ImmunoTools *special* Award 2014

Lucia Signorini, PhD-student

Supervisor: Dott.ssa Serena Delbue

Department of Biomedical, Surgery and Dental Sciences. Via Pascal 36, 20133 Milan, Italy

Generation of tumor-specific cytotoxic T-lymphocytes from peripheral blood of colorectal cancer patients for adoptive T-cell transfer

Colorectal cancer (CRC) is the third most common cancer worldwide and the fourth most common cause of death in the developed Western countries. Adoptive T-cell transfer (ACT) refers to an immunotherapeutic approach in which anti-tumor T lymphocytes, usually the tumor infiltrating lymphocytes (TIL), are identified, grown ex vivo and then re-infused into the cancer patient. ACT of Epstein-Barr Virus (EBV)-specific T-cell lines and T Cytotoxic Lymphocytes (CTLs) for the therapy of EBV-induced lymphomas is the best demonstration of clinically efficacious ACT, but there are many evidences also for leukemia and multiple myeloma. As regards to the solid tumors, ACT using autologous TIL, grown ex-vivo and then re-infused into the cancer patient, has emerged as an effective treatment for metastatic melanoma and renal cell carcinoma (RCC), that are the most immunogenic tumors in humans. Randomized clinical trials are ongoing for gastric cancer, hepatocellular carcinoma and lung cancer. These approaches mainly use the TIL and the definition of tumor associated antigen (TAA), tumor specific antigen (TSA) or cancer testis antigen (CTA), that are generally correlated with tumor progression and immunogenicity in various types of cancer. However these antigens are often found to be poorly expressed in CRC, and few is known about their relationship with this type of neoplasia. In addition, although a clear association between TIL and clinical outcome of CRC has been documented, active and adoptive immunotherapy do not play yet an important role in the treatment of advanced CRC.

In order to develop an ACT protocol for CRC treatment, we will design an experimental approach that does not require neither the definition of molecular defined tumor antigens, nor the availability of TIL. Our strategy is based on the *in vitro* stimulation of patient's $CD8^+$-enriched T-cells from peripheral blood mononuclear cells (PBMCs) with dendritic cells (DCs), pulsed with apoptotic tumor cells as a source of tumor antigens, in order to generate autologous CTLs with strong anti-tumor activity.

- Tumor samples will be obtained at surgery, together with 100 ml of heparinized peripheral blood (PB). Tumor samples will be mechanically dissociated to a single-cell suspension and cultured to obtain primary tumor cell line from each patient.
- $CD8^+$ T-lymphocytes (T CD8) will be isolated from PBMC via positive immunomagnetic selection. $CD4^+$ T-lymphocytes (T CD4) cells isolation will start from the negative fraction of the cell type previously isolated. DCs will be generated from the negative fraction recovered from T CD4 cells isolation using a magnetic positive selection of $CD14^+$ monocytes that will be cultured in presence of recombinant human Interleukin-4 (rh IL-4) and recombinant human Granulocyte-Macrophage Colony-Stimulating Factor (rh GM-CSF).

Anti-tumor CTLs will be elicited using patient-derived DCs, irradiated (200 Gray) autologous apoptotic tumor cells, irradiated T CD4 and patient CD8-enriched lymphocytes as effectors, supplementing the medium culture with 10 ng/mL recombinant human interleukin-7 (rh IL-7) and 10 pg/mL recombinant human interleukin-12 (rh-IL-12). After one week, the co-cultures will be recovered and re-stimulated in the presence of approximately adherent irradiated autologous PBMCs, apoptotic tumor cells and a low dose of human interleukin-2 (rh IL-2) (10 U/mL). The same protocol will be repeated for a total of 4 to 5 stimulation cycles, using increasing amounts of rh IL-2 (up to 100 U/mL).

- Lymphocytes will be analyzed using anti-CD45, anti-CD3, anti-CD8 and anti-CD4 mAbs;
- Monocyte-DCs phenotype will be determined using anti-CD14, anti-CD1a, anti-CD11c and anti-HLA-DR mAbs. DC maturation will be evaluated using anti-CD80 and anti-CD86 mAbs.

Isotype control antibodies of irrelevant specificities will be included as negative controls.

- After UV-B irradiation, the percentage of apoptotic primary tumor cells, PBMCs and $CD4^+$ lymphocytes will be evaluated using FITC-conjugated annexin V and Propidium Iodide (PI).
- CTLs Interferon-γ (IFN-γ) secretion will be assessed by flow cytometry and will be confirmed by the human ELISA-set for IFN-γ to evaluate their activation in response to autologous tumor.

In conclusion, to our knowledge, our approach is the first to examine the possibility of exploiting PBMC as a source of tumor-specific CTLs for adoptive cell therapy of CRC and this method may be considered a strategy clinical option. We believe that our results support the possibility of developing a new immunotherapeutic approach for CRC and may be further developed for this tumor, as for other solid and hematopoietic cancers.