The Effect of T-Regulatory Cells Separation From Blood Mononuclear Cells on the Generation of Lymphokine-Activated Killers

A. O. Sitkovskaya^a, *, E. Yu. Zlatnik^a, T. V. Shamova^a, E. S. Bondarenko^a, I. A. Novikova^a, L. N. Vashchenko^a, E. E. Kechedzhieva^a, I. R. Dashkova^a, T. V. Ausheva^a, and O. I. Kit^a

^a National Medical Research Center for Oncology, Rostov-on-Don, 344037 Russia *e-mail: grankina.anastasia@mail.ru

The aim of the work was to study the effect of different concentrations of interleukin-2-2 and interferon-y on the proliferation and immunophenotype of lymphocytes obtained from patients with locally advanced breast cancer (stage II–III) after immunomagnetic depletion of T-regulatory (T-reg) cells from a common pool of lymphocytes in vitro. The peripheral blood of 11 patients was used as the material. Peripheral blood mononuclear cells were isolated and T-reg lymphocytes were removed by immunomagnetic separation. Cells after separation were cultured in RPMI-1640 culture medium with 10% calf serum for seven days. Lymphocytes were activated on the first day of cultivation with one of the following cytokines: IFN-γ(10 IU/mL), IL-2 (0.1 or 1 μg/mL); IL-2 (0.1 or 1 μg/mL) and IFN-γ together. Lymphocytes without cytokine administration served as a control. Cells were counted using an automatic counter before cytokines were introduced and after 2, 4, and 7 days of cultivation with cytokines. The lymphocyte phenotype was examined. The results showed some phenotypic differences in a number of links of cellular immunity between control and experimental samples. Particular attention is paid to the description of changes in the expression of surface markers in subpopulations of natural killers. It was noted that the percentage of T-reg cells, despite their preliminary depletion, increases after exposure to cytokines. As a result, a preliminary decrease in the proportion of T-reg cells before stimulation of lymphocytes did not show the desired effect, so the use of depletion in such a methodical mode does not lead to significant results. However, the possibility of inhibiting T-reg cells in other ways should not be ruled out.

Keywords: lymphokine-activated killers, separation of T-regulatory cells, interleukin-2-2, interferon-γ