EXPANSION OF CD62L-EXPRESSING MYELOID-DERIVED SUPPRESSOR CELLS IN INFLAMMATION-RELATED TUMOR PROGRESSION

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Chronic inflammation contributes to the formation and progression of tumors by production of suppressor mediators and activation/accumulation of immunosuppressor cells [1]. The cells are capable of creating an immunosuppressive microenvironment, which favors tumor progression. Recently, a number of studies have reported expansion of MDSCs in chronic inflammation. Mouse MDSCs are characterized as cells with CD11b+Gr1+ phenotype and represented by two main subpopulations: granulocytic (G-MDSCs) with the CD11b+Ly6G-Ly6Chigh phenotype and monocytic (M-MDSCs) with the CD11b+Ly6G-Ly6Chigh phenotype. MDSCs possess an ability to suppress functions of immunocompetent cells through multiple mechanisms [2]. According to our previous study, an increase in the proportion of G-MDSCs and M-MDSCs in mice with adjuvant arthritis was observed. We hypothesized that the MDSCs induced by chronic inflammation can promote tumor progression. The purpose of this research was to study the role and phenotypic markers of MDSCs, induced by adjuvant arthritis, in growth of a transplanted tumor.

Materials and methods: We used CD1 mice (5-6 week old males, 23-27 g body weight). The experiments included healthy mice (control animals, CA), tumor-bearing mice (tumor animals, TA), mice with chronic inflammation (arthritic animals, AA), and inflamed animals with inoculated tumor (arthritic animals with tumor, ATA). Adjuvant arthritis was induced by a single subcutaneous injection of 100 ml of complete Freund’s adjuvant (CFA) into a hind limb footpad. Gemcitabine, antineoplastic drug, was administered intraperitoneally to ATA after injection of CFA on the 7th, 10th days at the dose of 75 mg/kg, 5×10⁶ Ehrlich carcinoma cells per mice were inoculated subcutaneously 2 weeks after injection of CFA (ATA) or PBS (TA). On the day 14 after tumor inoculation, mice were sacrificed by cervical dislocation. Surgically obtained tumors and spleens were weighted. For ELISA analysis, blood samples were collected from the orbital sinus. For cytometry analysis, splenocytes were obtained by homogenization of spleen in PBS by tissue grinder and stained with fluorescent antibody according to the manufacturer’s protocols and analyzed by FACS Calibur using Cell Quest software. For in vitro experiments, MDSCs were generated in vitro from bone marrow, as described [3]. TNFa was added to experimental cell culture at the final concentration 40 ng/ml. For both cultures, Cells were maintained in DMEM, supplemented with 2 mM L-glutamine, 20 µM 2-ME, 150 U/ml streptomycin, 200 U/ml penicillin, and 10% heat-inactivated FBS, at 37°C in humidified atmosphere containing 5% CO₂ for 4 days. Suppression activity of BM-derived MDSCs against ConA-stimulated proliferation of CD8+ T cells was determined using CFSE-based assay. Significance according to Student t-test was considered with p<0.05.

Results: ATA group showed considerably faster rate of tumor growth when compared to TA group not subjected to inflamed conditions. We detected no difference in the level of serum IL-1β, IL-10, IL-6 and GM-CSF, while ATA group was characterized by increased serum levels of TNFa and S100 among all groups. Further analysis detected a significant increase in the proportion of splenic G-MDSCs and M-MDSCs in AA, TA and ATA groups when compared to the control group. Administration of gemcitabine resulted in a significantly reduced tumor growth and a number of G-MDSCs and M-MDSCs in ATA when compared to untreated mice. The analysis of the pattern of migratory molecules demonstrated that both G-MDSC and M-MDSC subpopulations obtained from ATA group were characterized by up-regulated expression of CD62L (L-selectin) when compared to CA, AA, and TA. Addition of exogenous TNFa to MDSCs generated from bone marrow up-regulated membrane CD62L on both G-MDSCs and M-MDSCs and increased the suppressive potential of MDSCs towards CD8+ T cells as revealed in the test of mitogen-activated proliferation. The obtained results imply that chronic inflammation may facilitate tumor growth via induction of TNFa-mediated expansion of CD62L-expressing MDSCs. Obtained results may indicate the advisability of applying the blockade of TNFa for the treatment of inflammatory processes associated with cancer.

References:

Keywords: chronic inflammation, CD62L, myeloid-derived suppressor cells, adjuvant arthritis.