ImmunoTools *special* Award 2013

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Interleukin-15 transpresentation as attractive immunotherapeutic mechanism for the development and optimization of a dendritic cell-based vaccine against acute myeloid leukemia

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults. The primary treatment of *de novo* AML patients is intensive chemotherapy or 'small molecules' therapy and hematopoietic stem cell transplantation, resulting in a high rate of complete remission. However, approximately 75% of all patients relapse within two years, contributing to a five-year survival rate of less than 20%, which is even further decreased to 5% in patients older than 60 years. Relapse is caused by proliferation of residual cancer cells after primary treatment. Therefore, the challenge for AML therapy is to secure the complete remission condition of the patient and to prevent relapse by eliminating residual leukemia cells in an adjuvant setting.

This innovative basic research aims the *in vitro* generation and characterization of IL-15 transpresenting 'designer' DC to increase the immunostimulatory and antitumor effect of DC therapy as adjuvant cancer treatment. Hereby, we will study the cellular and molecular determinants of IL-15 trans-presentation by *in vitro* cultured DC. The focus is on maximizing the immunostimulatory properties of DC to raise both a NK-cell and a tumor-specific T-cell response. Our hypothesis is that DC loaded with IL-15 and IL-15Rα using mRNA electroporation, are able to induce a strong and effective NK-cell response that synergizes with the activation of adaptive immunity. Cotransfection of the DC with the leukemia-associated Wilms' tumor 1 (WT1) antigen, which is overexpressed in AML cells, has to ensure that also a strong WT1-specific T-cell response is generated.

In order to test this hypothesis, we will investigate the influence of IL-15 and/or IL-15Rα mRNA-transfected DC on (1) NK-cell activation with respect to their phenotype, cytotoxicity and cytokine production, (2) reciprocal NK-cell-mediated activation of DC, and (3) induction of autologous tumor-specific T cells in coculture systems. This strategy will be tested with cell material from healthy volunteers and

AML cell lines and in a later stage with cells from AML patients in remission. Our innovative strategy to obtain transpresentation of IL-15 by DC will be tested with two well-established DC preparation protocols, whereby autologous $CD14^+$ monocytes are *in vitro* cultured in the presence of recombinant human (rh) granulocyte macrophage colony-stimulating factor (GM-CSF) and rh IL-4 (i.e. IL-4 DC) or rh IL-15 (ImmunoTools) (i.e. IL-15 DC).

In detail, the first phase of this research project is the optimization of anti-human-IL-15 and anti-human-IL-15Rα antibody staining protocols for flow cytometric detection. Since the stable IL-15/IL-15Rα complex is expressed on the cell surface and within the cell, protocols for both membrane and intracellular staining will be optimized. In the second phase, IL-15 and IL-15Rα-mRNA is obtained after a T7 polymerase-based *in vitro*-transcription reaction. In the third phase, these mRNAs will be transfected by electroporation into mature monocyte-derived DC. The differentiation process requires rhGM-CSF and rh IL-4 or rh IL-15 (ImmunoTools), followed by a maturation step whereas the maturation cocktail contains rh IFN-γ (ImmunoTools). In the fourth phase, the IL-15 and/or IL-15Rα-transfected DC will be cocultured with autologous, purified $CD56^+$ NK cells, after which (1) the phenotypic activation profile of both DC (CD40, CD80, CD86, HLA-DR; ImmunoTools) and NK cells (CD7, CD16, CD56, CD69; ImmunoTools) will be determined by multiparametric flow cytometry analysis as compared to isotype control staining, and (2) the cytokine producing ability of both cell types is studied using ELISpot and/or ELISA, e.g. IL-12p40 ELISA (ImmunoTools). Furthermore, it will be examined whether the activation of the two cell types leads to a better killing of leukemia cells by NK cells/DC and an increased phagocytosis of leukemia cells by DC. Cell-mediated cytotoxicity and phagocytosis will be measured using a flow cytometry-based protocol that was optimized in our laboratory. In the fifth phase, we will check the *in vitro*-stimulation of WT1-specific T cells by IL-15/IL-15Rα-transfected and WT1-antigen loaded DC. In this context, detection of WT1-specific T-cell reactivity will be determined weekly by means of MHC class I tetramer analysis, antigen-specific cytokine production, upregulation of CD137 on $CD8^+$ T cells, and cytotoxicity in reaction to WT1-positive MHC-compatible target cells (tumor cell lines, and WT1-peptide-loaded T2 cells). To distinguish between $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, we will use CD3, CD4 and CD8 anti-human antibodies (ImmunoTools) and to promote their survival we use rh IL-2 (ImmunoTools).