

Primary Research

## Mass cytometry deep phenotyping of human mononuclear phagocytes and myeloid - derived suppressor cells from human blood and bone marrow

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### Abstract

The monocyte phagocyte system (MPS) includes numerous monocyte, macrophage, and dendritic cell (DC) populations that are heterogeneous, both phenotypically and functionally. In this study, we sought to characterize those diverse MPS phenotypes with mass cytometry (CyTOF). To identify a deep phenotype of monocytes, macrophages, and DCs, a panel was designed to measure 38 identity, activation, and polarization markers, including CD14, CD16, HLA - DR, CD163, CD206, CD33, CD36, CD32, CD64, CD13, CD11b, CD11c, CD86, and CD274. MPS diversity was characterized for 1) circulating monocytes from healthy donors, 2) monocyte - derived macrophages further polarized in vitro (i.e., M - CSF, GM - CSF, IL - 4, IL - 10, IFN -  $\gamma$ , or LPS long - term stimulations), 3) monocyte - derived DCs, and 4) myeloid - derived suppressor cells (MDSCs), generated in vitro from bone marrow and/or peripheral blood. Known monocyte subsets were detected in peripheral blood to validate the panel and analysis pipeline. Then, using various culture conditions and stimuli before CyTOF analysis, we constructed a multidimensional framework for the MPS compartment, which was registered against historical M1 or M2 macrophages, monocyte subsets, and DCs. Notably, MDSCs generated in vitro from bone marrow expressed more S100A9 than when generated from peripheral blood. Finally, to test the approach in vivo, peripheral blood from patients with melanoma ( $n = 5$ ) was characterized and observed to be enriched for MDSCs with a phenotype of CD14<sup>+</sup>HLA - DR<sup>low</sup>S100A9<sup>high</sup> (3% of PBMCs in healthy donors, 15.5% in patients with melanoma,  $P < 0.02$ ). In summary, mass cytometry comprehensively characterized phenotypes of human monocyte, MDSC, macrophage, and DC subpopulations in both in vitro models and patients.

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