MOLECULAR AND FUNCTIONAL HETEROGENEITY OF MYELOID CELLS IN CANCER

ABSTRACT

Myeloid cells play an important role during tumor progression. A systemic expansion of these cells in tumor-distal organs such as the spleen, and a high infiltration in the tumor-microenvironment indicate an involvement at different levels. However, myeloid cells are highly heterogeneous, and because of this heterogeneity many of their specific properties in cancer settings remain ill-defined. This work aimed to further characterize myeloid cells in cancer, in an attempt to deepen the biological understanding of these cells and potentially provide new therapeutic opportunities. More specifically, we focused on (i) Myeloid-Derived Suppressor Cells, an immunosuppressive population that expands in secondary lymphoid organs of tumor bearing hosts and (ii) Tumor-Associated Macrophages, which are a major component of the tumor microenvironment.

The induction of CD11b(+)Gr-1(+) myeloid-derived suppressor cells (MDSCs) is an important immune-evading mechanism used by tumors. However, the exact nature and function of MDSCs remained elusive, especially because they constitute a heterogeneous population that had not yet been clearly defined. In this work, we identified 2 distinct MDSC subfractions with clear morphologic, molecular, and functional differences. These fractions consisted of either mononuclear cells (MO-MDSCs), resembling inflammatory monocytes, or low-density polymorphonuclear cells (PMN-MDSCs), akin to immature neutrophils. Interestingly, both MO-MDSCs and PMN-MDSCs suppressed antigen-specific T-cell responses, albeit using distinct effector molecules and signaling pathways. Blocking IFN-gamma or disrupting STAT1 partially impaired suppression by MO-MDSCs, for which nitric oxide (NO) was one of the mediators. In contrast, while IFN-gamma was strictly required for the suppressor function of PMN-MDSCs, this did not rely on STAT1 signaling or NO production. Finally, MO-MDSCs were shown to be potential precursors of highly antiproliferative NO-producing mature macrophages. However, distinct tumors differentially regulated this inherent MO-MDSC differentiation program, indicating that this phenomenon was tumor driven. Overall, these data refine tumor-induced MDSC functions by uncovering mechanistically distinct MDSC subpopulations, potentially relevant for MDSC-targeted therapies.

Tumor-associated macrophages (TAMs) form a major component of the tumor stroma. However, important concepts such as TAM heterogeneity and the nature of the monocytic TAM precursors remained speculative. This work now shows for the first time that mouse mammary tumors contained functionally distinct subsets of TAMs and provides markers for their identification. Furthermore, in search of the TAM progenitors, we show that the tumor-monocyte pool almost exclusively consisted of Ly6C(hi)CX3CR1(low) monocytes, which continuously seeded tumors and renewed all nonproliferating TAM subsets. Interestingly, gene and protein profiling indicated that distinct TAM populations differed at the molecular level and could be classified based on the classic (M1) versus alternative (M2) macrophage activation paradigm. Importantly, the more M2-like TAMs were enriched

in hypoxic tumor areas, had a superior proangiogenic activity in vivo, and increased in numbers as tumors progressed. Finally, it was shown that the TAM subsets were poor antigen presenters, but could suppress T-cell activation, albeit by using different suppressive mechanisms. Together, these data help to unravel the complexities of the tumor-infiltrating myeloid cell compartment and provide a rationale for targeting specialized TAM subsets, thereby optimally "re-educating" the TAM compartment.

Our results also show that the Macrophage Mannose Receptor (MMR), an endocytic membrane molecule, was expressed on TAMs but not on other cell types present in the tumor. Furthermore, the M2-like TAMs expressed the highest levels of MMR, suggesting that this is a marker that can potentially be used for the in vivo targeting of this cell population. Therefore, we have now created camelid heavy-chain antibody fragments or "nanobodies" raised against the MMR and show that these nanobodies efficiently bind to TAMs ex vivo. In addition, our results show that anti-MMR nanobodies can be used for the in vivo imaging of MMR+ cells in solid tumors using pinhole SPECT/micro-CT imaging.

Finally, our work emphasizes that monocytes play a primordial role in tumor-bearing hosts, be it as MDSCs in the spleen/circulation or as progenitors of TAMs in the tumor. A more thorough understanding of monocyte activation and function, especially in tumor settings, therefore remains important. However, the lack of a straightforward and reliable method for the isolation of mouse blood monocytes remains a hurdle. We now describe a fast and easy method for isolating monocytes from mouse blood based on immuno-magnetic labeling.

In conclusion, this work brings new insights in the biology of myeloid cells in cancer, highlighting the existence of molecularly and functionally distinct subsets of MDSCs and TAMs and potentially being relevant for future therapeutic interventions.