Introduction

The efficacy of immune-modulating anti-cancer therapeutic antibodies that have been FDA-approved in recent years, such as anti-CTLA-4 and anti-PD-1, has driven growing interest in methods that provide a mechanistic understanding of drug function. Development of new mono- and combination therapies with immune-modulatory effects requires more powerful immunophenotyping techniques capable of in-depth cell characterization. To this end, using the CT.26 murine syngeneic colorectal cancer model we have developed a 10-color flow cytometry antibody panel that focuses on the identification of tumor-infiltrating immune cell subsets derived from myeloid lineage precursors using the high-throughput-capable 4-laser, 14-color Attune™ NxT Flow Cytometer. The panel includes a combination of antibodies against CD45, CD3, CD19, CD49b, CD11b, CD11c, Ly-6G, Ly-6C, F4/80 and CD115. By excluding cells of lymphoid lineage, we show that this panel facilitates analysis of myeloid-derived cells including natural killer (NK) cells, macrophages, neutrophils, dendritic cells (DCs) and monocytes or granulocytic myeloid-derived suppressor cells (mMDSCs and gMDSCs) subsets in tumor and peripheral blood. In addition, this antibody combination allows for a more complete analysis of MDSC cells which can differentially express several disease-relevant myeloid specific markers including Ly-6G, Ly-6C, F4/80, CD11c and CD115. This panel was utilized to characterize changes in the myeloid subset between control and anti-PD-L1 treated mice.

Materials and Methods

Balb/c mice (Envigo) were subcutaneously implanted with CT.26 tumor cells and treated with either anti-mPD-L1 antibody (10F.9G2; BioXCell) or anti-rat isotype control antibody (LTF-2; BioXCell). On the last day of treatment, mice were terminated for immunophenotypic analysis of tumor-derived cells and blood by flow cytometry. Tumors were processed into single-cell suspensions using gentleMACS™ Dissociators (Miltenyi Biotec). Samples were acquired on an Attune™ NxT Flow Cytometer (Life Technologies) and data were analyzed using FlowJo software (Tree Star).

Results and Conclusions

- Treatment with anti-PD-L1 increased the abundance of CD45+ cells and NK cells in tumors.
- Treatment with anti-PD-L1 altered the composition of the MDSC milieu from mMDSC dominant to gMDSC dominant.
- Treatment with anti-PD-L1 resulted in a reduction of immature dendritic cells circulating in blood and increased the number of detected NK cells.

#3242. In-Depth Myeloid Cell Characterization in the Murine Syngeneic CT.26 Colon Carcinoma Model by 10-Color Flow Cytometry

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