Abstract

Tumor microenvironment is an area, where the local immunosuppressive effects dominate and prevents the immune system to perform its physiological functions. The cells infiltrating the microenvironment have an important function among many cell types since they produce a large quantity of factors suppressing the immune response. In our work, we monitored the immune changes in the microenvironment during tumor growth and chemotherapy. For these purposes, we utilized the methods for analysis of the proportion and phenotype of the distinct populations of immunocytes and for analysis of the total level of expressions of selected genes associated with immunosuppression or with distinct populations of immunocytes.

The aim of our work was to discover, using two types of mouse tumors (TRAMP-C2 and TC-1/A9), how 5-azacytidine (5AC), a cytostatic drug with epigenetic activity, affects the proportion of leukocytes infiltrating the tumor microenvironment and, further, whether these changes are accompanied by decreased expression of immunosuppressing genes. In addition, we have also focused on the changes of relative expression of genes encoding markers of lymphoid lines and, on other immunoregulating genes, encoding IL-6, IL-10, IL-12, IL-4 and IFNγ cytokines, in the microenvironment of these tumors.

Furthermore, we were interested in the effects of 5AC on tumors pretreated with the cyclophosphamide (CY), a DNA alkylating agent widely used in the treatment of tumors. A higher dose of CY suppressed the immune reaction by inducing accumulation of myeloid-derived suppressor cells (MDSC). It has been found that 5AC significantly suppressed the growth of established TRAMP-C2 tumors, while this effect was not noticed on TC-1/A9. The relative expression of immunosuppressed genes for ARG-1, iNOS, ROS and percentage of MDSC in microenvironment of both types of tumors was significantly reduced.

Combined therapy with CY and 5AC significantly inhibited growth of both TRAMP-C2 and TC-1/A9 tumors. After the treatment with CY only, accumulation of MDSC and dendritic cells was increased. In TC-1/A9 tumors, increased accumulation of tumor-infiltrating macrophages was also observed. This increased accumulation was associated with the reduction of relative expression of the immunosuppressing genes for ARG-1, iNOS and ROS. Further, subsequent therapy of CY-pretreated mice with repeated 5AC administration, led to lower accumulation of MDSC and reduced the relative expression of immunosuppressive genes.