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The study of the Activation of T lymphocytes and Natural Killer (NK) Cells after immunotherapy of cancer patients.

Cancer immunotherapy attempts to stimulate the immune system of cancer patients to reject and destroy tumors or metastasis. The effector functions of T- and NK (natural killer) cells are regulated by positive and negative signals integrated from multiple stimulatory and inhibitory receptors as well as soluble factors with we investigate before and after immunotherapy. NK cell receptor NKG2D (CD314) is a peculiar activating. In humans, NKG2D binds to MHC class I–related chain (MIC) A, MICB, and ULBP (UL16-binding proteins) whose expression is restricted or absent on normal tissues but is induced in situations of stress and disease. The expression of MICA/B has been described in multiple types of tumors. The MICA/B can be shed from tumor cells. Recent studies reveal that release of soluble MICA (sMICA) compromises NKG2D-dependent NK-cell cytotoxicity leading to tumor escape from immunosurveillance. NK cells and T-cells are involved in various biological processes in the organism; they play a critical role in immune surveillance and can be applied for cancer therapy. We use adoptive cell transfer uses T- and NK-cell-based cytotoxic responses to attack cancer cells. T- and NK-cells were generated in vitro with stimulatory cytokines (IL-2, IL-12, IL-15) and then transferred back into the 13 patients with metastatic colon cancer. IL-12 plays an important role in the activities of NK-cells and T lymphocytes. IL-12 mediates enhancement of the cytotoxic activity of NK-cells and $CD8^+$ cytotoxic T lymphocytes. There also seems to be a link between IL-2 and the signal transduction of IL-12 in NK cells. IL-2 stimulates the expression of two IL-12 receptors, IL-12R-β1 and IL-12R-β2, maintaining the expression of a critical protein involved in IL-12 signaling in NK-cells. Enhanced functional response is demonstrated by IFN-gamma production and killing of target cells. IL-2 is necessary for the growth, proliferation, and differentiation of T cells to become 'effector' T cells. IL-15 was found to be similar to IL-2. Both cytokines are able to facilitate production of immunoglobulins made by B cells and induce the differentiation and proliferation of NK-cells.

In our work we study the phenotypical characteristics, of specific type of cells mainly the NK cells ($CD16^+CD56^+$) and T lymphocytes (CD3, CD4, CD8, $CD4^+CD25^+$) are isolated from the peripheral blood of the 13 cancer patients before and 2-4 weeks after immunotherapy. Also we study the changing in expression of activator receptors on NK- and T-cells (CD25, CD38, CD68, HLA-DR, CD314).

After an immunotherapy at all patients initially low level of Treg (average 7.58%) and B-lymphocytes (average 5.15%) remained. The average quantity of NK-cells (decreased from

22.1% to 14.8%) was normalized, initially lowered absolute number of cytotoxic T-lymphocytes raised with 0.40 to 0.45x109/l. Other subpopulations of lymphocytes were in limits of referensny values though after an immunotherapy the increase in the average maintenance of a activation receptor of NKG2D ($CD314^+$) on all lymphocytes from 40.93% to 45.17%, insignificant decrease in the maintenance of the activated NK-cells ($CD16^+CD314^+$) in the blood from 16.47% to 10.25% and increase in the activated T-cells ($CD3^+$HLA-DR^+ on 1.56%) was observed. The relative and absolute number of T-, Th- and Tc-lymphocytes was within norm though initially lowered absolute maintenance of NKT-cells remained. The quantity of cages of $CD38^+$ increased on 4.3%, and $CD38^+CD3^+$ on 6.4%. In our future work we plan to study the physiological characteristics of cytotoxic lymphocytes after activation (production of IL-2, IL-6, FNO-alpha, IFN-gamma).

During research we found out that lymphocytes are well activated and proliferated in vitro. The immunotherapy the autological cytokine activated lymphocytes characterized by lack of side effects and can be recommended as a additional to basic radio- and chemotherapy, for the purpose of improvement of quality of life of patients. Modulating the balance between activating and inhibitory signals through NK- or T-cell receptors may open a new approach to cytotoxic lymphocytes-based biotherapy for cancer diseases.