ImmunoTools *special* Award 2013

Marizina Yulia, PhD student, Research Assistant

Supervisor: Prof. Dr. Romanko Yu.S

Federal state budget institution, Medical radiological center, Ministry of Health of the Russian Federation. Koroleva-4, Obninsk, Kaluga region, Russia, 249036

The study of phenotype of human lymphocytes before and after activation *in vitro*.

In connection with the development of molecular biology and immunology, a growing importance in the treatment of cancer immunotherapy method takes. In our study, we use the peripheral mononuclear cells isolated from the blood of cancer patients that activated *in vitro* by IL-2. It is known that IL-2 is widely used for the activation of both allogeneic and autologous lymphocytes. Lymphocytes are allocated by the standard method for Ficoll density gradient. The color of lymphocytes produce fluorescent dyes and evaluate the expression of surface markers on this for further work. Activated cells we injected intradermally back to cancer patients. The advantage of such a direct antitumor immunotherapy due to the action of activated killer cells by their ability to kill tumor cells.

In order to examine the change of surface activation markers on lymphocytes, it is necessary to analyze the phenotype of peripheral blood lymphocytes, and culture *in vitro* LAK-cell by flow cytometry. It can to help us to use activated lymphocytes for further studies and immunitherapy of cancer patients. We have developed a panel of markers for optimal estimation pool activated lymphocytes before and after *in vitro* culture. Phenotype was rated B-, T-, NKT-, NK-cells in peripheral blood and activator markers (HLA-DR, CD25, CD38, CD69, CD314) on T- and NK-cells.

To assess changes in expression of activator markers in lymphocytes after culture on day 3 was conducted phenotype of LAK-cells. Considering the average of the pool of lymphocytes increase on 10% in by the third day of cultivation can be concluded that the lymphocytes are activated and proliferate at the same time. Among LAK-cells predominate in patients with cancer of abdominal region significantly increased expression of CD314 (on 21%) and CD69 (on 40%) at all the lymphocytes including NK-cells (CD314+CD16+ - on 9%), and T-cells (CD69+CD3+ - on 26%, CD3+HLA-DR+ and CD3+CD38+ on 16%), somewhat less increased expression HLA-DR (on 11%) and CD25 (on 12%). The number of naive T-cells (RA+) decrease on 8% and increased the number of mature lymphocytes (RO+). The tendency to increase the activation markers was shown on T-and NK-cells of patients with melanoma and subpopulations of Tc-lymphocytes, NKT-cells and NK-cells. Significantly increased expression of CD314 (on 18 %), CD69 (on 46 %), HLA-DR

(on 26%) and CD38 (on 25%) at all the lymphocytes including NK-cells (CD314+CD16+ - on 7%), and T-cells (CD69+CD3+ - on 32%, CD3+HLA-DR+ and CD3+CD38+ - on 26%), somewhat less increased expression CD25 (on 8 %). The number of mature lymphocytes (CD45RO+) increased on 11%. We can conclude that all the values of the activation markers in the two groups of patients increased significantly. No change in the cell contents slightly change the number of activated NK-cells (CD314+CD16+, CD25+CD16+). In patients with melanoma was more pronounced activation of lymphocytes in comparison with cancer patient of abdomen (HLA-DRall - 26% against 11% CD38 - 25% against 13%; CD69 - 46% against 40%) and T-cells (CD3+HLA-DR+ and CD3+CD38+ - 26% against 16%; CD69+CD3+ - 32% against 26%). The expression of IL-2 receptors on NK-cells (CD25+CD16+CD56+) slightly increased on 4.4%. In cancer patients of the abdominal region increased the expression of receptor NKG2D on NK-cells (CD314+CD16+ on 9%) and CD25+ lymphocytes on 12%, while the group of melanoma patients on 8%. The marker of mature lymphocytes CD45RO+ have tendency to increase in both groups. It shows that the lymphocytes isolated from the blood of the cancer patients on day 3 of culture with IL-2 well activated *in vitro* and differentiated to mature forms. To continue the research work we should more detail study the process of activation and functional activity of T- and NK-cells.

ImmunoTools *special* AWARD for **Marizina Yulia** includes 25 reagents

FITC - conjugated anti-human CD3, CD4, CD8, CD16, CD20, CD25, CD56, CD69, CD71, HLA-DR, Control-IgG2a,

PE - conjugated anti-human CD3, CD11c, CD16, CD56, Control-IgG2a,

PerCP - conjugated anti-human CD3, CD4, CD45

ImmunoTools recombinant human cytokines: rh IFNgamma, rh IL-12, rh IL-15, rh IL-2

human IL-12p40 ELISA-set for 96 wells,
human TNFa ELISA-set for 96 wells