Profiling immune cell populations in the tumor microenvironment with complementary capillary-based and single-cell Western assays

Charles H. Haitjema[1], Rainer Grant[2], Lindsay Wentz[2], Stefan Luhowskyj[2], Jeff Cooper[2], Annegret Boge[1], Kathy Brumbaugh[2], Kelly Gardner[1], Christopher D. Heger[1]

[1]ProteinSimple, a Bio-Techne Brand, 3001 Orchard Parkway, San Jose, CA 95134, USA; [2]Bio-Techne, 614 McKinley Place NE, Minneapolis, MN 55413, USA

Poster # 1596

INTRODUCTION

The tumor microenvironment (TME) is a complex mixture of cancerous and non-cancerous cells, including immune cells like T-cells, macrophages, and neutrophils. The TME plays a key role in tumorigenesis and metastasis, and it has recently been recognized that it can dramatically shape a response to therapy. Thus, there is a pressing need to accurately identify and quantify the variety of cell types in any given TME. However, studying the TME presents major challenges. For example, the heterogeneity of the environment requires sensitive and high-resolution techniques to parse subpopulations of different cell types. This challenge is compounded by the severely limited sample size that can be obtained from donor tissues. To address these challenges, we use an in-capillary immunoassay with small sample sizes (3 µL) to identify immune cells commonly found in the TME. We also leverage single-cell Western to uncover trends in population heterogeneity. Human peripheral blood mononuclear cells (PBMCs) were differentiated into dendritic cells (DCs) and regulatory T cells (Tregs), and natural killer (NK) cells were expanded from isolated NK cells. These samples were then analyzed by in-capillary immunoassay and single-cell Western. These analyses revealed the identification and characterization of cell types, at both the single-cell and population level, based on the differential expression of protein biomarkers. Specifically, in-capillary immunoassay analysis identified mature populations by CD209 for DCs, a CD56+/CD3- phenotype for NK cells, and CD25 and Foxp3 expression for Tregs. Analysis of single cells provided further detail within these populations, for example, we observed FoxP3^{low} and FoxP3^{high} subpopulations in Tregs, and an unexpectedly large (81%) CD56-/CD3- subpopulation in undifferentiated PBMCs, suggesting the presence of other cell subtypes. We anticipate that the small sample size, automation, single-cell resolution, and multiplexing ability of these assays collectively will enable a more efficient and deeper characterization of the TME not possible with traditional immunoassays like Western blot and flow cytometry.

METHODS

An overview of the cell-type specific differentiation and identification workflow used in this study is shown in **Figure 1**. All cell types were analyzed by single-cell Western assay (Milo) and in-capillary immunoassay (Wes).

A pool of PBMCs was differentiated to DCs (left), NK cells (middle) and Tregs (right). The same sample of differentiated cells was analyzed on Wes and Milo for markers indicative of the differentiated cell type. PBMCs were differentiated to DCs by isolation of CD14+ monocytes, followed by induction and expansion to DCs (left). NK cells were expanded from isolated NK cells (middle). PBMCs were differentiated to Tregs by isolation for naïve CD4+ T cells, followed by induction and expansion to Tregs (right).

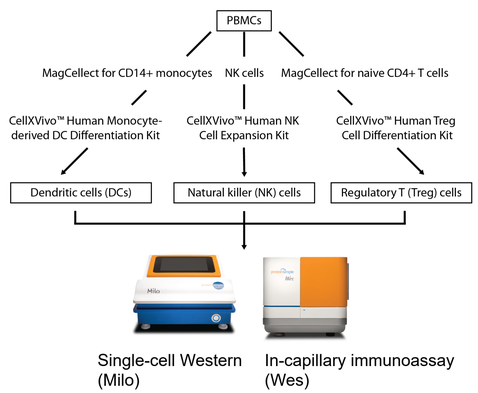
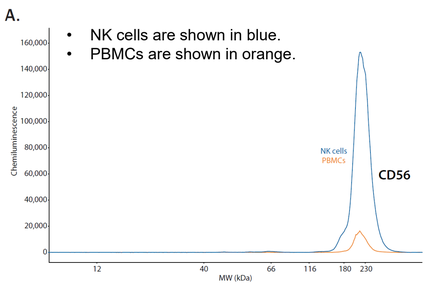


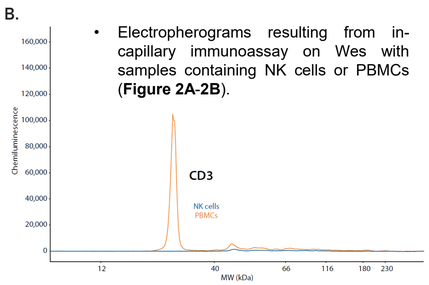
FIGURE 1. Experimental workflow performed in this study.

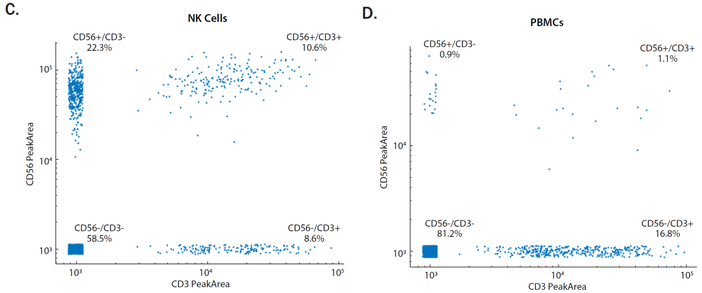
RESULTS

Natural killer cells

CD56 is the archetypal phenotypic marker of NK cells, and NK cells do not express cell-surface CD3. To test this, we probed NK cells and PBMCs with antibodies targeting CD56 and CD3 (**Figure 2**).







The single-cell analysis revealed subpopulations not revealed by the in-capillary immunoassay, including an unexpectedly large (81%) CD56-/CD3- subpopulation in undifferentiated PBMCs, suggesting the presence of other cell subtypes (**Figure 2C-2D**).

FIGURE 2. Identification and characterization of NK cells on Wes (A-B) and Milo (C-D). NK cells yield a stronger CD56 signal compared with PBMCs when 0.5 mg/mL of cell lysate for each sample is loaded (A). CD3 is detected in PBMCs but not in NK cells (B). Single-cell Western analysis showed that 22% of NK cells were enriched for CD56+/CD3- (C). 16.8% of PBMCs are enriched for CD56-/CD3+ (D). The largest cell subset revealed within both NK and PBMC samples of 10,000 cells/mL analyzed is of the CD56-/CD3- phenotype (C and D).

Dendritic cells

DCs are characterized by the absence of CD14, and the presence of the CD209 receptor, which bind several pathogens. To test this, we probed DCs and CD14+ untreated cells with CD14 and CD209 (**Figure 3**).

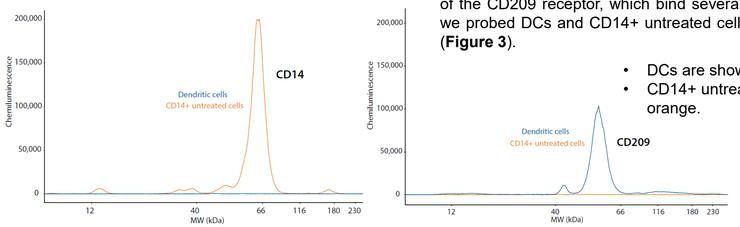


FIGURE 3. Monocyte-derived DC marker expression analysis on Wes. CD14 positive monocytes were enriched from PBMCs and differentiated into mature DCs. DCs were profiled according to the absence of CD14 expression and presence CD209 when 0.5 mg/mL of each cell sample lysate was loaded. The electropherograms show that CD209 (right) are enhanced in DCs compared with the CD14+ monocyte cell sample control. Conversely, and as expected, CD14 is expressed in the monocyte sample, but not in the differentiated DC population analyzed (left).

RESULTS (CONT.)

Dendritic cells (cont.)

- As with NK cells, single-cell analysis revealed subpopulations that were not detectable by in-capillary immunoassay (**Figure 4**). In addition to CD209, we probed DC markers CD80, CD86, and CD83.

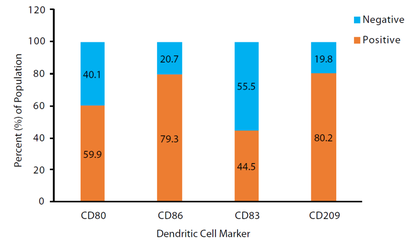


FIGURE 4. Characterization of monocyte-derived DCs on Milo. Bar graph defining the subpopulations of DCs that are positive for CD80, CD86, CD83 and CD209. Histone H3 was used as a loading control to determine the total number of cells in the population.

Regulatory T cells

- Lane view resulting from in-capillary immunoassay on Wes using samples containing PBMCs and Tregs (**Figure 5**).
- FoxP3 is a master regulator for the development and function of Treg cells, which also express CD25 and CD4.

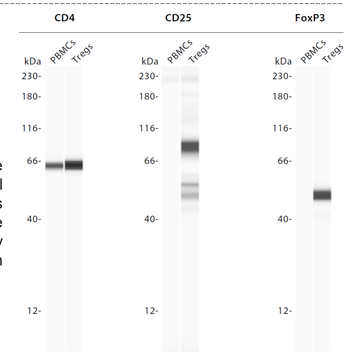


FIGURE 5. Treg marker expression analysis on Wes. CD4+ T cells were enriched from PBMCs and differentiated into Tregs, 0.2 mg/mL of each cell sample lysate was loaded for analysis of FoxP3 and 0.04 mg/mL for analysis of CD25 and CD4. CD4 expression is detected in PBMCs, but much more so post-enrichment in the Treg population (left). Tregs are characterized by the presence of FoxP3 and CD25 markers, which were not detected in PBMCs (middle, right).

- Again, single-cell analysis revealed subpopulations in these samples that were not detectable by in-capillary immunoassay, including FoxP3^{low} and FoxP3^{high} subpopulations (**Figure 6**).

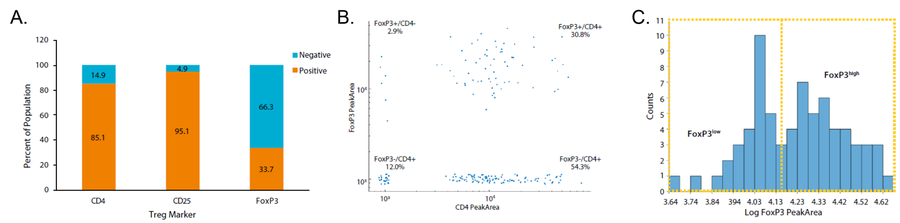


FIGURE 6. Characterization of CD4+ PBMC-derived Tregs on Milo. Bar graphs illustrating the quantification of cell subpopulations present within our Treg sample according to CD4, CD25 and FoxP3 (A). Two-dimensional scatter plot of the subpopulations present based on the presence or absence of FoxP3 and CD4 (B). Histogram showing classification of Treg populations based on intracellular FoxP3 expression (C).

CONCLUSION

In this study, DCs, NK cells, and Treg cells were detected and characterized with antibodies to cell-specific biomarkers. In-capillary immunoassay and single-cell Western analysis were complementary techniques to profile cells commonly found in the tumor microenvironment. In-capillary immunoassay had the advantage of small sample size (3 µL) and automation. Single-cell Western analysis could reveal subpopulations within samples that were not detectable by in-capillary immunoassay.

CONTACT: charles.haitjema@bio-techne.com

bio-techne.com