Characterization of the tumor microenvironment of key suppressive myeloid populations in 11 commonly used preclinical syngeneic cancer models

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Background

The immune infiltrating populations in the tumor microenvironment (TME) is crucial for therapeutic outcome. Immune infiltrating cells may be either anti- or pro-tumorigenic (typically referred to as type 1 or 2, respectively) depending on phenotypes. Comprehensive literature has been made describing what phenotypes are advantageous for survival in both clinical and pre-clinical settings. Monocytic myeloid-derived suppressor cells (Mo-MDSCs) are associated with poorer prognosis due to their potent cell-inhibitory function by secretion of arginase, suppressive cytokines, and reactive oxygen species induction.

The present work characterizes the most prevalent tumor-infiltrating myeloid cells across 11 syngeneic mouse cancer models in relation to both abundance and cellular markers associated with anti- or pro-tumorigenic function using flow cytometry.

Study design and methods

1x10^6 cancer cells were inoculated s.c. in the flank of BALB/cJRJ, C57BL/6JRJ, or DBA2 mice. In the LJ2 model, 3x10^6 cells were inoculated. Tumors included in the study ranged 100-500 mm^3 and were excised and processed for flow cytometry or IHC. Tumors were weighted and total cells in tumors were counted on a Muse Cell Analyzer. Following staining for flow cytometry, all samples were analyzed on a 4-color FACSVerse X-20 with 2 panels (samples) (see Figure 1). Fluorescence minus One (FMO) controls were done for all markers in three models.

Phenotyping:
- Tumor associated macrophages (TAMs): C11b/Cd11b/Mhc11
- Myeloid-derived suppressor cells (Mo-MDSCs): Cd11bLy6Chigh
- Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs): Cd11bLy6C^lo
- PMN-MDSCs: Cd11bLy6C^lo
- T cells: CD3^hi

Leukocyte infiltration and viability

1. Macrophages

- TAM: F4/80+Ly6C^hi
- Mo-MDSC: F4/80^-Ly6C^hi
- PMN-MDSC: F4/80^-Ly6C^lo

- Cells/100mg tumor x 10^6

Figure 2. Infiltration characteristics in syngeneic flank cancer models. A) Cell count of the population per 100 mg tumor (10^6). The numbers were calculated using a FACSVerse X20 and gate on all the viable cells and the total number of events in the scatter gate to give a cells/100 mg count for syngeneic flank cancers. B) Tumor associated Macrophages (TAM), polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) and mononuclear myeloid-derived suppressor cells (Mo-MDSCs) for the plot with 50 values of the expression of viable cells and the subpopulation viable CD45+ leukocytes in syngeneic cancer models.

Figure 3. Expression of scavenger receptors, checkpoint inhibitors and Co-stimulatory molecules across the syngeneic myeloid populations. The data was analyzed with flow cytometry and used to determine the immune infiltrating populations for several classical markers used in the evaluation of anti-tumor/suppressive state of the cells. The intensity was evaluated in tumor associated macrophages (TAM) for some of the markers the TAMs were gated out using a MHCII^high^F4/80^-^Ly6C^-^F4/80^-^. Fifty mice were used from the xenograft tumor model and data are shown to show difference in expression comparing murine (m) macrocytic myeloid-derived suppressor cells (Mo-MDSCs) and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs).

Figure 4. Expression of MHCII across the syngeneic cancer models. The data was analyzed with flow cytometry and used to determine the immune infiltrating populations for several classical markers used in the evaluation of anti-tumor/suppressive state of the cells. The intensity was evaluated in tumor associated macrophages (TAM) for some of the markers the TAMs were gated out using a MHCII^high^F4/80^-^Ly6C^-^F4/0^-^.

Summary

Characteristics of the most prevalent tumor-infiltrating myeloids subsets were investigated in 11 syngeneic cancer models commonly used for preclinical anti-cancer therapy.

CT26 infiltration spans by a factor 50 from the hottest TME in CT26 to the coldest in 4T1.

Comparable trends were observed in expression of selected markers across different cancer models.

See handouts for examples of treatment effects of radiation, chemotherapy (cisplatin), or aPD-L1 on the selected tumor models.