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Identification and characterization of an unusual monocyte subpopulation

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Abstract

Personalized immunotherapy for cancer has become an effective treatment choice in certain oncology indications. To successfully develop such therapeutic candidates requires a deep understanding of the biology including the interaction between a variety of hematopoietic cells, mesenchymal cells, and the microenvironment of bone marrow and peripheral blood. Flow cytometry offers unparalleled advantages in immunophenotyping, functional analysis, and receptor occupancy determination. For example, flow cytometry is capable of simultaneously analyzing millions of leucocytes and identifying populations of regulatory T cells, cytotoxic T cells, B cells, NK cells, monocytes, macrophages, and dendritic cells with multiple surface and intracellular biomarkers. Because of the diversity of clinical samples, variable protocols, and storage conditions, new challenges arise during the development and flow cytometric analysis of immunotherapeutic drug candidates. One of these challenges is the observation, upon flow cytometric analysis, of an unexpected pattern of immune cells. For example, monocytes are one of the important components in the immune system, but they are also readily modulated during an immune response. In our clinical studies, we found that some peripheral whole blood samples exhibit an extra population paralleling normal monocytes during flow cytometric analysis. To correctly categorize these cells, we applied a panel of 18 fluorochrome conjugated monoclonal antibodies, such as CD34, CD11c, CD1b, to define this uncharacterized cluster. Peripheral whole blood samples were maintained at ambient temperature as well as refrigerated at 4˚C. The monocyte subpopulation was most evident in samples maintained at refrigerated temperature. We used a gating strategy to separate the two clusters, normal monocytes and the additional high forward scatter (FSC) monocyte subpopulation in FCS Express analysis software. Using custom designed data analysis templates, we performed side-by-side comparison of these two populations. The results indicated that the two clusters demonstrated a similar profile in this panel. In conclusion, we found that this extra cluster phenotyped as an altered subpopulation of monocytes, and was most evident following cold stimulation. The significance of this monocyte subpopulation remains unknown at the current time. The importance of panel design in flow cytometry was highlighted in this work. The results and subsequent bioinformatics proved informative in characterizing this unusual subpopulation by profiling an array of cellular biomarkers.

Advanced single cell assay by flow cytometry: opportunity and challenges in immunotherapy study

Figure 1. Multiple channels in Flowcytometry. The system is capable of detecting multiple parameters (including FSC and SSC) for a single cell.

Advantages:

1. High speed analyses (100,00 events/droplets per second.
2. Measures single cells and a large number of cells.
3. Simultaneous analysis of multiple parameters.
4. Identification of rare populations, e. g. tetramer assay.
5. Quantification of fluorescence intensities.

Disadvantages:

1. Complex instruments are prone to problems.
2. Need single cell suspension.
3. Expensive.
4. No standard reference or normal range.
5. Massive data set with too many possible combinations.

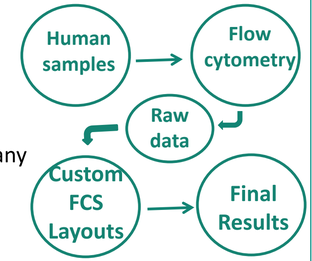


Figure 2. Complex system . This ecosystem has never been seen before. During the long process of a clinical study the line between machine generated data and human produced data is increasingly blurred.

Defining unknown population by a panel of antibodies

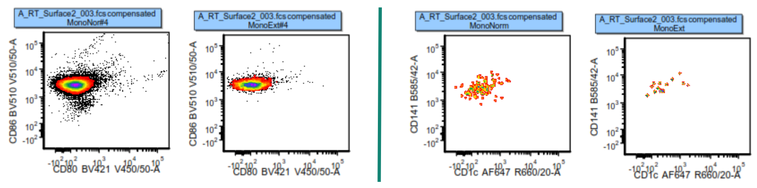
Antibodies	Fluorochrome	Source
CD11b	PerCP-Cy5.5	BioLegend
CD11c	PE	Biolegend
CD123	AF647	BD
CD14	APC	BD
CD141	PE	BD
CD15	PerCP-Cy5.5	BD
CD16	BV421	BD
CD1b	FITC	BD
CD1c	AF647	BD
CD33	BV421	BioLegend
CD34	PE-Cy7	BD
CD56	FITC	BD
CD64	BV510	BioLegend
CD80	BV421	BD
CD86	BV510	BD
PD-1 (CD279)	PE	BD
HLA-DR	BB515	BD
HLA-DR	APC-H7	BD
HLA-DR	PerCP-Cy5.5	Biolegend
CD14	PE-Cy7	BioLegend
CD3	APC-H7	BD
Lin3 Cocktail	FITC	BD
CD45	V500	BD

A. Antibody list for the study

B. Panel design

TUBE	Staining	FITC	PE	PERCP	PE-Cy7	APC	APC-H7	BV421	V500
1	FMO	HLADR BB515				CD14		CD16 BV421	CD45
2	Surface1	CD1b	CD11C	CD11b	CD34 PE-Cy7	CD14	HLADR	CD16 BV421	CD45
3	Surface2	Lin3	CD141	HLADR	CD14	CD1c AF647	CD16	CD80 BV421	CD86 BV510
4	Surface3	CD56	PD-1	CD15	CD14	CD123 AF647	HLADR	CD33 BV421	CD64 BV510

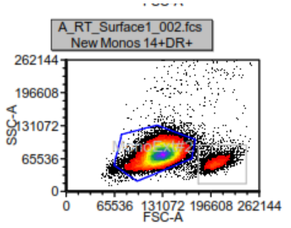
Figure 4. Applying a panel to define the target population. Because of the size of unknown population, several Abs specific for stem-cell properties were included to check if these cells are stem-like.



Biodiversity of samples in clinical study

In clinical studies the biodiversity of samples is a common phenomenon because of the diversity of genetic background, variable protocols, and storage conditions. One of these challenges is the observation, upon flow cytometric analysis, of an unexpected pattern of immune cells. For example, monocytes are one of the important components in the immune system. The biologic functions of these cells interacting within living individual makes them highly adaptive.

Figure 3. Extra population of CD14+. Some peripheral whole blood samples exhibit an extra population paralleling normal monocytes during flow cytometric analysis.



Clinical study: different solutions for a variety of challenges

Challenges	Solution	Outcome
Unexpected CD14+ population (this study)	Panel of antibody and custom FCS layout analysis	Monocytes showing biodiversity, similar characteristics as regular monocytes.
Unexpected CD8+ lymphocytes	Run two scenarios of analyses, to include and to exclude the population	The unexpected CD8+ lymph were demonstrated to be part of a normal population

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