responses. To this purpose, a strategic interaction between Dr. Belardelli's group at the ISS and Dr. F. Marincola's laboratory at the NIH was established. To foster the interaction between the two groups, a researcher from the ISS (Dr. E. Aricò) spent two years in Dr. Marincola's laboratory at the NIH, with the specific aim of developing new microarrays technologies particularly suitable for a molecular tracking and prediction of the response to IFN of cytokine-treated patients.

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

– *Studies on gene expression profiles in the peripheral blood mononuclear cells (PBMCs) from patients treated with IFN-a*

  The first phase of the project was focused on applying microarray technology to the immunological monitoring of two clinical trials, promoted by the ISS group to evaluate the adjuvant activity of IFN-α. In particular, gene expression profiling analysis was carried out on PBMCs isolated 24 hours after the treatment from melanoma patients vaccinated with melanoma peptides (Di Pucchio *et al*., 2006) and healthy donors receiving the anti-HBV vaccine (Rizza *et al*., 2008). Of note, the results of the experiments, performed on the cDNA microarray platforms previously developed and validated in Dr Marincola's laboratory, revealed the presence of a signature of IFN-induced genes common to both the clinical settings, and including genes involved in immunological pathways, such as antigen processing and presentation, cytokine and chemokine activity. Our study provided a global description of the *in vivo* effects of IFN-α and its molecular signature, thus opening perspectives into the comprehension of the mechanisms of action and, possibly, into the identification of molecular markers of the clinical response to IFN. The gene expression data obtained with the microarray platform have been fully validated using more conventional molecular biology methods, such as TaqMan Real Time RT-PCR. A manuscript reporting these results is under preparation.

  The achievement of such a deep knowledge about the signature of IFNα *in vivo* represent an important tool that can be exploited to other clinical studies to investigate the possible involvement of IFNα1 in the mechanisms of action of other therapies or compounds. During this project, we took advantage of the database of genes known from the previous study to be modulated by IFNα in humans PBMCs to interpret microarray data obtained in the context of other clinical trials. In particular, in a study in which we used microarray analysis to explore the mechanisms of action of chemotherapy in the enhancement of the immune response to a peptide vaccine in melanoma patients (Nisticò et al., 2008, in press), the discovery of many Interferon-induced genes in patients PBMC suggested a possible role of the cytokine in the induction and sustainment of peptide-specific effector

memory cells observed after DTIC-vaccine administration. The same approach was applied to a blinded, randomized, placebo-controlled study to assess the role of IFNα in the rejection of Basal Cell Carcinoma after Imiquimod administration. In particular, the early transcriptional events induced by the Toll-like receptor-7 agonist were analyzed, and the list of modulated genes was matched with the database of previously characterized *in vivo* Interferon Stimulated Genes to evaluate the timing and the entity of the involvement of IFNα in the rejection process.

– *Development and validation of a SNP platform for cytokine and cytokine-related genes*
The two laboratories are also cooperating on the development of a new microarray platform for measuring single nucleotide polymorphisms (SNP) in several cytokine and cytokine receptors genes, with particular interest in the role of the SNP present in the genes encoding for IFNα and IFNα receptor, with the final aim of establishing a novel and validated strategy for predicting the response to IFNα using DNA samples from patients.
The platform has been designed and obtained in Dr. Marincola's laboratory, and its functional validation is being carried out in the ISS by Dr. Aricò in collaboration with other ISS researchers involved in microarray-based studies. At the moment, the group is making a big effort to identify an effective protocol for genomic DNA labeling and hybridization, due to many technical difficulties associated with handling such a complex genetic material, very likely to produce non-specific hybridization signals.
The discovery of functional SNP present in the IFN or other cytokine related genes and involved in the response to IFNα will be exploited to the SNP analysis on the DNA collected in the clinical trials mentioned above as well as in the context of new clinical trials based on the use of IFN-α in melanoma patients (which will be shortly activated in the context a collaborative effort promoted by the Italian Network of Bio-immunotherapy, NIBIT). The possible correlation between the presence of SNP and different patterns of gene expression profiles observed in different individuals after the administration of the IFN α will be also evaluated with the final aim of identifying molecular markers capable of predicting and monitoring the response to IFN.

Publications of the project

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