A method for non-overlapping identification of human myeloid derived suppressor cells

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Abstract

The function of human myeloid derived suppressor cells (MDSCs) cannot be precisely measured without the proper identification of homogeneous populations. Indeed, many published accounts of human MDSCs are really describing multiple distinct populations confused either by sample processing, poor gating strategies, and/or lack of appropriate cell surface markers. To improve the standardization of the identification of human myeloid derived suppressor cells and other myeloid phenotypes, we developed a method for the identification and analysis of human myeloid populations by the use of several 10-color flow cytometric protocols in combination with novel software analyses. This method utilizes the direct staining of peripheral blood that allows for the quantitation of myeloid cells and the delineation of non-overlapping immunophenotypes. The 10-color protocols, tested in both healthy volunteer controls and cancer patients, allow us to define diverse phenotypes that include mature monocytes, granulocytes, circulating dendritic cells, immature myeloid cells, in addition to MDSCs. We demonstrate that human MDSC subsets fall into three distinct populations: $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes, $LIN^{-}CD33^{+}HLA\text{-}DR^{-}$ cells, and $CD15^{+}$ granulocytes. We additionally identify CD123 as a marker uniquely expressed on $LIN^{-}CD33^{+}HLA\text{-}DR^{-}$ MDSCs. Our method permits us to measure myeloid and MDSC phenotypes in relation to total mononuclear cells, total myeloid cells, total leukocytes and as cell counts. The data generated from our method will allow for more uniform reporting of myeloid and MDSC phenotypes for biomarker development.

Methods

Whole blood immunophenotyping and flow cytometry:

100 ul of fresh whole blood from healthy volunteers (HVs) and cancer patients was added to 50 ul mouse serum (Sigma-Aldrich) prior to staining to block non-specific antibody binding. Immuno-staining and lyse/wash steps were performed as previously reported[1,2] with the exception that RBCs were lysed with Versa-Lyse (Beckman Coulter). Peripheral blood mononuclear cells were isolated by density gradient centrifugation. Samples were run on the Gallios flow cytometer (Beckman Coulter). List Mode Data files analyses including multi-parameter radar plots and bivariate plot overlays were performed using Kaluza software (Beckman Coulter). Data graphing and statistical analyses were performed using Prism v5.0 software.

Protocol	FL1-FITC	FL2-PE	FL3-ECD	FL4-PC5.5	FL5-PC7	FL6-APC	FL7-AA700	FL8-AA750	FL9-Pac Blu	FL10-KrO
Myeloid	LIN2*	CD123	HLA-DR	CD11c	CD11b	CD33	CD16	CD66b	CD15	CD45
Myeloid-2	CD16b	CD14	HLA-DR	CD33	CD11b	LIN2*	CD16	CD66b	CD15	CD45

Table 1.Gallios protocols for ten color antibody staining of human myeloid cells. *LIN2 cocktail includes CD3, CD14, CD19 and CD56.

Results

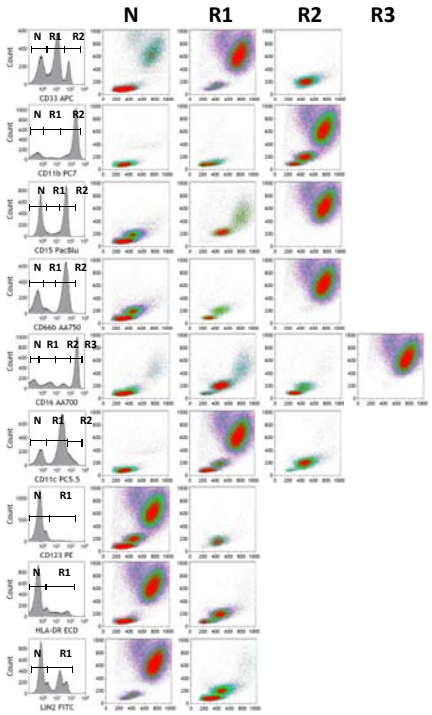

Figure 1. Characterization of myeloid marker staining patterns on whole blood. Peripheral blood from a healthy control sample was processed via the lyse/wash Myeloid protocol. Histograms were generated from each of 9 antibodies used to delineate myeloid populations. In most cases regions (R1, R2, and R3) were created for each peak of expression including peaks with no expression (N). Other regions represent populations of cells falling between the negative/positive peaks or populations that do not have a clear peak. Density gradient plots were created from cells gated from each region plotted by forward and side scatter. A representative example from a healthy volunteer is shown.

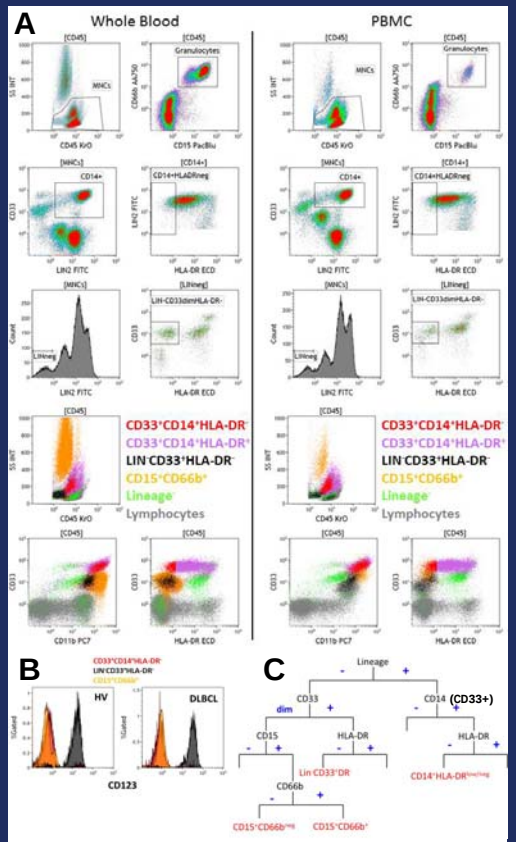

Figure 2. Delineation of human MDSC phenotypes. A. Gating strategies of MDSCs. Peripheral blood and PBMCs from a healthy volunteer (HV) sample was processed via the lyse/wash Myeloid protocol. Each MDSC population and other indicated populations were colored and plotted on the same dot plot with commonly used markers for MDSC identification (CD33, CD11b, HLA-DR). **B.** Representative histograms of CD123 expression on 3 MDSC populations from a HV and a patient with diffuse large B cell lymphoma (DLBCL) **C.** Schematic diagram of the phenotypic relationships of MDSCs.

References

1. Gustafson MP, Lin Y et al. Strategies for improving the reporting of human immunophenotypes by flow cytometry. J ImmunoTher Cancer 2014; 2:18.
2. Gustafson MP, Lin Y et al. A method for identification and analysis of non-overlapping immunophenotypes in humans. PLoS One 2014;

Conclusions

- **Our ten color flow panel allows for the quantification of distinct, non-overlapping human MDSC phenotypes as well as other myeloid cell phenotypes.**
- **Reporting results in absolute values (in contrast to relative fractions of parent/grandparent populations) is critical to the use of immune phenotypes as biomarkers.**